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SPECIAL ISSUE

THE NEW HARVEST

Plant translational biology gives fresh skills to familiar crops

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Camelina sativa seeds (length: 2 to 3 millimeters) in their pods, floating in oil extracted from the seeds. This antioxidant-rich oil can become a source of heart-healthy omega-3 fatty acids. Research on *Camelina* and other plants is exploring the means to improve food quality, build sustainability, discover pharmaceuticals, and reduce the environmental impact of deriving specialty chemicals. For more on challenges and opportunities in plant translational biology, see page 1218. *Photo: © Sasha Gitin*

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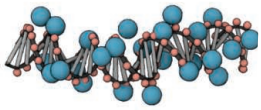

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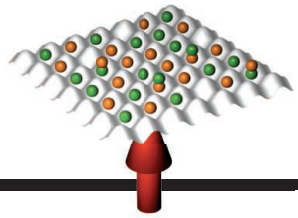

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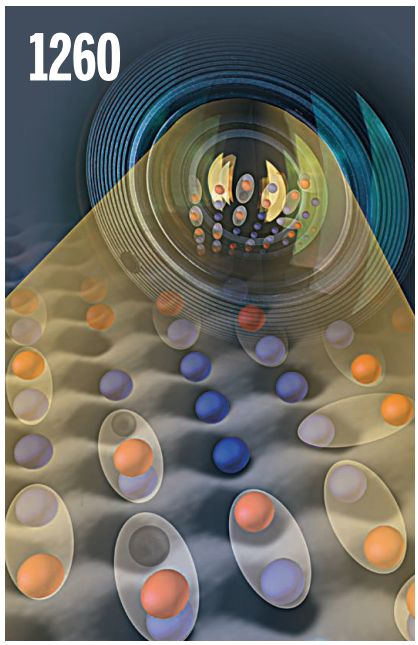

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EDITORIAL

GM crops—lessons from medicine

Earlier this year, the U.S. National Academy of Sciences released a report on genetically engineered crops that is comprehensive but offers few recommendations in terms of regulatory guidance. Although it acknowledges that a process-based regulatory system is becoming less technically feasible, the report suggests that regulators focus on safety aspects of technology when considering approval. We believe that products of new technologies should be regulated not only on the basis of their benefit-risk profiles, but also on their societal context and need.

Currently, a new crop variety is handled under European Union regulations dependent on the process used to generate it. A conventionally bred crop primarily has to show that the variety is uniform, distinct, and stable, whereas a genetically modified (GM) crop has to undergo an additional evidence-based risk assessment. With the advent of new technologies, such as genome editing with the clustered regularly interspaced short palindromic repeats (CRISPR)–Cas system, the boundaries between GM and non-GM techniques will become increasingly blurred, and in many cases there will be no way to tell whether a variety was arrived at by conventional breeding and/or use of new methods.

In Canada, a trait-based regulatory system is used in which the actual trait, such as drought or disease resistance, rather than the method used to derive it, is the basis for regulation. Such a trait-based system is analogous to the regulation of new agents in medicine, which takes into account the context in which the product will be applied. For example, therapeutic antibodies for diseases as diverse as cancer and arthritis are not regulated simply on the basis that they are antibodies—rather, they are assessed in terms of the proteins they target, the benefit to patients, and the risks of adverse events. The focus is on a benefit-to-risk ratio that is reassessed throughout the life cycle of the product (as evidence accumulates). With new medicines for life-threatening diseases, there will usually be a greater acceptance of risk in the absence of existing effective treatments—that is, the consequences of doing nothing are taken into account. Therefore, patients, as well as regulators, accept a lower benefit-to-risk ratio than would be considered appropriate for a disease that is self-limiting and rarely life-threatening, such as the common cold.

"...a contextual framework... should aid the translation of new research into application."

The contextual notion used in regulating new medicines may also be helpful in debates around the assessment of new varieties of crops or other engineered products. It is important to consider their benefit-to-risk ratio in the context of the likely harm of making no intervention to combat the problem that the new product is aimed at solving, such as fungus resistance. Context might also change with time. For example, the risk posed by doing nothing in terms of the threat of swine fever, and therefore the acceptability of approving disease-resistant pig strains, might be very different if there were a low incidence of the disease and the existence of a vaccine to prevent it, versus a situation in which there was a high incidence of a virulent strain causing the disease and no vaccine.

Of course, in many cases, the evidence provided will not be complete because proving absence of adverse effects is subject to "real-life" data collection, as is the case in the postapproval surveillance process of new medicines. Indeed, absence of evidence is not evidence of absence. However, such a contextual framework will at least facilitate a more constructive debate that is more consistent with other forms of regulation in Europe and elsewhere and which should aid the translation of new research into application.

–Jackie Hunter and Gordon Duff

Jackie Hunter is the chief executive officer of Benevolentai Bio Ltd., London, UK, and is the former chief executive of the U.K. Biotechnology and Biological Sciences Research Council. Email: jackie.hunter@benevolent.ai

Sir Gordon Duff is principal of St Hilda's College, University of Oxford, UK; was formerly chair of the U.K. Medicines and Healthcare products Regulatory Agency; and is chair of the U.K. Biotechnology and Biological Sciences Research Council. Email: principal@st-hildas.ox.ac.uk

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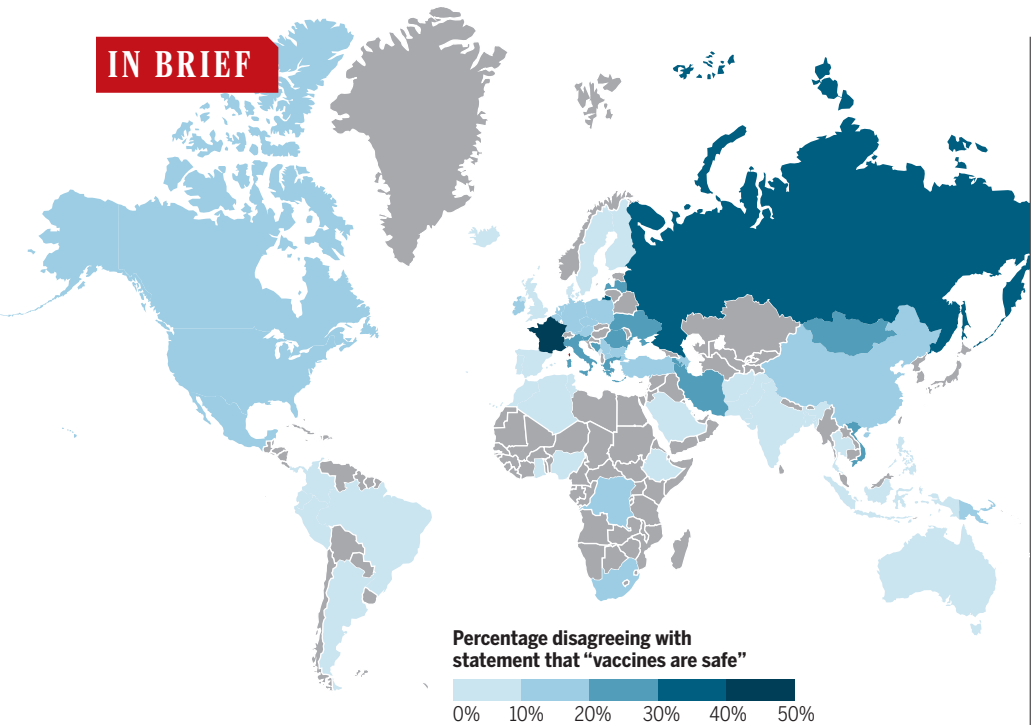
121,000 : 1

Odds against space having any spin axis or other special direction, according to a new study of the cosmic microwave background (*Physical Review Letters*). **http://scim.ag/spacedirect**

IN BRIEF



France most skeptical of vaccine safety

The French are less confident in the safety of vaccines than the residents of 66 other countries, a new survey suggests. A team led by anthropologist Heidi Larson of the London School of Hygiene & Tropical Medicine conducted what it contends is "the largest survey on confidence in immunization to date," interviewing more than 65,000 people between September and December 2015. Some 41% of respondents in France disagreed with the assertion that vaccines are safe, the researchers reported online last week in *EBioMedicine*, compared with just 12% of respondents in other nations, on average. "I didn't expect France to be as negative as it was," says Larson, who runs The Vaccine Confidence Project, a nonprofit that monitors public concerns about immunization. However, Larson notes that France recently has experienced "anxiety" about suspected but unproven links between, for example, the hepatitis B vaccine and multiple sclerosis. Meanwhile, Russians had the greatest skepticism about the importance of vaccines for children, at 17.1%. Bosnia and Herzegovina had the most serious doubts about the effectiveness of vaccines, with a whopping 27.3% of respondents expressing a "negative sentiment." Both numbers were about three times higher than the global average. **http://scim.ag/vaccsafemap**

AROUND THE WORLD

Mixed marks for monkey meeting

BETHESDA, MARYLAND | Depending on whom you ask, a 7 September National Institutes of Health workshop on the ethics of nonhuman primate research was either a raging success or a complete fiasco. The event—requested by Congress in response to a campaign by People for the Ethical Treatment of Animals—brought together dozens of scientists, veterinarians, and bioethicists to discuss how research on monkeys and related animals contributes to human medicine—and to review ethical policies related to this research. Proponents of animal research hailed the workshop as "a great showcase of the importance nonhuman primates have played and continue to play in human health," but animal rights groups called it "a wasted opportunity" that did not address Congress's concerns about the use of these animals in biomedical research. **http://scim.ag/NIHmonkeyethics**

Debate over new Nobel Center

STOCKHOLM | A plan to build a major new home for the Nobel Prize in central Stockholm is creating controversy. The eight-story, $141 million building, designed by U.K. architect David Chipperfield, would replace a historic customs house built on the Blasieholmen peninsula in 1876—and, critics say, would ruin Stockholm's skyline. Currently, the Nobel Museum and Nobel Library share space in a small former stock exchange building in Stockholm's old town. Stockholm's City

An artist's conception of the planned Nobel Center.

CREDITS: (MAP) J. YOU/*SCIENCE*; (MAP DATA) HEIDI LARSON *ET AL.*, *EBIOMEDICINE*; (PHOTO) HANDOUT/REUTERS/NEWSCOM

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Planning Committee approved the plan last spring, but a protest group is appealing the decision in court; the group, which has the support of Swedish King Carl XVI Gustaf, also planned to hold a demonstration at the proposed building site on 16 September. The center will be the venue of the Nobel Prize ceremony, held every year in December, but will also host a museum, exhibitions, meeting spaces, and a restaurant; proponents say it will become a major draw for Stockholm. Construction is scheduled to begin next year and finish in 2019. http://scim.ag/NobelCtr

Medical isotope shortage looms

WASHINGTON, D.C. | Global supplies of a vital medical radioisotope could founder when an aging research reactor ceases routine production next month. The 59-year-old National Research Universal (NRU) reactor near Chalk River, Canada, is one of a handful of facilities that produces molybdenum-99 (Mo-99); its decay product, technetium-99m, emits gamma rays that can be used to image the brain, heart, and other organs; it is used in roughly 80% of all nuclear medicine procedures worldwide. In a 12 September report, a U.S. National Academies of Sciences, Engineering, and Medicine committee warns of a "substantial likelihood" of severe supply shortages until new sources of Mo-99 come online around 2018. After October, the panel notes that the Canadian government will keep NRU on "hot standby," allowing it to be a "supplier of last resort" until permanent shutdown in 2018. Complicating matters, about 75% of Mo-99 now is made by irradiating weapons-grade uranium targets. Efforts by European suppliers over the next 2 years to convert to low-enriched uranium targets could further crimp Mo-99 supplies, the panel warns.

Nobelist against supercollider

BEIJING | Nobel laureate and particle physicist Chen Ning Yang has come out strongly against plans to build the world's largest particle accelerator in China. The Circular Electron Positron Collider (CEPC) is a contender to succeed the Large Hadron Collider (LHC) in Geneva, Switzerland. But debate is intensifying over whether the CEPC's science questions are compelling enough to justify the estimated $6 billion price tag, 70% of which China would bear, with as-yet unidentified partners covering the rest. On 4 September, Yang posted an article on the social media platform WeChat, stating that the science does not justify the cost, as the LHC confirmed the existence of the Higgs boson but has not discovered new particles or inconsistencies in the standard model of particle physics. The prospect of an even bigger collider succeeding where the LHC has failed is "a guess on top of a guess," he wrote. Wang Yifang, director of the Chinese Academy of Sciences's Institute of High Energy Physics in Beijing, which is leading the charge for the CEPC, responded on the institute's public WeChat account, criticizing Yang for rehashing arguments made in the 1970s against building a much smaller collider in Beijing.

PHOTO: KEN BOHN/SAN DIEGO ZOO GLOBAL

The 'alalā uses and makes tools, like its distant cousin the New Caledonian crow.

Crowing about a new tool-using bird

An endangered Hawaiian crow has joined a very select group: The 'alalā (*Corvus hawaiiensis*) is one of fewer than a dozen bird species known to use tools. 'Alalās, which are extinct in the wild and survive only in captivity, are distantly related to the famous tool-using New Caledonian crows (*C. moneduloides*). Researchers tested 104 of 109 captive 'alalās for their tool-using smarts; most of them (78%) spontaneously used sticks to extract food from crevices in pieces of wood, much as New Caledonian crows use their tools to pry grubs and insects from rotted logs. Many of the Hawaiian crows also shortened the sticks the scientists supplied to make more suitable tools, and some manufactured their own tools by cutting sticks from ferns and bushes, as do the New Caledonian birds. The scientists don't think the 'alalās acquired their talent while in captivity, but instead use tools as part of their natural behavior; all the tested adults adeptly wielded their sticks, and young chicks picked up the skill without observing the adults or being trained. The discovery, reported in this week's issue of *Nature*, may help scientists understand the evolution of tool manufacture and use, which is extremely rare in the animal kingdom.

NASA may rejoin LISA mission

ZURICH, SWITZERLAND | Earlier this year, scientists announced they had detected gravitational waves—Einstein's ripples in spacetime. Similarly, ripples are now reverberating through NASA, nudging the agency to mend fences with the European Space Agency (ESA) and rejoin an ambitious mission, called the Laser Interferometer Space Antenna (LISA), to study gravitational waves from space sometime after 2030. Last week, at the 11th LISA symposium in Zurich, Switzerland, Paul Hertz, director of NASA's astrophysics division, said he was ready to rejoin the LISA mission, which the agency left in 2011. The mission includes three separate spacecraft, flying millions of kilometers apart from each other and monitoring the distance between them using sensitive lasers. Tiny distance changes between them would reveal low-frequency ripples in spacetime—emitted by, for example, orbiting supermassive black holes at the centers of distant galaxies. LISA was conceived as a $4 billion joint ESA-NASA

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mission, but NASA dropped out because of budgetary problems. ESA gave the green light to a trimmed-down €1 billion version of LISA, dubbed eLISA, in 2013, with the launch expected in 2034. The United States planned to kick in about $150 million in technical support, but in 2015, a National Academy of Sciences decadal report strongly recommended that NASA rejoin the project as a partner. http://scim.ag/rejoinLISA

NEWSMAKERS

Three Qs

Astronomers have detected more than 3000 planets beyond our solar system—but most are unlikely to harbor life. Recently, theoretical physicist **Claudius Gros** of Goethe University Frankfurt in Germany argued in *Astrophysics and Space Science* that we should put it there ourselves. *Science* spoke with Gros about his proposed Genesis Project, which would send artificially intelligent (AI) probes to lifeless worlds to seed them with microbes that might evolve into multicellular organisms over millions of years, and, perhaps eventually, into plants and animals. http://scim.ag/seedlifeQA

Q: What would starting microbes look like?
A: AI could create specifically adapted microbes for each planet's conditions, like a very hot planet would be given extremophiles. Or, the AI could just send down the same kind of microbes on many planets. The first would have better survivability, but the second would have more opportunities to branch off and create different species—though many would perish in harsher climates.

Q: Why is AI important?
A: The robots will have to decide if a certain planet should receive microbes and the chance to evolve life. The AI would be aboard the [smartphone-sized] Genesis probe, [which] would fall into orbit around the planet and, after double checking the planet was lifeless, begin the seeding process. Microbes would be inside small capsules and shot down to the exoplanet's surface. First, the AI would seed with photosynthesizing microbes. When oxygen levels were high enough, eukaryotic microbes would be sent down, too.

Q: What organisms would you like to see evolve from these seeds?
A: The dream would be something intelligent. There is a theory that we became an intelligent species when we began to develop language. So I think that any animal that would evolve to be what is referred to as intelligent life would have to be a social being.

Psittacosaurus's skin pigments hid it from forest predators.

Dinosaur 'wore' forest camouflage

Cleverly placed skin pigmentations help many modern animals hide from predators in plain sight—and that was true in the Cretaceous, too. Now, researchers studying the pigmentation patterns preserved in the 120-million-year-old fossil of a small horned dinosaur called *Psittacosaurus* report that it had a kind of camouflage called countershading, with darker coloring on the back and lighter on the belly. The team, which included a paleoartist, painstakingly reconstructed the animal's shape and pigmentation to create a detailed, life-sized, 3D model from the 2D fossil, and then observed the play of shadows over the model in different lighting environments—a bright, open savanna versus the dappled light of a forest. Its pigmentation, they report this week in *Current Biology*, would have made it particularly hard to see in a forested environment. http://scim.ag/3Ddinocamo

They said it

The nonprofit ScienceDebate.org asked the U.S. presidential candidates 20 questions about science issues. This week, they answered. Two excerpted responses to a question on how to "improve federal research and our public health system" are below. Want more? Visit http://scim.ag/scidebate2016.

"In a time of limited resources, one must ensure that the nation is getting the greatest bang for the buck. We cannot simply throw money at these institutions and assume that the nation will be well served."

Donald Trump (R)

"We are not investing in public health preparedness and emergency response in the way we should to keep our families and communities safe. ... I will create a Public Health Rapid Response Fund."

Hillary Clinton (D)

CREDITS: (ILLUSTRATION) JAKOB VINTHER, UNIVERSITY OF BRISTOL AND BOB NICHOLLS/PALEOCREATIONS. COM; (PHOTOS, TOP TO BOTTOM) MICHAEL VADON; GAGE SKIDMORE/WIKIMEDIA COMMONS

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Burning pig carcasses failed to support the government's contention that 43 students were incinerated.

PHOTO: SCHOOL OF CIVIL ENGINEERING, THE UNIVERSITY OF QUEENSLAND

FORENSIC SCIENCE

Pyre experiments cast doubt on students' fate

Findings suggest investigators in Mexico should take new tack in unsolved case

By **Lizzie Wade**, *in Mexico City*

In September 2014, 43 university students disappeared in Guerrero state in southern Mexico. The Mexican government has maintained that a drug cartel murdered the students and burned their bodies at a trash dump. But forensic investigators and human rights groups were doubtful, citing gaps in the evidence and a federal investigation that they contend fell short of international standards. Now, a renowned fire scientist says his latest experiments rule out the government's explanation once and for all.

Using pig carcasses as a proxy for human bodies, José Torero, a fire scientist at the University of Queensland, St. Lucia, in Brisbane, Australia, incinerated up to four pigs at a time and determined that the inferno necessary to consume 43 bodies could not have occurred at the dump. "José knows what he's doing," says John Lentini, an independent fire investigator in Islamorada, Florida, who wasn't involved in the research but has participated in other high-profile cases. "It doesn't make any sense that you can make 43 people disappear like that."

Torero's experiments "are one more element that says the so-called 'historical truth'"—how a former attorney general labeled the government's theory of the crime—"is impossible," says Francisco Cox Vidal, a lawyer in Santiago and member of an expert group (known in Spanish as the GIEI, or Interdisciplinary Group of Independent Experts) convened by the Inter-American Commission on Human Rights in Washington, D.C., to examine the disappearance and the official inquest. Eber Betanzos Torres, deputy attorney general for human rights, did not respond to requests for comment.

> ***"It doesn't make any sense that you can make 43 people disappear like that."***
>
> **John Lentini**, independent investigator

The missing students studied at the Ayotzinapa Normal School, a rural teacher's college near Tixtla in Guerrero. On 26 September, a larger group of Ayotzinapa students hijacked commercial buses to travel to a protest here in Mexico City, a common practice at the politically active school. According to confessions from members of the Guerreros Unidos cartel, the gang, abetted by local police, ambushed the students, possibly mistaking them for members of a rival cartel. Some students were killed by gunfire, some escaped, and 43 were kidnapped and allegedly executed. Cartel members said they incinerated the bodies in a municipal dump outside the town of Cocula. Six weeks later, federal investigators said they had found bags of human remains, burned to ash, in the dump and in a nearby river.

The ash was sent to a lab at the University of Innsbruck in Austria. Almost all the organic matter in the ash had burned away, but the lab eventually recovered DNA of two missing students in remains from the river. In April, the lab announced that it had not been able to link any of the other 41 students to the remains, nor to hair and clothing samples recovered from the dump.

From the start, doubts plagued the attorney general's reconstruction of events. Basic facts, even the number of buses commandeered, differed in the official reports and the escaped students' own accounts. (GIEI has theorized that unbeknownst to the students, one of the hijacked buses may have been used for drug running.) A group monitoring the investigation on behalf of the victims' families has questioned the provenance and chain of custody of the ash bags. Human rights groups also suspect that the cartel members' confessions were

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extracted under torture. Faced with international criticism, the Mexican government agreed to allow GIEI to investigate.

The expert group called on Torero. Born in Peru and trained at the University of California, Berkeley, Torero has investigated many high-profile fires, including those that brought down the Twin Towers in New York City. The cartel members had testified that they incinerated the bodies on a pyre of wood and tires in the open air. Torero's calculations suggested that fully incinerating 43 bodies in the manner the cartel described would have required a staggering amount of wood: between 20,000 and 40,000 kilograms. He also doubted that it would be possible to nearly eliminate organic matter from the remains with an open-air fire, rather than with a furnace. And when he visited the Cocula dump in July 2015, he saw no evidence of a massive fire. He concluded that it was impossible the students had been burned there (*Science*, 11 March, p. 1141).

In an 8 June report, the attorney general's office called for experimental verification. Torero independently took up the challenge. He and a dozen students simulated the alleged pyres at Cocula in a field at his university's Gatton campus, outside Brisbane. They used bone-dry wood, stacked precisely, and left out tires, which would have made the fire less efficient. The experimental setup, Torero says, represented "the ideal scenario."

His team systematically burned pig carcasses. Even when using 630 kg of wood for a single 70-kg pig, 10% of the pig's flesh remained after the fire burned out, Torero told *Science*. Forty-three bodies of a similar weight, therefore, would have required more than 27,000 kg of wood, and organic matter would have survived the fire. Even if the cartel had been able to find that much wood in Cocula, the intense bonfire would have scarred nearby tree trunks, Torero says. Visiting the dump 10 months after the disappearances, he saw no such scars.

Torero also burned up to four pig carcasses at once to explore whether body fat would fuel the fire and promote total incineration. Each added carcass weakened fire intensity, the team found. Burning 43 bodies together, therefore, would require much more wood than burning each separately. "Bodies are a large percent water," Lentini says. "They're not great fuel."

Torero plans to submit his findings for peer review in the fall. In the meantime, he hopes his experiments will prod investigators in the grisly case to move beyond Cocula. "We should stop looking into the dump," Torero says, "because that's not what happened." ■

Turkey has closed Fatih University in Istanbul and more than a dozen others.

PHOTO: AP PHOTO/LEFTERIS PITARAKIS

ACADEMIC FREEDOM

Turkey shakes up universities as coup fallout continues

Grants are frozen, campuses closed, and staffers fired

By **John Bohannon**

For Turkish researchers and educators, the aftershocks of the failed 20 July military coup are continuing. After it crushed the uprising, the government launched a political purge, firing or suspending some 50,000 public employees, including many academics. Now, just weeks before the start of the academic year, Turkey has launched a broader reorganization of higher education.

On 1 September, using powers granted under an ongoing state of emergency, the government fired an additional 2346 university staff and revamped the employment contracts of some 10,000 teaching assistants at public universities, most of them Ph.D. students; the assistants are now contract employees instead of permanent hires, making them easier to fire. Officials have also closed 15 private universities and launched a fresh wave of firings at secondary schools. Turkey's science funding agency, TÜBİTAK, has postponed new calls for proposals. And to the dismay of international collaborators, officials shut down a major archaeological dig.

There was one sign that the turmoil may be abating: Officials reinstated 1386 of the 1577 university deans they had suspended after the coup. Still, "this purge in academia will have dire consequences for science in Turkey," predicts Mehmet Ali Alpar, a physicist at Sabancı University in Istanbul, Turkey.

The government says the changes will help root out members of an Islamic group led by Fethullah Gülen, a preacher now living in the United States, who they allege organized the coup. Ironically, Alpar notes, Turkish President Recep Tayyip Erdoğan was once allied with Gülen's movement, which "has infiltrated the police, army, judiciary, and universities systematically."

Many Turkish academics describe an atmosphere of suspicion and paranoia. "All of us who are for science and reason are afraid of losing our jobs," says one university biologist, who requested anonymity because he fears reprisals. Officials recently escorted him into a campus office for what he calls a "ridiculous interrogation." A lawyer representing the university read a series of questions about his ideology and politics, and the dean of his department and faculty from several others listened to his answers. Officials later notified the biologist that he was cleared to work, but he was denied permission to attend two scientific conferences in Europe where he was planning to present his research. "I am at the very center of the chaos," he says.

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One of the biologist's friends, an archaeologist, didn't fare as well. She was dismissed because she earned high marks last year on a national exam, he says. Turkish media have reported that the answers to the exam were distributed in advance to Gülen followers. Ipso facto, those who scored well on the exam are Gülenists, the biologist says. "But I know that she is a highly intelligent person and that is the only reason for her high grade."

Scholarly societies outside Turkey are closely watching events. At a 6 September meeting in Brussels, members of the European University Association (EUA) raised concerns about academic freedom with officials from Turkey's Higher Education Council (YÖK), and questioned them about university closures. "YÖK has reassured us that all [students from the closed private universities] have the option of being transferred to public institutions," says Michael Gaebel, EUA director of higher education policy in Brussels. However, "given the start of the new academic year, and the relatively large number of students, this is of course a big challenge." He says more changes are in the works, including plans to merge three universities in Izmir into a gigantic new public university.

Most critics of the government within Turkey have gone silent, but some academic organizations are speaking out. The government is using "the purge of Gülenists as a pretext to reorganize universities," reads a statement issued last week by Turkey's Association of University Councils. The statement cites the suspension of TÜBİTAK fellowships and funding programs "in addition to the ongoing unlawful sackings and intimidations [of] scientists." Alpar, who is president of the Turkish Science Academy, says the turmoil will end up "discouraging young researchers from staying in Turkey or returning."

Archaeologists, meanwhile, are absorbing the government's surprise decision to shut down a long-running international dig at Ephesus, a Greek and Roman site near Selçuk, as well as a smaller excavation in nearby Limyra. Turkish officials notified the Austrian Archaeological Institute in Vienna, which has been involved in the Ephesus project for more than a century, that they had withdrawn permits. They gave no reason, but relations between Turkey and Austria have been strained since Austria's chancellor, Christian Kern, said in August that Turkey was not fit to join the European Union. Austrian science minister Reinhold Mitterlehner bemoaned the breach in a 4 September statement. "With this step," he said, "the freedom of science is continuing to decline." ■

With reporting by Erik Stokstad.

SCIENTIFIC MISCONDUCT

Former star surgeon's disgrace rocks Swedish science

Investigations into the Paolo Macchiarini scandal highlight misconduct and institutional failings

By **Gretchen Vogel**

What seemed a coup has turned into a nightmare for Sweden's most prestigious university, the Karolinska Institute (KI). In 2010, KI and its associated hospital in Stockholm managed to recruit star surgeon Paolo Macchiarini, who had made international headlines when he implanted artificial windpipes into patients. With his groundbreaking tissue engineering work, KI leaders hoped he would propel the university to the top of a hot field.

Instead, Macchiarini has plunged Swedish science and KI into their most serious misconduct scandal in decades, with allegations ranging from faking scientific data to subjecting patients to a risky procedure without the necessary approvals, in at least two cases leading to their deaths. Over the past 2 weeks, four investigations have released their damning findings. One confirms that Macchiarini committed misconduct, and the other three paint an unflattering image of the roles KI and the hospital have played in the affair. The entire episode "is a sad failure of the academic community to take care of its own mistakes," says Hans Rosling, a retired international health professor at KI.

The case has already led to a wave of resignations and dismissals, both at KI and the Nobel Assembly, the august body of 50 KI professors that selects the winners of the Nobel Prize in Physiology or Medicine. But restoring trust won't be easy. A recent survey by a leading Swedish polling company found that KI's reputation had plunged as a result of the scandal.

Macchiarini created artificial tracheae to help patients with a missing or damaged windpipe. He "seeded" them with a patient's own stem cells, hoping the cells would proliferate and cover the artificial scaffold to create a fully functional organ. But the implants didn't work. Two of his Stockholm patients died; a third has been in intensive care since 2012. Several Russian recipients died as well.

In 2014, colleagues from the hospital asked KI to investigate possible misconduct in Macchiarini's papers and in the ethics and consent documents for the surgeries. The recipients were not as seriously ill as Macchiarini claimed, they said, and his descriptions of patients' improvement after surgery were falsified. KI officials asked Bengt Gerdin, a retired professor of surgery at Uppsala University in Sweden, to investigate. Gerdin found that the allegations had merit, but KI stood by its wunderkind: In August 2015, then–Vice-Chancellor Anders Hamsten said that Macchiarini's rebuttal to the report was convincing and dismissed the charges.

The case was rekindled in January by a three-part TV documentary that painted a troubling picture of Macchiarini's treatment of patients in Sweden and Russia and the way KI had handled the allegations. After the final episode aired, KI announced it would cut ties with Macchiarini when his contract ended in November and asked the Swedish Central Ethical Review Board (CEPN) to reinvestigate the misconduct charges. It also commissioned an independent investigation into its own role, as did the hospital. In March, the university disciplinary board decided it had learned enough about Macchiarini's conduct to fire him.

The hospital's report was made public

Shakeup in Stockholm

Since February, the following people were dismissed (●), resigned (●), or were asked to resign (●) as a result of the Macchiarini scandal.

KAROLINSKA INSTITUTE	STATUS
Anders Hamsten, vice-chancellor	●
Hans-Gustaf Ljunggren, dean of research	●
Jan Carlstedt-Duke, adviser to the vice-chancellor	●
Paolo Macchiarini, senior researcher	●
Lars Leijonborg, chairman of the board	●
All other board members	●

NOBEL ASSEMBLY	STATUS
Urban Lendahl, secretary-general	●
Anders Hamsten, member	●
Harriet Wallberg-Henriksson, member	●

SWEDISH HIGHER EDUCATION AUTHORITY	STATUS
Harriet Wallberg-Henriksson, chancellor	●

DATA: KAROLINSKA INSTITUTE/NOBEL ASSEMBLY

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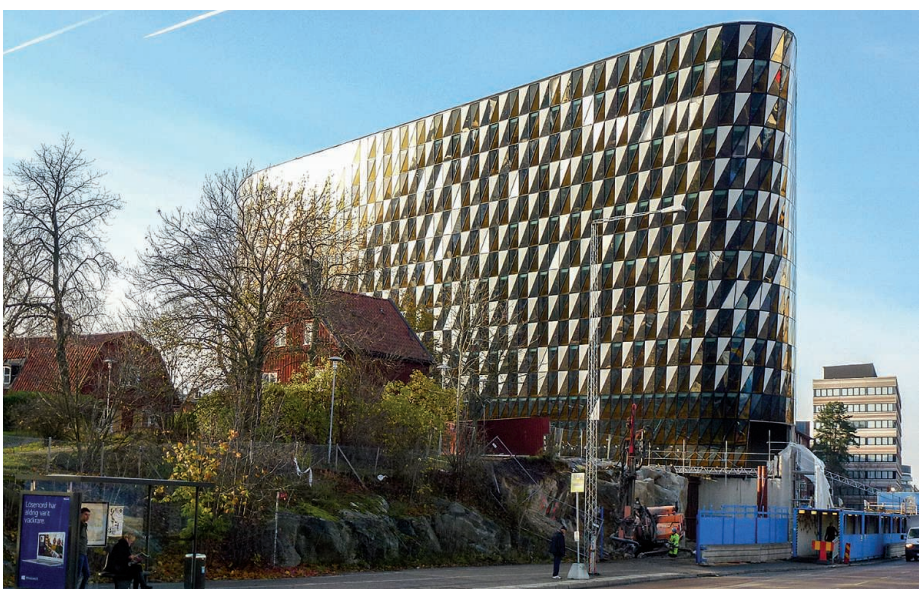
on 31 August, and KI's own report a few days later. Both concluded that Macchiarini should never have been hired; administrators ignored "strikingly negative" references from Macchiarini's previous employers as well as irregularities in his CV. Once he was on board, the reports found, loose oversight allowed him to skirt ethical regulations. "There was a stunning lack of interest in learning more about his work before extending his contract," said Sten Heckscher, a former president of Sweden's Supreme Administrative Court who headed the KI investigation.

In response to the reports, Swedish Minister for Higher Education and Research Helene Hellmark Knutsson fired the country's chancellor in charge of all public universities, Harriet Wallberg-Henriksson, who was KI's vice-chancellor when Macchiarini was hired. The minister also dismissed all remaining KI board members who were active during Macchiarini's tenure. (Five board members had already stepped down.) Nobel Assembly leaders asked Wallberg-Henriksson and Hamsten to resign their memberships.



The Karolinska Institute in Stockholm fired Paolo Macchiarini last March.

Meanwhile, a CEPN report issued on 9 September found scientific misconduct in a paper on rat esophagus implants that Macchiarini published in *Nature Communications* in 2014. Among other problems, the panel found no data to support the claim that the recipient animals had recovered and gained weight. (The journal says it's investigating.) As *Science* went to press, the agency was about to release another report, on papers describing the artificial scaffolds. Conclusions about two key *The Lancet* papers describing successful human implant surgeries are expected later this year.

Macchiarini, who faces criminal inquiries, including possible manslaughter charges, did not respond to *Science*'s requests for comment last week. Swedish television, however, reported on 5 September that he again denied any wrongdoing.

Swedish scientists are left wondering how things went so terribly wrong. Macchiarini was exceptionally persuasive, notes Anders Ekbom, a clinical epidemiologist at KI. In January, *Vanity Fair* reported that Macchiarini had told an NBC news producer with whom he had an affair that he had treated many world leaders, including two popes; he also convinced her that Pope Francis would marry them. Rosling says the *Vanity Fair* story helped KI leadership see that they, too, had been deceived: "It pinpointed that there was a deep personality problem with Macchiarini."

Another part of the problem, according to the KI report, was "a growing fixation on excellence" at the university and "the aspiration to close the gap between research and its application in healthcare." The fixation is natural, Rosling says: "There is nothing more wasteful and boring than mediocre research. We need spectacular new research findings," he says. But although hiring Macchiarini may have been understandable, he says, "the serious thing here is the failure to rectify the mistake."

Ekbom hopes the dismissals and resignations will give KI a fresh start. "If something good comes out of it—better routines, better safety—the net outcome after this drama will be a better Karolinska," Gerdin adds. "That is what we hope." ■

BIOMEDICINE

Cystic fibrosis foundation opens drug discovery lab

Funded by a drug royalty bonanza, lab seeks novel ways to target mutations

By **Bijal P. Trivedi**

The Cystic Fibrosis Foundation (CFF) is once again breaking new ground. In 2000, with little beyond symptomatic relief available for the inherited, life-threatening condition, the Bethesda, Maryland–based foundation hired a biotech company to develop more effective treatments. The move, unprecedented for a disease advocacy organization, paid off in two new drugs, the first to target the molecular root of the disease. But there is still no cure, and cystic fibrosis (CF) patients continue to die—467 in the United States alone in 2014. So on 19 September, the foundation is setting another precedent by officially opening its own independent laboratory in Lexington, Massachusetts, to speed drug development, funded primarily from the success of the two drugs it helped bring to fruition.

The goal of the new CFF Therapeutics Lab, says Preston W. Campbell III, the foundation's CEO and president, is to generate and share tools, assays, and lead compounds, boosting its partners' chances of finding treatments. Frustration with academic technology transfer agreements was a key motivation, he notes. University-based researchers funded by the foundation have to seek approval from their institution's legal department before sharing assays, cells, or any intellectual property, a hurdle that can take a year to negotiate. "This was killing us," Campbell says, "[but] if we created our own laboratory, we could not only focus on the things we wanted to focus on, we could also share them freely."

Margaret Anderson, executive director of FasterCures, a Washington, D.C.–based think tank and arm of the Milken Institute, believes other disease philanthropies will be watching the experiment. "I'm excited … and I would venture to say that the disease foundation community is going to be excited about it, too."

CF, which afflicts 30,000 people in the

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PHOTO: AINALI/WIKIMEDIA COMMONS

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For many with cystic fibrosis, available treatments only address symptoms, such as lung mucus buildup.

United States and some 70,000 worldwide, results from mutations in the gene for the CF transmembrane conductance regulator (CFTR)—a cell membrane protein that is crucial for salt-water balance in the lungs, pancreas, and gastrointestinal (GI) tract. The most common of the 1800 or so known mutations disable the ion channel, causing viscous mucus to clog the lungs and leading to serious infections and—without a lung transplant—early death.

The picture started to brighten with the arrival of the two drugs that CFF codeveloped with a California firm later acquired by Vertex Pharmaceuticals, headquartered in Boston. Orkambi, approved by the Food and Drug Administration in 2015, partially corrects a misfolding in the CFTR protein in people who carry two copies of F508del, the most common and deadly CF mutation. But Orkambi doesn't work alone—it requires another drug to make the CFTR protein functional. Kalydeco, approved in 2012, opens ion channels wedged shut by a rarer mutation, but it is also given with Orkambi. In 2014, CFF sold the rights to future royalties for these drugs to Royalty Pharma for $3.3 billion—money that it plans to fold back into research. Part of this windfall is funding the new lab, which has a budget of $6 million for 2016.

Its research is potentially critical because although the two recent drugs are game changers for some with CF, neither cures the disease, and they help only about half of all CF patients (see chart, right). In addition, both are extremely expensive, costing hundreds of thousands of dollars a year or more per patient—a price tag that has raised eyebrows, given CFF's role in their development.

To seek out new and better therapies, the lab—which has been up and running since December 2015—devotes about 70% of its activity to original research, with the remainder centered on developing resources for the rest of the scientific community. Its first priority is screening compound libraries to find a second generation of drugs targeting the F508del mutation; at least 90% of CF patients carry a single copy of this mutation.

The lab's director, Martin Mense, is an electrophysiologist who began studying the CFTR protein at The Rockefeller University in 2000 and then ran CF biology programs at two biotech firms. His team is also using high-throughput screening to identify compounds that suppress "nonsense mutations," which truncate the CFTR protein enough to cause the ion channel to malfunction. "We definitely have a number of molecules with interesting activity," says Mense, who is hoping to find something that corrects multiple CF nonsense mutations. That's a practical approach, because for some mutations the patient populations are so small that it is unrealistic to develop a drug for each one. If the lab succeeds, the benefits would go beyond CF, Campbell says, as many other diseases are caused by similar mutations.

The lab now employs about 23 scientists, drawn from both academia and the drug industry, but it has room for twice that number. Mense and William Skach, vice president of research affairs at CFF who leads its basic research, drug discovery, and preclinical therapeutic development, plan to add staff to bolster the lab's expertise in gene editing and stem cells. They envision editing stem cell genomes to reproduce rare patient mutations; the altered cells would then be differentiated into airway epithelium cells and used to screen for promising drug candidates.

Skach is also hoping to explore possible treatments: extracting patients' own stem cells, editing out their CF mutation, and repopulating their lungs, pancreas, and GI tract with healthy cells. Ultimately, he would like to edit out the mutation right in a patient's body. "If we can use gene-editing tools to fix the mutations in CF patients, then we can basically cure CF once and for all. That's where we want to go," he says. The foundation is moving swiftly in that direction. In 2015, CFF funded 50 labs and several companies to develop editing technology for the condition. "This is all rather futuristic by today's terms," Mense admits. "But you have to think a little bit ahead."

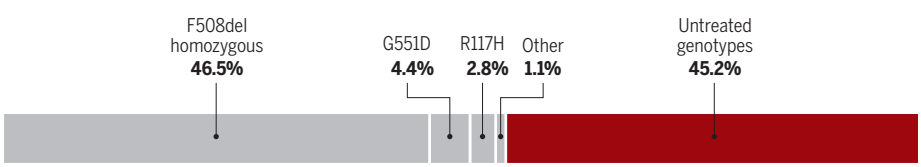
The new lab's in-house expertise will help the multibillion-dollar foundation evaluate the many outside projects it is asked to fund each year, says Eric Olson, the former head of Vertex's CF program, who oversaw the development of Kalydeco and Orkambi. "If you don't have people who have done those kinds of experiments, generated that kind of data, know the pluses and minuses of the experimental system, it is tougher to do true due diligence and evaluate," says Olson, now chief scientific officer at Syros Pharmaceuticals in Cambridge, Massachusetts, and a member of the board of trustees for CFF and the CFF Therapeutics Lab.

The foundation says it has no intention of developing drugs from scratch and stresses that it doesn't want to crowd other academic labs or companies out of CF research with its new venture. "We don't care who's successful," Campbell says. "We want success." ■

Bijal P. Trivedi, a writer based in Washington, D.C., is working on a book on cystic fibrosis.

A drug cabinet half-empty

Although two recently developed cystic fibrosis drugs can treat several mutations causing the disease (gray), nearly half of the people in a 2014 patient registry had mutations that don't benefit from either drug.



CREDITS: (PHOTO) BURGER/PHANIE/SCIENCE SOURCE; (DATA) CYSTIC FIBROSIS FOUNDATION PATIENT REGISTRY, 2014

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Zimbabwe is eager to sell its copious stockpile of ivory.

PHOTO: AP PHOTO/TSVANGIRAYI MUKWAZHI

CONSERVATION BIOLOGY

Study takes sharp tusk to effort to legalize ivory trade

Slow-growing elephants can't be harvested sustainably

By **Virginia Morell**

Is killing elephants—legally—the best way to save them? The controversial idea will get a hearing next week at a major conservation meeting in South Africa, where elephant-rich African nations will renew a push to scrap a long-standing global ban on ivory sales and replace it with a limited legal trade in tusks taken from carefully managed elephant populations. A legal market, they argue, will undermine the poaching that is depleting herds and provide a financial incentive for protecting them.

There's just one problem, a new study says: African elephants grow and reproduce too slowly to support a robust global trade. "The demand for ivory is simply too great; it outstrips what elephants can produce," says biologist David Lusseau of the University of Aberdeen in the United Kingdom, the study's lead author. Trying to essentially farm African elephants for their tusks will likely "kick them into extinction," he says.

The study is certain to fuel debate at the 17th meeting of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which runs from 24 September to 5 October in Johannesburg, South Africa. Many African herds are in serious danger: A recent survey of savanna elephant populations estimated that poachers killed 30,000 animals annually between 2007 and 2014, reducing the population to fewer than 400,000. Overall, researchers estimate that African elephant numbers have plummeted more than 95% over the past century.

In the late 1980s, similar trends prompted CITES members to institute the ivory ban. It passed over the objections of southern African nations with relatively healthy elephant populations, which argued for a regulated trade. But with the exception of limited ivory sales that CITES approved in 1999 and 2008, the ban has held firm and has helped rebuild some populations despite the overall decline.

Next week, three nations—Zimbabwe, Namibia, and South Africa—are expected to offer proposals for restarting a legal ivory trade. All argue that some elephant populations are healthy enough to be managed for ivory production. The proposals envision taking tusks from both animals that are intentionally killed—sometimes because they become nuisances, trampling crops and threatening people—and those that die naturally.

The idea prompted Lusseau and animal behaviorist Phyllis Lee of the University of Stirling in the United Kingdom to develop a model to predict how much ivory might be sustainably harvested. Because they doubt that naturally dead elephants could fill the demand, they based their model on hunting. Using fertility, mortality, and growth data collected in Kenya's Amboseli National Park, where researchers have studied elephants since 1972, they constructed a virtual herd of 1360 elephants. Then they calculated how much ivory the herd could sustainably produce under various harvest scenarios.

Not much, the researchers report in the 15 September issue of *Current Biology*. In a best-case scenario, in which neither poachers nor a natural disaster like a drought shrinks or stresses the herd, only 100 to 150 kilograms of ivory could be harvested annually. That's roughly equivalent to removing just a single large male each year.

Any attempt to increase or even maintain ivory production would run into two problems, Lusseau says. One is that managers would "quickly run out of the biggest males [which produce the most ivory], and so you have to take several smaller animals to harvest the same amount of ivory," leading to a kind of death spiral. Another is that large males are typically 45 to 55 years old, so it takes decades to replace them.

Such issues mean ivory yields from managed herds would be "far below" current global demand, estimated at 210 metric tons annually, predicts Samuel Wasser, a conservation geneticist at the University of Washington, Seattle. "It becomes clear that a legal ivory trade could never be sustainable," Wasser wrote in an email. Even at low harvests, managed herds would ultimately face extinction.

Advocates for legalization say the study has flaws. One is that Amboseli's relatively small elephant herd is "far from the real situation" elsewhere in Africa, says Rowan Martin, a wildlife consultant in Ruwa, Zimbabwe. (He is part of Zimbabwe's CITES team and helped craft its legalization proposal.) Instead, he says, modelers should draw data from managed populations of some 20,000 animals, like those in southern African. He estimates that by culling females, managers could skew the herds in favor of males and in 40 years boost ivory production to 17,500 kg annually, mostly from natural deaths.

Martin's large herd assumption is "not at all realistic," Lee responds. Zimbabwe, she notes, had some 82,000 elephants according to the recent census, but many of the animals "live in small herds of only 5000 to 7000" animals each, closer to the study scenario.

Other researchers fear that even discussing legalization will make matters worse for elephants. "It gives a positive signal to would-be buyers and the chain of criminals involved in the trade," says elephant behaviorist Joyce Poole, who co-directs ElephantVoices, a conservation group in Nairobi. Lee says: "We should retain the ban, and eliminate the demand, not try to fill it." ■

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SCIENTIFIC PUBLICATIONS

In Iran, a shady market for papers flourishes

New law would tame the practice of selling theses and research articles—if enforced

By **Richard Stone**, *in Tehran*

"Can you write me a thesis?" asks the woman, who has called a number from a flier taped to the main gate of Iran's prestigious University of Tehran. The woman, an actress, is posing as a botany graduate student from Islamic Azad University (IAU), Abadeh, in Fars province. Her topic is the flora of the Khuzestan region, she explains with a Fars accent to the salesman at the other end of the line. He obligingly lays out a schedule for delivery of thesis chapters. "If your subject doesn't need lab work," he says, the cost will be a mere 1.8 million tomans ($600), plus another $400 if she desires a paper, published under her name in a reputable journal.

The firm is one of a veritable army of outfits in Iran that offer to write theses and scientific papers for a fee, advertising on the internet, through fliers, and via the placard-carrying touts who line the sidewalk outside the University of Tehran. The actress, a movie star in Iran, was helping out a friend at the university who is infuriated with the firms—but the call she made on his behalf was hardly a sting. The transactions may be unethical, but they are legal. For now.

This autumn, Iran's parliament, the Majlis, is expected to take up work on a bill that would outlaw shady practices in scientific publishing. That's none too soon, says Javad Rahighi, director of the Iranian Light Source Facility here. "It's very bad for Iran's science image," he says. "This is one of the problems of an oil and gas country," adds Sorena Sattari, Iran's vice president for science and technology. "We think we can buy everything."

It's unknown how many papers and theses are ginned up under false pretenses. In 2014, a member of Iran's Academy of Sciences estimated that each year as many as 5000 theses—roughly 10% of all master's and Ph.D. theses awarded in Iran—are bought from dealers. In a recent Google search, Behzad Ataie-Ashtiani, a civil engineering professor at Sharif University of Technology here who has shined a light on the practice (*Science*, 18 March, p. 1273), says he found 330,000 links to paper sellers in Farsi. He estimates there are at least a couple of thousand such operations in Iran. Iranian scientists publish about 30,000 papers a year in international journals, a 20-fold increase since the 1979 revolution (*Science*, 4 September 2015, p. 1029). Purchased publications "damage the reputation of large numbers of Iranian scientists who don't cheat, and erode the trust of the international scientific community," possibly endangering collaborations, says Hossein Akhani, a biologist at the University of Tehran.

Iran's paper bazaar echoes a similar phenomenon in China, where firms sell authorship slots on manuscripts (*Science*, 29 November 2013, p. 1035). But Iran's industry has its own roots. Since 1979, the government has opened scores of universities, among them IAU and Payame Noor University, both of which have hundreds of branches across the country. Many politicians and diplomats solicit advanced degrees from these universities without earning them, says one scientist here who has tracked the issue. "They need someone to sell them ISI [Institute for Scientific Information] papers or theses." Possessing an advanced degree allows well-connected individuals to land important jobs, and by law credentialed employees earn a higher salary for the same job description. "Now, getting a degree this way is very fashionable," Rahighi says.

Ghost authorship is often hard to detect because many paper-writing companies strive to produce quality work. The authors apparently are often experts in the subject material who are adept at avoiding plagiarism and even conduct experiments when needed to complete the job.

A call to a different firm, this time by a University of Tehran professor posing as a biochemistry graduate student, revealed a meticulous approach. The firm, in its flier, advertises a knack for placing manuscripts in journals published by Springer and Elsevier. But there's no guarantee, the salesman says. "If you use unreal data … your work will be invalid and they will find it out," he warns. The salesman offers to share the supplicant's data with "a specialist in your field of study, to see what we can do." Publication in an ISI journal, he says, will cost at least 5 million tomans ($1660), with 30% down and the precise amount fixed after expert evaluation.

It does not surprise Rahighi that scientists are involved in the trade. Science jobs are scarce in Iran, leading many able young graduates to drift into the paper-selling business, he says. "These are parasitic jobs, and an unhealthy way of doing things." But he questions whether many paper buyers really benefit. "If you can't find a job with a good thesis," he asks, "how can you find one with a bad thesis?" The problem, he predicts, should go away after Iran's economy improves, bringing more job opportunities in science.

Although paper sellers brazenly ply their trade just outside the University of Tehran, scientists here think the problem is most acute at second-tier institutions. Universities with weaker standards should do a better job vetting theses, Rahighi says. "Examination systems should be set up in such a way to detect students who purchase their thesis."

The proposed law, if enforced, should solve the problem decisively. Drafted by the science ministry, the bill would make it a crime to produce academic works "for another party who wants to use the work to achieve an official educational degree or to get credit for promotion in academia." It calls for financial penalties and even jail time for paper sellers and customers. But the bill's fate is uncertain: One issue, Iranian scientists note, is whether legislators who obtained degrees in this manner will recuse themselves from the upcoming debate. ■

Along a row of bookshops just outside the main gate of the University of Tehran, touts openly advertise for firms that write scientific papers and theses.

PHOTO: FARHAD BABAEI

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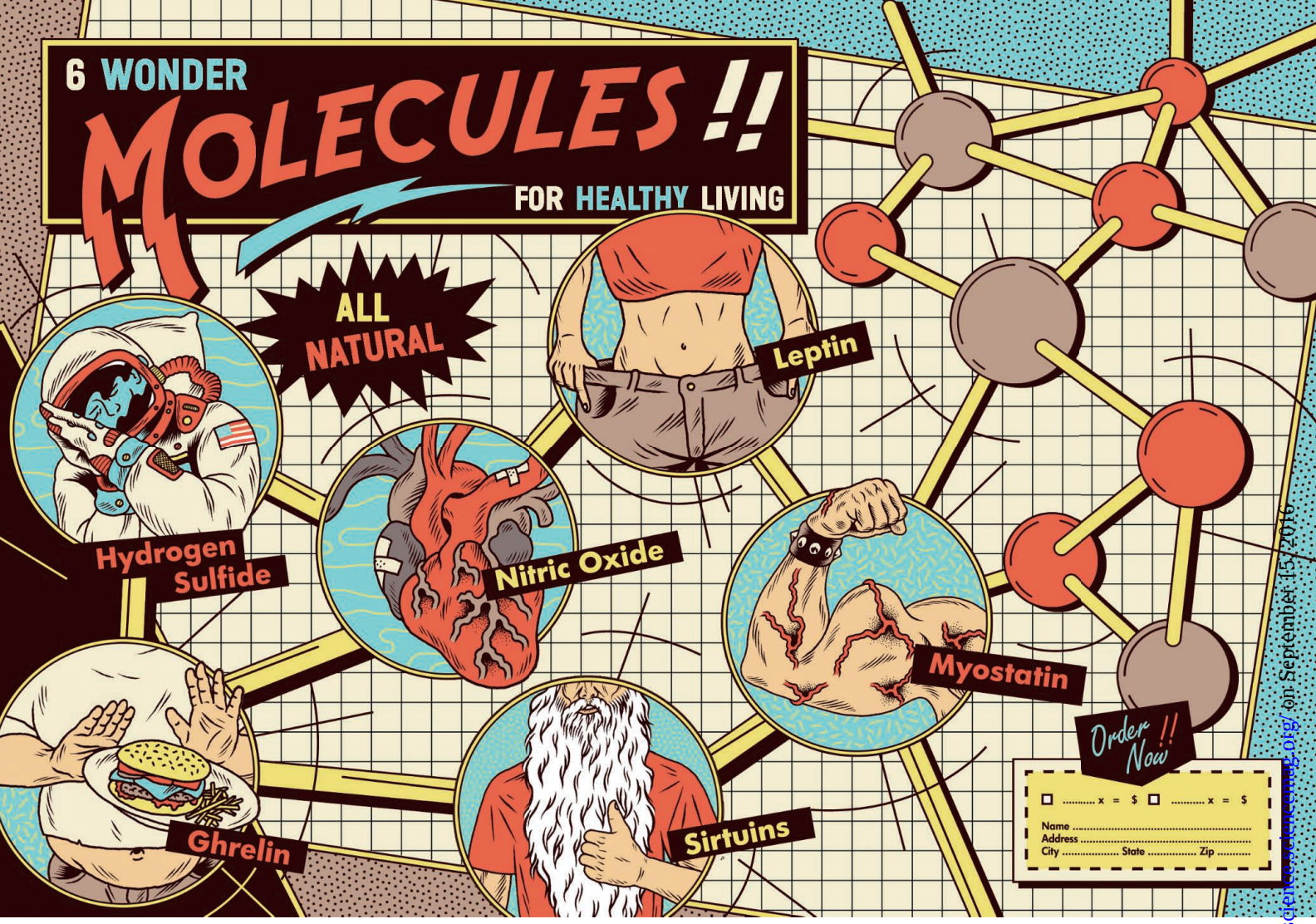

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FEATURES

WHATEVER HAPPENED TO ...

Celebrity molecules promised to transform our health, but haven't always lived up to their billing

By **Mitch Leslie**; *Illustrations by* **Freak City**

Hydrogen sulfide's short career as a panacea ended one day in 2011. Ikaria, a company founded 6 years earlier to develop medical treatments based on hydrogen sulfide (H_2S), announced that it was giving up on the gas. Toxic and smelly, H_2S wafts from rotten eggs and volcanoes. It is also one of a handful of molecules made naturally in our bodies that, if you believed press stories and some researchers in recent years, were going to trim our waistlines, spare our hearts, build up our muscles, and even allow deep space travel.

But the molecular circuits that control our physiology are almost always more complex than they appear at first glance. Plucking out a single molecule and turning it into a drug or drug target is an ambitious goal. So *Science* decided to check in on whether some of these substances delivered on their initial promise. We found that although a few have spawned drugs that patients are now taking, none has lived up to its hype.

That doesn't mean they failed, however. Mark Roth of the Fred Hutchinson Cancer Research Center in Seattle, Washington, who co-founded Ikaria, maintains that its H_2S research didn't strike out; the company's move was a business decision, not a scientific one, he says. "We never swung the bat."

These powerful molecules may yet prove to be useful. "There's typically a 10- to 20-year delay in the impact of basic science on everyday life," says vascular biologist Mark Gladwin of the University of Pittsburgh

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Medical Center in Pennsylvania. "We can't be impatient."

SOURING ON ROTTEN EGG GAS

Just a decade ago, H_2S seemed to brim with potential. Only the third gas to be recognized as a molecular signal in our bodies (more on the first, nitric oxide, later), it appeared to produce a range of beneficial effects, from soothing inflammation to protecting against reactive molecules to reducing blood pressure (*Science*, 30 May 2008, p. 1155). When Roth and colleagues discovered that H_2S sends rodents into suspended animation, wildly futuristic applications seemed right around the corner. Researchers and news stories spun scenarios that involved putting trauma patients and wounded soldiers into a kind of dormancy and enabling people to hibernate on long space flights.

These notions soon crashed to Earth when scientists tested larger mammals such as pigs and sheep and found that the gas didn't have the same effect on them. "You won't be putting astronauts into H_2S pods and sending them to Mars," says Csaba Szabo, the former chief scientific officer of Ikaria, who is now a pharmacologist at the University of Texas Medical Branch in Galveston.

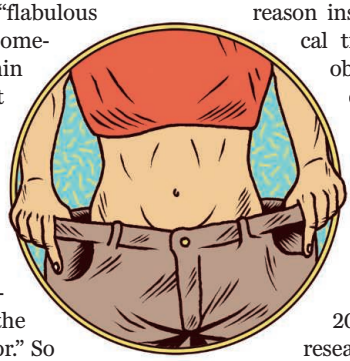
The gas has also proven difficult to work with. For one thing, scientists still can't accurately measure the concentrations in our cells, so tracking H_2S and analyzing its impact are difficult. In addition, Roth says, H_2S "has a narrow therapeutic index." That is, the difference between a helpful dose and a harmful one is slim. Partly as a result, Roth has cut back on H_2S research, and Szabo calls the early enthusiasm premature. "At the time when we jumped all over H_2S, we didn't understand a lot of what we do now. We needed another 5 to 10 years of research."

The H_2S-releasing compounds that have reached clinical trials reflect more down-to-earth goals. One drug, developed by Antibe Therapeutics of Toronto, Canada, pairs naproxen, a nonsteroidal anti-inflammatory drug (NSAID), with another molecule that emits H_2S. Popular painkillers, NSAIDs can cause gastrointestinal (GI) problems such as bleeding. The hope, says pharmacologist John Wallace, Antibe's founder, is that the gas will quell these side effects. "A GI-safe NSAID has great potential," he says. In August, the company announced positive results from a phase II trial of the drug in people with knee arthritis.

A second H_2S-boosting compound, identified by cardiovascular physiologist David Lefer of the Louisiana State University School of Medicine in New Orleans and colleagues, has undergone a phase I trial in healthy people and heart failure patients who have low levels of H_2S in their blood. Boosting quantities of the gas may, among other benefits, prevent heart muscle cells from dying. The drug's Ohio-based manufacturer, SulfaGENIX, plans to launch phase II trials, Lefer says.

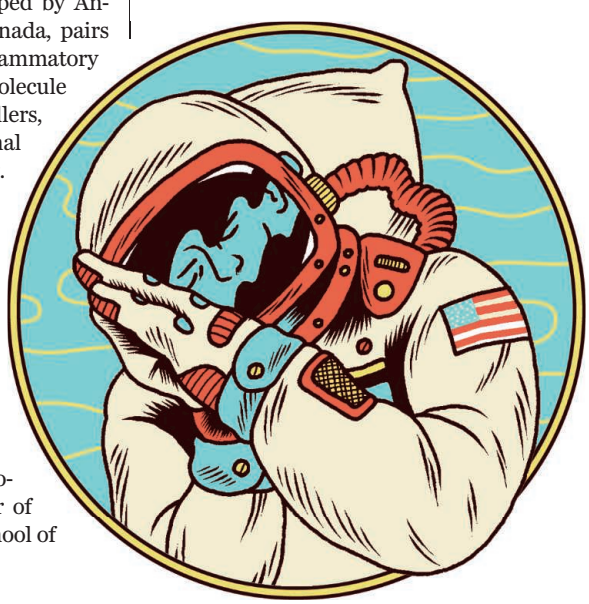
IS LEPTIN A LIGHTWEIGHT?

In 1995, *The New York Times* described the newly identified hormone leptin as a potential "magic bullet against obesity." *The Washington Post* hailed it as a "flabulous discovery" that could be "something like the mythical 'thin pill' that many overweight Americans have fantasized would cause their bodies to shed pounds effortlessly." But the excitement has faded, says physiologist Michael Cowley of Monash University, Clayton, in Australia. "It didn't deliver the magical therapy we hoped for." So what happened?



Exuded by fat cells, leptin notifies the brain to dial down our appetite when we have ample stored energy. In 1994, molecular geneticist Jeffrey Friedman of The Rockefeller University in New York City and colleagues revealed that the leptin gene was defective in mutant mice that become monstrously obese. The next year, his team and two other groups reported in *Science* that injections of the hormone transformed the animals' physiques—some mice dropped about 40% of their body weight in just over a month.

Photos showing one of the slimmed-down animals next to its corpulent counterpart ran with many news articles, a dramatic testament to leptin's apparent power. The images may also have skewed the public's expectations of the hormone's potential, Friedman now says. "You take a fat mouse and make it skinny—people would assume that would work for everyone."



Researchers made that assumption, too. "All the evidence at the time was that this could be a treatment for obesity," says molecular biologist Martin Myers of the University of Michigan Medical School in Ann Arbor. "But we didn't understand the biology as well then as we do now." Researchers now know that unlike the mutant mice, many obese people make plenty of leptin but are for some reason insensitive to it. Several clinical trials have determined that obese patients who receive additional leptin typically drop only a few kilograms. "You just can't squirt leptin into 'garden variety' obese people and get them to lose weight," Myers says.

Does that make leptin a disappointment? More than 20 years after its flashy debut, researchers still regard leptin as a scientific bonanza because "it was the key to helping us peel back the onion on how the brain regulates appetite," Cowley says. And Friedman notes that the hormone has saved lives. A handful of people worldwide are equivalent to his obese mice, carrying mutations that prevent them from producing leptin. Injections of a synthetic version of the hormone can reverse their morbid obesity and correct their diabetes. In addition, the U.S. Food and Drug Administration (FDA) has approved synthetic leptin for treating some kinds of lipodystrophy, in which patients have almost no body fat, develop diabetes, and have other metabolic problems.

Some researchers remain convinced that leptin could benefit a larger portion of the population. Some obese people do make less than normal amounts of leptin, and evidence suggests that the hormone might spur them to lose weight, Friedman says. Because it curbs appetite, leptin could also help successful dieters keep the weight off, Cowley says—though no one has yet tested this approach in a formal trial.

NITRIC OXIDE HEARTACHE

Physiologist Nathan Bryan of Baylor College of Medicine in Houston, Texas, is willing to suffer for science. As he explains the difficulties of developing drugs that harness the gas nitric oxide (NO), he has a needle stuck in his arm so he can draw blood samples every few minutes. He recently drank a glass of beetroot juice, which contains nitrate that gets converted to NO in the body; the samples will show how long the NO surge lasts.

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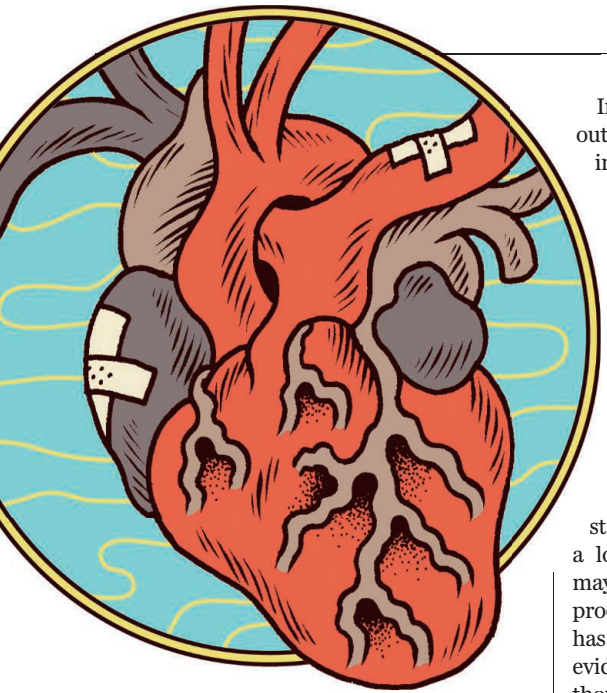


Researchers are willing to go to such lengths in hopes of improving NO's mixed record as a therapy. The discovery in the 1980s that the molecule relaxes blood vessels and triggers other helpful responses in our bodies led to the development of Viagra, one of the most prescribed drugs. NO also underlies the benefits of nitroglycerin, which patients have been taking since the 1870s to ease chest pain. "When I look at the NO field, I wouldn't say it was a failure," Gladwin says.

Yet scientists wanted to go further and deploy NO against high blood pressure, heart disease, stroke, and other conditions. The biggest obstacle to using the gas medically, Bryan says, is that the half-life of a molecule of NO in the body "is only a matter of seconds." Furthermore, the effect of NO on blood vessels wanes quickly as cells become accustomed to it. The molecule's widespread influence—it's involved in everything from transmission of nerve impulses to blood clotting—also poses a challenge, says clinical investigator Alan Schechter of the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland. When a molecule "has dozens or hundreds of effects," he notes, "it is very difficult to base a therapeutic program on."

So far, FDA has sanctioned inhaled NO gas only for newborns suffering from pulmonary hypertension, or high pressure in the arteries that shunt blood to the lungs. Doctors also often furnish the gas off-label to adults who are about to undergo organ transplants or other surgeries—it may increase the amount of oxygen in the blood and protect the transplant—or who have conditions such as respiratory distress syndrome. "I've had a myriad of patients who have survived" because of NO, says anesthesiologist John Lang of the University of Washington (UW), Seattle.

Inhaled NO gas hasn't proven useful outside the operating room and neonatal intensive care unit, however, so researchers have been searching for other drugs that might work better. NO prompts an enzyme to generate cyclic guanosine monophosphate (GMP), a messenger molecule that is responsible for many of its effects. One second-generation drug, riociguat, also promotes the production of cyclic GMP by a different mechanism; FDA approved it in 2013 for some adults with pulmonary hypertension.

Bryan's research inspired yet another strategy, spawning a company that markets a lozenge containing beetroot extract that may reduce high blood pressure and other products derived from the vegetable. The firm has sponsored clinical trials that offer some evidence for those therapeutic effects, although FDA has deemed the products nutraceuticals, which it doesn't need to approve.

New data suggest that the old standby nitroglycerin, which releases NO, may also be worth a second look. Last year, the largest study to test the impact of NO on stroke, which involved more than 4000 people, found that nitroglycerin patches weren't helpful overall. But patients who received the patches within 6 hours of suffering a stroke were less likely to die or become disabled. Study co-author Philip Bath, a clinical stroke researcher at the University of Nottingham in the United Kingdom, says that a larger trial that started this year will try to confirm this result.

HUNGER HORMONE DOESN'T SATISFY

After nearly 2 decades of setbacks and disappointments, next year some ailing patients will begin receiving the first FDA-approved drug based on the hormone ghrelin. They might howl or whimper when they go in for the treatment, but they'll start wagging their tails again in no time. That's because the drug, which goes by the trade name Entyce, targets dogs that aren't eating enough.

Boosting the appetites of pets is not what researchers once envisioned for ghrelin, a molecule dubbed "the hunger hormone" and touted as a potential breakthrough in the war against obesity. "It's a huge comedown from what we thought it would be," Cowley says.

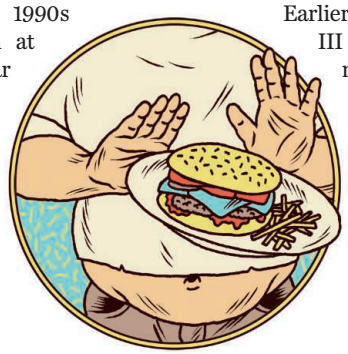
Discovered in the late 1990s by Masayasu Kojima, then at the National Cardiovascular Center Research Institute in Osaka, Japan, and colleagues, ghrelin grabbed the attention of obesity researchers because it makes lab animals and people ravenous. In one study, volunteers who received a single injection of the hormone downed 28% more calories from an all-you-can-eat buffet. Other evidence reinforced the idea that it is our appetite's gas pedal. The biggest source of ghrelin in the body, the stomach, pumps out more of the hormone when it's empty. Moreover, ghrelin stimulates receptors in the hypothalamus, a part of the brain that manages hunger.

But studies in the early 2000s undercut hopes that inhibiting ghrelin would make us thinner, says obesity researcher Mark Sleeman of Monash University, Clayton. At the time, he headed the metabolic research division at Regeneron Pharmaceuticals in Tarrytown, New York, which attempted to confirm the ghrelin findings by creating genetically modified mice that were missing the hormone. Ghrelin's absence had little effect on how much the animals ate, however. Other scientists saw a similar picture in genetically altered rodents that lacked ghrelin's receptor. "These results led us to think that [ghrelin] was going to have very little therapeutic value," Sleeman says.

In retrospect, we were probably asking too much of ghrelin, says endocrinologist Jenny Tong of Duke University School of Medicine in Durham, North Carolina. The mechanisms that regulate our metabolism and weight are so complex that "I always question how much a single peptide can do."

Even today, notes endocrinologist Jeffrey Zigman of the University of Texas Southwestern Medical Center in Dallas, scientists aren't sure whether ghrelin normally regulates hunger. Although boosting its levels in the blood ramps up appetite, researchers still argue over whether the smaller amounts that are naturally present dictate when we want to eat. "What's less debatable," Zigman says, "is that ghrelin protects against life-threatening falls in blood sugar." If food is scarce and our blood glucose starts to decline, ghrelin intervenes, activating mechanisms that restore the levels to normal.

Ghrelin may still find a medical niche in people, says endocrinologist Jose Garcia of UW. For example, researchers are testing whether a synthetic form of the molecule can counteract cachexia, the currently untreatable wasting that often afflicts people who suffer from cancer, chronic obstructive pulmonary disease, and other illnesses. Earlier this year, a pair of phase III trials showed that a ghrelin-mimicking molecule could increase muscle mass in lung cancer patients who have cachexia. But the drug didn't boost the patients' grip strength, so whether FDA will approve it remains unclear.



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MYOSTATIN SHOWS WEAKNESS

The rodents in molecular biologist Se-Jin Lee's lab at Johns Hopkins University School of Medicine in Baltimore, Maryland, looked like they had been sneaking out of their cages to spend hours in the weight room. With their bulky legs, brawny shoulders, and huge jaw muscles, they made normal mice look even wimpier than usual. Lee says he knew the mice were remarkable "from the first minute" he saw them.

He and his colleagues created the ripped rodents by inactivating the gene for the protein myostatin, which curbs muscle growth by controlling the number and size of muscle fibers. When the animals made their public debut in 1997, they set off a rush to develop drugs that would target myostatin and potentially rebuild muscle in people who have diseases such as muscular dystrophy and cancer. In the past decade, at least a dozen clinical trials have put potential myostatin-inhibiting molecules through their paces. However, Lee says, "so far, none of the trials have shown clear clinical benefits."

The first drug to falter was the antibody MYO-029, produced by Wyeth (now part of Pfizer), which latches onto and inactivates myostatin. A different drug that blocks myostatin showed a little more promise, slightly increasing lean body mass and strength in boys who had Duchenne muscular dystrophy. But several of the patients began bleeding from their noses or gums, spurring the company conducting the trial, Acceleron Pharma, to stop it.

Undaunted, companies have started three other trials of antimyostatin antibodies for muscular dystrophy. Yet muscle physiologist Nathan LeBrasseur of the Mayo Clinic in Rochester, Minnesota, says they may be targeting the wrong condition. Muscular dystrophy patients "already have compromised cellular architecture in muscles," and blocking myostatin might not overcome this defect. The strategy may work better, he says, in wasting diseases such as cachexia and sarcopenia, the deterioration of our muscles as we age, where adding bulk can be beneficial.

A hint of promise comes from a study of "older weak fallers," men who were at least 75 years old and had recently taken a tumble. Treatment with an antimyostatin antibody not only bulked up the men's muscles, it also reduced the time they took to rise from a chair. Other measures of strength, such as the time required to climb a flight of stairs, didn't improve, however. Lee says he's confident that some company "will reach the finish line," if it can find the right drug and condition to treat. LeBrasseur agrees that "it's far too soon to give up on myostatin as a viable therapeutic target."

NEW LIFE FOR SIRTUINS?

Vintners, liquor stores, and supplement manufacturers have certainly profited from the excitement generated by sirtuins. But can the rest of us count on any rewards from the molecules, a family of proteins that extend the lives and improve the health of lab animals? FDA has not approved any drugs designed to stimulate activity of sirtuins, as the resveratrol found in red wine reportedly does, and the nutraceuticals that some researchers say switch on the molecules remain unproven. "It's still unknown how important sirtuins are going to be as a therapeutic target," says Matt Kaeberlein, who studies aging at UW. "Certainly the enthusiasm got ahead of the science."

That enthusiasm started to build in the late 1990s when molecular biologist Leonard Guarente of the Massachusetts Institute of Technology in Cambridge and two colleagues, including Kaeberlein, discovered that yeast cells with an extra copy of one sirtuin gene have longer life spans. Researchers then found that additional sirtuin gene copies increase longevity and trigger positive metabolic responses in other species. But the field exploded in the mid-2000s when studies by molecular biologist David Sinclair of Harvard University and other scientists suggested that resveratrol sparks some of the effects of sirtuins—at least in certain mice and other lab organisms.

Sirtuin-activating drugs seemed one step closer to reality in 2008 when pharmaceutical giant GlaxoSmithKline (GSK) paid $720 million for Sirtris Pharmaceuticals, the company Sinclair co-founded to investigate therapies targeting the protein family. Just 5 years later, however, GSK folded its Sirtris division. James Ellis, GSK vice president and head of the sirtuin discovery performance unit in Philadelphia, Pennsylvania, says the company was merely reorganizing to better develop the molecules. But as molecular biologist Johan Auwerx of the École Polytechnique Fédérale de Lausanne in Switzerland sees it, the field "fell on its face."

After dozens of clinical trials for conditions from Alzheimer's disease to skin aging, resveratrol hasn't produced the striking results in people that it did in mice. For example, the molecule can restore blood sugar to normal in some mice that have diabetes. Yet, "we haven't seen anything like that in humans," notes molecular biologist Joseph Baur of the University of Pennsylvania.

In spite of the disappointments in the field, Auwerx says, "now it's starting to recover." Ellis says GSK plans to have two sirtuin-activating molecules ready for clinical trials within 18 months—although he declined to specify what conditions they will target.

Whether resveratrol, sold in supplements that now rake in more than $40 million a year, will play a role in the sirtuins' second act isn't clear. As Sinclair acknowledges, the compound is "dirty" and targets many molecules besides sirtuins. So scientists are looking for other mechanisms to activate sirtuins.

One is NAD, a partner required for sirtuins to function. NAD is essential for key metabolic reactions, and in many tissues of our bodies its levels plunge as we get older, suggesting that boosting its quantities might fend off age-related diseases. Elysium Health, a company Guarente co-founded last year, already sells capsules containing a NAD precursor and a relative of resveratrol. The firm doesn't have to prove to FDA that the combo has health benefits because they're natural substances. Nonetheless, Guarente says the company is completing a placebo-controlled, randomized study of the mixture. Such trials are just what the much-hyped field needs, says cardiologist Christian Matter of the University of Zurich in Switzerland. "You may not be as quick, but you walk on safe ground."

A DOSE OF REALISM

If NO has made you feel deflated or ghrelin has left a bad taste in your mouth, who's to blame? The press has to take some responsibility for breathless news articles that stoked unrealistic expectations, researchers say. But scientists are just as susceptible to the cycle of enthusiasm and letdown as everyone else, Cowley points out. "It's very rare that something lives up to all of our expectations—you can see that in the divorce statistics."

And instead of breaking up with myostatin or leptin, researchers may eventually be able to turn their disappointment into a better understanding of the molecules, which could in turn pay off with new treatments. "The process of having a clinical impact takes time," UW's Garcia says. ■

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FOOD

Plating up solutions

Can eating patterns be both healthier and more sustainable?

By **Tara Garnett**

The food system—that is, all the processes involved in feeding the global population—is responsible for ~25% of global greenhouse gas (GHG) emissions. It also drives deforestation and biodiversity loss, land degradation, water overuse, and pollution, and creates and perpetuates inequalities within and across societies. And it does not even feed us effectively: While obesity and diet-related noncommunicable diseases escalate, hunger and micronutrient deficiencies persist. As the global population grows, becomes wealthier, and demands more resource-intensive foods, these problems are likely to worsen. A systemic approach that jointly addresses problems related to production, consumption, and inequity is needed.

So far, policy-makers have mostly adopted a production-based approach to these interconnected challenges, focusing on technologies and strategies that increase food output in ways that do less environmental damage. Cleaner production techniques are clearly essential. But by itself, this "more food with less impact" approach cannot deliver on the deep cuts in emissions needed for the food system to help deliver on the Paris climate commitment (*1*). Nor does it address the systemic imbalances that cause food insecurity, rising obesity and noncommunicable diseases, and high levels of food waste.

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Healthy diets may have high environmental impacts—for example, if food is transported by air. Yet, less environmentally damaging diets are not necessarily nutritionally adequate—for example, if they include processed foods high in sugar.

To address these imbalances, policy-makers must also consider the driver of production: human eating patterns. Scientists are now starting to explore these drivers. Could dietary alterations achieve multiple benefits? Is it possible to identify diets that are sustainable and also benefit our health? Almost all researchers who study sustainable healthy diets highlight the high environmental impacts of meat and dairy consumption and the complex associations between high meat intakes and poor health outcomes. But beyond this, their research approaches and scope vary.

Some studies compare the environmental impacts of diets differing in their meat content with those of average national dietary patterns. The nutritional impacts of these diets are not examined; nutritional adequacy is assumed (*2*). Other studies pay closer attention to the health implications of lower-emitting diets, considering either

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the nutrient contents of differing diets or associations with health outcomes (*3*). An additional approach reverses the question to consider the environmental impacts of healthier diets (*4*–*6*). Linear optimization models seek to identify "best-fit" diets that deliver adequate nutrition at the lowest environmental cost, while maintaining some semblance of normality (*7*, *8*). Finally, a few global studies use life cycle assessment and nutritional epidemiological data sets comparing average diets in different regions with defined "healthy," Mediterranean, vegetarian, and vegan diets (*9*, *10*).

What conclusions can be drawn? Differences in methodologies, data sources, and question-framing muddy the waters. Furthermore, most studies focus on the country level, mainly in Northern and Western Europe. Less affluent country contexts are almost entirely unexamined, barring a few exceptions; for example, studies for China and India confirm the importance of meat consumption as a determinant of overall GHG emissions (*11*, *12*). Global studies are still scarce.

However, findings to date do allow the following observations to be made. The typical Western eating patterns that are growing in prevalence across the world have high environmental impacts and damage health. These "lose-lose" diets are the point of departure when considering the merits of alternatives. Diets containing fewer or no animal products generally emit fewer GHGs than the high-meat Western norm: On average, the lower the animal component, the lower the GHG emissions, although this varies by production context and choice of substitute foods. Conclusions on water impacts are less clear because fruits and vegetables can require high levels of irrigation. Reducing overall energy intakes in line with dietary recommendations also reduces impacts overall.

"Win-win" diets that are less land-, water-, and GHG-intensive than the Western default and that broadly adhere to nutritional guidelines can be identified. Micronutrient deficiencies are a risk, however, and some studies suggest that sugar intakes can increase. To avoid these outcomes, reduced meat intakes need to be compensated for with increases in the quantity and diversity of whole grains, legumes, fruits, and vegetables. Critically, although synergies are possible, they are not guaranteed. Healthy diets may have high environmental impacts if rich in dairy, lean meats, and fresh produce grown under protected conditions or transported by air, while less environmentally damaging diets are not necessarily nutritionally adequate (see the figure).

There are, moreover, many more questions to answer. First, work on sustainable diets does not generally account for differences in production systems. These vary hugely, such as between industrial feedlot beef and sub-Saharan pastoral cattle herds, and have very different impacts. Food processing affects both environmental and nutritional impacts of a given food, as exemplified by chicken breast and bread-crumbed nuggets. Future innovations in production and processing methods could alter the environment-health relationship; for example, lower-impact animal feeds could address some concerns around livestock production. These factors make it difficult to identify an optimal level of animal product consumption.

Second, studies largely focus on only a few environmental aspects. Most assess the impact on GHG emissions; some additionally consider aspects of land and water use. Other environmental dimensions are less examined. Impacts on biodiversity are a notable gap, reflecting difficulties of identifying appropriate metrics, diversity of agroecological production contexts, and perhaps the fact that whereas GHG emission reductions may lead to cost savings, this applies much less to biodiversity protection.

This reliance on just one or a few indicators of sustainability matters because the metrics used influence the conclusions drawn (*13*). For example, poultry has much lower GHG emissions than beef. However, poultry production relies heavily on feeding cereals that could potentially be consumed directly by humans. This is also true of ruminants in the very intensive systems that are increasingly prevalent. But ruminants in mixed and less extensive systems rely more on coarse residues and by-products or on grass from land that may be unsuited to cropping, thereby recycling nutrients and making them available as manure for crops. In certain limited contexts, and provided that they are well managed, grazing animals can also help maintain biodiversity in grasslands.

Third, a full definition of sustainability would include broader societal concerns, encompassing livelihoods, affordability, ani-

Health and environmental impacts of different diets

This figure compares "win-win" diets that are both healthy and have a low environmental impact with other dietary patterns seen around the world today. The typical Western diet, which is both unsustainable and unhealthy, is growing in prevalence around the world.

	Dietary diversity	Food energy intake/expenditure	Animal products	Fish & related	Vegetables & fruits	Whole grains tubers & legumes	Processed foods	Food losses & waste	Cooking fuels
Healthier & more sustainable diets	High	Balanced	Low (all parts eaten)	Low to moderate (sustainable)	High (minimally processed)	High	Avoided	Low	Efficient
Diets of the healthy wealthy	High	Balanced	Moderate to high (lean meats)	High	High	Low to high	Avoided	High (consumer)	Heavy use
Western-type diets (global)	Low	Excessive	High	Low to high (unsustainable)	Low	Low	High	High	Inefficient
Diet of the poor in poor countries	Low	Insufficient	Low	Low	Low	High (legumes often low)	Low (but growing)	High (spoilage) Low (consumer)	Inefficient

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mal welfare, and non-nutritional health issues. There will inevitably be trade-offs. For example, livestock intensification may lower GHG emissions per kilogram of meat or milk output but raise animal welfare concerns, increase antibiotics use, or cause local job losses. Additionally, many social and economic objectives are difficult to agree upon and measure. Food should be affordable, but should it be cheap? Is smallholder agriculture or large-scale production preferable? Is equality an end in itself or can its pursuit stifle innovation? How far are radical changes in the workings of the economy possible, legitimate, or desirable?

Some of these questions can be addressed using methodologies drawn from specific disciplines. Attempts are being been made to develop frameworks for incorporating socioeconomic dimensions into environmental life cycle assessment (*14*). This is, however, still a new and evolving area of research. Moreover, not all issues are considered equally important by all—for example, some people care more about animal welfare than others—and visions of what a sustainable food system might look like vary based on individual aesthetics and ideologies.

It is in this arena of values that questions about effecting change become most contested. We know little about how to move eating patterns in healthier, let alone more sustainable, directions, except that education and awareness-raising alone achieve little. This ignorance reflects the chronic privileging of the natural and physical over the social sciences and policy reluctance to interfere with the market, risk votes, or displease powerful corporations.

"...most studies focus on... Europe. Less affluent country contexts are almost entirely unexamined..."

But if, as evidence suggests, we cannot address our environmental problems without altering diets, we need a research program that encompasses more structural approaches to exploring what mix of regulatory and economic measures, industry actions, and education programs could be effective and acceptable, and at which scales, from local to transnational. Policymakers must also be willing to test promising approaches where evidence is scarce; experimentation builds evidence.

It will also be necessary to investigate possible unwanted rebound effects. Country-level interventions to cut meat intakes may not lead to global emission cuts if national production is maintained and meat exported. Interventions will also affect population subgroups differently. For example, a meat tax could potentially incentivize a switch to fish, with environmental problems swapped rather than eliminated; to refined, nutritionally poor carbohydrates; to cheaper processed, less healthy meat, resulting in no environmental gain and worse health; or to meat with lower animal-welfare credentials. Policy-induced changes in diets may also affect broader nonfood consumption through price rebounds, with knock-on environmental impacts.

Ultimately, to address these questions, researchers need to focus more on the values that underpin food systems research and advocacy (*15*). A critical appraisal of the assumptions shaping our own research questions is warranted. More generally, values frame and inform which issues or options are considered or ignored, which sustainability dimensions prioritized, which interventions considered researchable and fundable, and whose voice counts. These questions go beyond the natural sciences. But since food is entangled in almost every aspect of our lives, understanding how sustainable food systems and diets look and how to achieve them will require deeper collaborations across disciplines and beyond academic boundaries. ■

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NANOMATERIALS

Designer nanorod synthesis

Modified cellulose strands create versatile reactor compartments

By **Andrew Houlton**

One-dimensional (1D) rodlike nanostructures are of fundamental interest for examining size- and shape-dependent phenomena and can have applications that include next-generation electronics and sensing elements (*1*–*3*). The growth of such nanostructures poses considerable challenges for synthetic chemists and materials scientists. However, because few materials naturally grow in such an anisotropic manner, linear pores (molds) or surface templates (such as DNA) are used to guide their formation. On page 1268 of this issue, Pang *et al.* (*4*) describe a highly versatile approach that combines both multicompartmentalization and surface modification using cellulose-based materials and that can control nanorod surface chemistry. This work conceptually extends the level to which chemical reaction space can be designed.

Methodologies for 1D material synthesis must direct growth by defining the reaction space in which product formation occurs. A common approach is to use porous matrices, such as alumina and polycarbonate membranes, as molds (see the figure, left panel) (*5*, *6*). The linear pore channels in these matrices are filled with soluble precursors, such as metal ions, that are chemically transformed into insoluble material that conforms to the pore shape. However, the matrix needs to be removed to isolate individual rods. Diameters have been limited by the range of available pore sizes (tens of nanometers), but the recent development of molecular-based molds may overcome some of these problems (*7*).

The smallest-diameter nanorods have been prepared by using naturally occurring linear biopolymers, such as DNA (see the figure, middle panel) (*8*). The rodlike double helix of individual DNA molecules is

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just 2 nm in diameter and available in precise lengths with subnanometer resolution. Although DNA intrinsically lacks many useful optoelectronic properties, its ability to bind a wide range of chemical precursors enables templated synthesis of 1D materials. This mechanism relies on precursor nucleation on the DNA followed by growth guided by the template shape (*9*). The approach is adaptable to a wide range of material types, including metals, inorganic compounds, and even soft molecular-based conductors (*8*). It can also take advantage of the unprecedented structure-building available with so-called DNA origami (*10*, *11*). However, the limited solubility of DNA in nonaqueous solvents restricts the types of chemical reactions, and hence materials, compatible with this method. Furthermore, the growing material entombs the template, so the surface chemistry of the final nanostructure is dictated by its growing material and not the template, a situation that is not always desirable.

Pang *et al.* combined these approaches of defined reaction spaces and linear biopolymer templates by turning to the carbohydrate cellulose and adding the precision of modern synthetic polymer chemistry. In doing so, they create multicompartmentalized reaction vessels that can be adapted to a wide range of reaction conditions. A key step was the development of a procedure to size-sort the cellulose into individual molecular strands of well-defined length. Individual cellulose molecules are then used as the spine of a "bottlebrush" structure to form a nanosized reaction vessel (see the figure, right panel).

Chemical functionalization of the cellulose provided sites for the growth of synthetic polymer strands from the central backbone. This step introduces a high density of polymer chains that contain distinct regions, or blocks, with different chemical groups. With careful selection, the block types can be tuned in such a manner that the internal block is solvophilic, favoring the reaction media, but the outermost block is not well matched and solvophobic.

"Individual cellulose molecules are...used as the spine of a 'bottlebrush' structure to form a nanosized reaction vessel."

Standard chemical reactions can then be performed in solutions containing the bottlebrush structures, each of which acts as an individual smart test tube and defines a discrete reaction space. The material growth occurring within the nanoreactor is confined to the solvophilic inner block, which importantly is isolated from the bulk solvent by the solvophobic outer block. The resulting nanorods can be separated by means of simple centrifugation from the unwanted material that also forms in the bulk solvent.

The approach is readily adaptable to synthesizing nanostructures with desirable properties (such as metallic, semiconducting, magnetic, or luminescent) and of increased complexity (for example, multimaterial core-shell structures). By increasing the block count and introducing protection-deprotection strategies, different chemical functionalities can be sequentially revealed to enable multistep syntheses. The final and perhaps most farsighted aspect of this approach is that the nanorods have a surface, or exterior sheath, that is defined from the outset by the outermost polymer block. This control of surface chemistry can provide stable suspensions of nanorods in different solvents, which both facilitates their use in experiments as well as in the development of processing routes.

The drive for new technologies to address societal needs, ranging from renewable energy to novel drug systems, challenges synthetic methodologies to fashion materials in ever more complex ways. Creative thinking is needed to extend existing approaches and conceive ones that are conceptually new. The single-molecule, multicompartmentalized reactors of Pang *et al.* provide a powerful addition to the synthetic arsenal and bring chemists closer to the dream of controlling all aspects of the assembly of matter. ■

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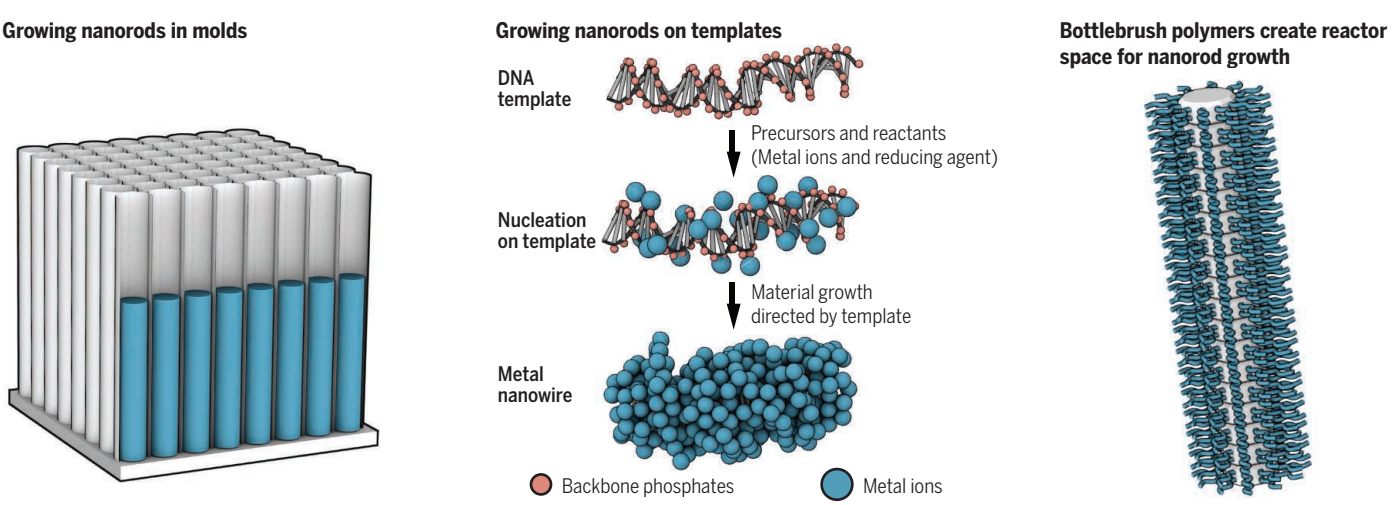
Three routes to nanorods

Nanorod growth can be controlled with nanoscale molds, templates such as DNA, and designed nanoscale chemical reactors based on cellulose fibers.



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MOLECULAR BIOLOGY

Harnessing mutation: The best of two worlds

Combining vertebrate and bacterial immunity components enables targeted mutagenesis

By **Silvestro G. Conticello**[1] *and* **Cristina Rada**[2]

Rapid evolution requires high rates of mutation that come at the cost of reduced viability. This is true at the single-cell level in bacteria as well as in the highest complex vertebrates. Immunity relies on rapid evolution to enhance the recognition of external threats and to neutralize them, often by inflicting damage to the genomes of potentially harmful invaders. On page 1248 of this issue, Nishida *et al.* (*1*) report combining a polynucleotide cytosine deaminase (PmCDA1)—an enzyme involved in antigen receptor diversification in vertebrates—with the bacterial immune restriction system clustered regularly interspaced short palindromic repeat (CRISPR)–Cas9 to enhance point mutation with almost single-nucleotide precision to levels that, if occurring genome-wide, would otherwise result in lethality. With localized mutagenesis, also achieved through a similar approach by Komor *et al.* (*2*), comes the promise of gene correction by means of targeted editing. Both studies show that not only is targeted mutation feasible but that its efficiency, and even the type of mutation, can be driven by manipulating or engaging the various cellular pathways involved in repairing edited bases.

Targeted editing of large vertebrate genomes is a bioengineer's dream that has been on the horizon since the early 1970s. But the prospects of high throughput and high versatility have only become a tantalizingly imminent possibility since the RNA-guided bacterial Cas9 entered the game in 2012 (*3*–*5*). Bacteria and archaea acquire adaptive immunity by incorporating snippets of genetic material from foreign DNA into their own genome during the course of an infection or invasion. This piece of foreign DNA is used to produce RNA guides for the Cas9 endonuclease to recognize further attacks by the same threat. The small RNA molecules associate with Cas9 and use homology to displace the double helix of foreign DNA and direct cleavage of the two DNA strands. The stable displacement of one DNA strand by the RNA guide creates a perfect substrate for cytosine deaminases, such as AID (activation-induced cytidine deaminase) or the APOBECs (apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like), that act on single-stranded substrates (*6*).

The CRISPR-Cas9 complex has greatly facilitated targeted editing by means of homologous recombination (*4*), which requires exogenous addition of a DNA template bearing the correction. Although the frequency of recombination at a specific site is increased by locally induced DNA breaks, the process remains inefficient and at risk of creating genes marred with deletions or insertions. The coupling of a catalytically inactive Cas9 (dCas9) with a deaminase that can only act on single-stranded DNA triggers localized cytosine-to-uracil conversion but avoids collateral damage and insertions or deletions (indels) brought about by the repair of breaks.

For both Nishida *et al.* and Komor *et al.*, tricks learned from unraveling the mechanisms of AID (the mammalian homolog of PmCDA1) during antibody diversification proved useful (*7*): By inhibiting the

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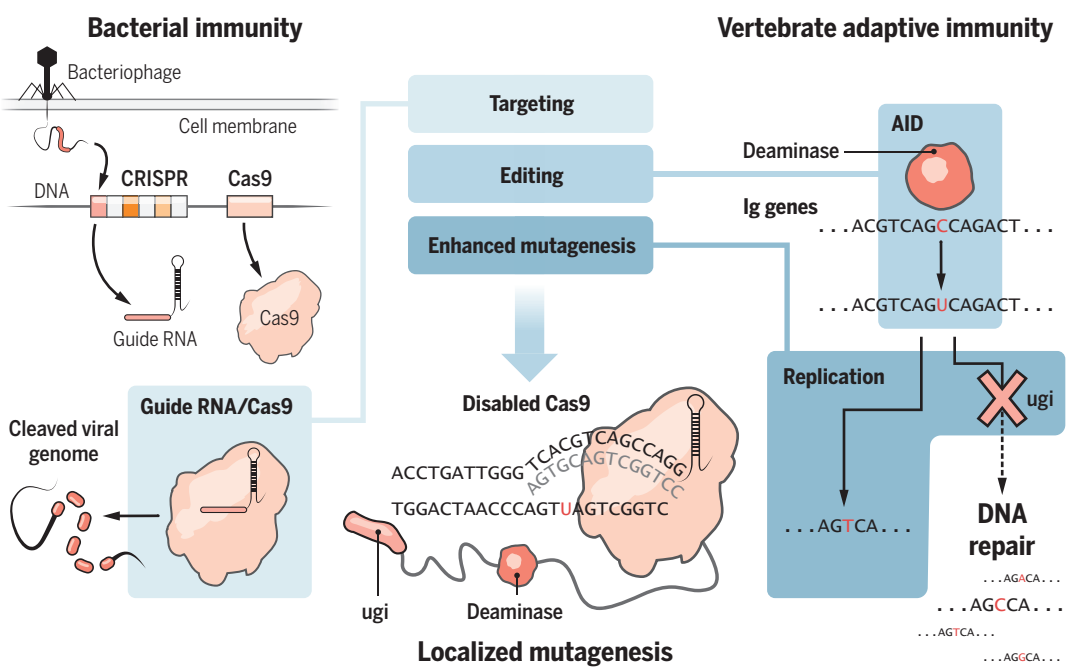
Localized mutagenesis tool

Elements from bacterial and vertebrate adaptive immunity can be combined in a new approach. The bacterial endonuclease Cas9 contributes the targeting module by using a homology-guided small RNA and by creating a single-stranded substrate for a vertebrate polynucleotide cytosine deaminase (AID) to initiate editing through a C-to-U change. Addition of a bacteriophage inhibitor of uracil glycosylase (ugi) prevents the removal of the edited base, thus increasing mutagenesis efficiency. The use of a disabled Cas9 that lacks endonuclease activity prevents unwanted collateral mutagenesis in the form of insertions and deletions. Ig, immunoglobulin.



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removal of uracil with a bacteriophage peptide (ugi) that inactivates the cellular repair enzyme uracil glycosylase, mutation frequencies are enhanced to levels that obviate the need for additional selection (see the figure). The sequence of such targeted yeast genomes contains negligible off-target changes, but up to 10% localized mutation. By using rat APOBEC1, a stronger DNA-RNA deaminase than lamprey PmCDA1 (*8*), Komor *et al.* obtained mutation frequencies up to 20% in human cells, with a further increase to 37% achieved by biasing the cellular mismatch repair toward correction of the unedited DNA strand—at the slight cost of increasing indels (from <0.1 to 1.1%). APOBEC1, like the related human APOBEC3 deaminases, is a powerful genome mutator in cancer cells (*9*, *10*), and even in physiological conditions, AID off-target activity is a frequent cause of oncogenic mutations and translocations (*11*).

"With localized mutagenesis...comes the promise of gene correction..."

Although testing known off-target sites of CRISPR-Cas9 for APOBEC-dependent mutations in the study of Komor *et al.* suggests low collateral damage, whole-genome or -exome sequencing will still be a necessary proposition for human gene therapy. Physically tethering the deaminase to Cas9 might be the key strategy that allows both groups to minimize unwanted editing activity in mammalian cells, where transient single-stranded DNA is unavoidably associated with transcription. It could well be that "less is more" in this case and that the less efficient deaminase AID might be a better choice in mammalian cells.

Transient use of dCas9-PmCDA1-ugi practically eliminates off-target mutations and indels but limits the genes amenable for correction because only transitions from GC to AT pairs can be reliably achieved. This is not a major limitation to technological applications; the obvious combination of dCas9-AID with yeast display immediately comes to mind as a tool for accelerated antibody evolution, but many other examples are ripe for its use, from genome evolution to conventional genetics.

Aside from the technical innovation, the methods described by Nishida *et al.* and Komor *et al.* could provide insights into the physiology of these deaminases. Coexpression of dCas9 and PmCDA1 is sufficient to increase the frequency of localized mutation even in trans; indeed, the main requirement for deamination is the presence of a small but persistent single-stranded region of DNA (*12*). In vivo, other factors contribute to editing efficiency, such as the sequence context preferences of the edited cytosine and its position within the open DNA. In both the Nishida *et al.* and Komor *et al.* studies, distal sites were more frequently edited, a difference that was exacerbated for cytosines that did not fall within the sequence consensus of the deaminase. As exemplified by Nishida *et al.*, a more relaxed sequence context favored by AID [WRC (where W is either A or T, and R is either A or G) versus NTC (where N is any base)] can be an advantage for mutagenesis applications, whereas hybrid deaminases with different sequence context preferences could be part of the targeted mutation toolkit (*13*).

Although powerful, the system is still short of the promised targeted base editing. One reason is that mutations are biased to GC pairs and are promiscuous within the single-stranded bubble created by the Cas9-RNA guide. More importantly, the short homology required for RNA-guided recognition is an adaptation that provides bacterial CRISPR defense the flexibility to accumulate immunity against a large number of viruses or foreign plasmids while retaining a compact genome, but results in promiscuous targeting in larger genomes. As in the case of the deaminase component, a less efficient Cas9 with a more fastidious and longer homology requirement would be a desirable development.

Harnessing mutation, a dream long anticipated by geneticists and pioneered by early molecular biologists, was still haunting Michael Smith in his 1993 Nobel Prize lecture: "The ignis fatuus of genetics has been the specific mutagen, the reagent that would penetrate to a given gene, recognize it, and modify it in a specific way." He was quoting Joshua Lederberg's 1959 Nobel Prize lecture. By the looks of it, the dreamt future of genetics is now. ■

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VACCINES

Vaccine trust and the limits of information

Understanding trust in local contexts is key to communication about vaccination

By **Heidi J. Larson**

Over the past decade, there has been growing recognition and increasing research around the phenomenon of vaccine reluctance and refusal (*1, 2*). More recently, there has been a flurry of articles on what is being referred to as "vaccine hesitancy," depolarizing the earlier characterization of individuals or groups as being outright pro- or antivaccine, and instead recognizing the liminal state between becoming aware of, and deciding whether or not to accept, vaccination. Episodes of waning public confidence around vaccines have become so global that the World Health Organization's Strategic Advisory Group of Experts on Immunization convened a working group (*3*) to better understand and recommend actions to address this growing challenge of vaccine hesitancy, which the group defined as "delay in acceptance or refusal of vaccination despite availability of vaccination services." Indeed, vaccine hesitancy is complex and context specific (*4*). How can we better understand the circumstances that influence this state to ensure more effective uptake of vaccines and secure public health?

Faced with an ever-growing portfolio of new vaccines and combinations of vaccines, parents—and society more broadly—are becoming more questioning as they assess whether vaccines for themselves or their children are too many or too new, better given individually or in combination, or worth even the smallest risk. The landscape of information and misinformation about vaccines, as well as the varied and sometimes divisive views of legitimate and self-espoused experts, is further complicating the public's genuine interest in making the right decision. A broader environment of distrust in institutions (*5*) and "experts" (*6*) additionally prompts pub-

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lic questioning, with some trust issues so tense that refusal of health interventions is uncompromising, with little room for reason. For example, this happened in the early days of the West Africa Ebola response when some families in the Ebola-affected countries hid sick family members for fear that they would go to treatment centers and never return. In Guinea, some even resorted to violence against health workers.

Various studies have examined the positive and negative nudges along the increasingly complex path to a vaccination decision, and a number of them point to the importance of trust as a key lever in the decision-making process (*7, 8*). Recent research by Scherer *et al.* (*9*) investigated one of the identified drivers of distrust—lack of trust in vaccine information—which is perceived as being incomplete, inadequately researched, or not fully transparent. Another recent study of 67 countries found that there was overall positive sentiment about the importance of vaccines, but lower confidence reported in the safety of vaccines (*10*). Furthermore, countries with high levels of schooling and good access to health services reported some of lowest confidence in vaccine safety.

Scherer *et al.* explored whether or not providing more information on vaccination against human papilloma virus (HPV) made study participants more confident and willing to accept the vaccine. HPV infections can cause cervical, vaginal, anal, throat, and penile cancers. Vaccination is recommended at ages 11 and 12, when children have a robust immune response. The timing protects individuals before most become sexually active and at risk of exposure to HPV. The study reports on three surveyed groups—one given the standard HPV vaccine information sheet from the U.S. Centers for Disease Control and Prevention (CDC); another presented with the standard sheet supplemented with more information about the Vaccine Adverse Event Reporting System (VAERS) database (which is cosponsored by the CDC and U.S. Food and Drug Administration) and the goals of VAERS in lay language; and a third group shown guidance on how to interpret the cases reported to VAERS as well as a summary of deaths and permanent disabilities reported in 2013. Interestingly, the group given the most information was the least willing to be vaccinated.

However, it is difficult to generalize from the findings of Scherer *et al.* for a number of reasons. One point, which the authors note, is that the U.S. sample that was surveyed was largely white, more educated than the general population, and more conscientious in participating in surveys. Also, and perhaps more important, the group that received the most information also received the least certain information—that is, they received information from VAERS. VAERS is a passive online reporting system where anyone can report a suspected adverse event following immunization, none of which have yet been investigated to confirm whether or not the event was coincidental or caused by the vaccination. As noted on the VAERS website, "The report of an adverse event to VAERS is not documentation that a vaccine caused the event…Reports vary in quality and completeness. They often lack details and sometimes can have information that contains errors" (*11*). Although Scherer *et al.* acknowledge this characteristic of VAERS and, from a trust-building perspective, VAERS is a proactive effort on the part of the government to listen to the concerns of the public, it also risks being misinterpreted. Given that the HPV vaccine is one of the more challenging vaccines in terms of public acceptance (as of 2015, state-level data for three doses of HPV vaccination ranged from 24 to 68% for girls and 16 to 58% for boys) (*12*), it is not surprising that after seeing additional information exposing possible, albeit uncertain, risks, the third group in the study would be less inclined to vaccinate. The results may have been different had the third group received more information than the second group, albeit a type of information that was more complete and less ambiguous.

"Building trust and confidence in those who provide the information and the vaccines, as well as the system that delivers them, is also key."

More information alone, however, does not build trust. As Dempsey *et al.* (*13*) found, "attitudes and life experiences," along with the opinions of their doctor and their peers, were stronger influencers on survey respondents than information. In other research (*5*), trust in the medical profession and in one's personal health care provider, were important. In other words, the source of information matters as much as the content.

Trust is relational, and is often defined as "competence to do what one is trusted to do" (*14*) and also implies trust in the motivations of the trusted. Do they have my—or my child's—best interest in mind?

The issue of motive was writ large in the local anxieties around the Ebola virus outbreak control measures (such as quarantine) and regarding the various stages of the clinical trials of multiple Ebola vaccine candidates. The trust building for both issues was critical to the eventual deployment of both interventions. Siegrist *et al.* (*15*) point to trust as a key lever of public compliance in outbreaks, and it will certainly be key to ongoing Ebola vaccine trials as well as the recently approved start of human trials for Zika vaccine candidates. Understanding the dynamics of existing trust relationships, and building on them, will be essential to support clinical trials as well as eventual deployment of new vaccines. Building trust and confidence in those who provide the information and the vaccines, as well as the system that delivers them, is also key. For example, in India in 2010, women's groups had called for a public forum to discuss HPV vaccination but were ignored for months; this resulted in stronger activism against vaccination, and the program was eventually suspended. In Kenya, the Conference of Catholic Bishops, which had always been an invited partner in national vaccination campaigns, became suspicious when they were initially excluded from the tetanus vaccination campaign, and mobilized resistance in response. Local understanding of trust networks and weak points is required, as well as understanding the key issues that inhibit trust. Only then can genuine trust building begin, allowing for more openness to interventions, even in times of risk and uncertainty. ■

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POLICY FORUM

U.S. President Barack Obama signs the Every Student Succeeds Act into law 10 December 2015.

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SCIENCE EDUCATION

Will latest U.S. law lead to successful schools in STEM?

Despite shortcomings, there is room for cautious optimism

By **Adam Gamoran**[1,2]

Remarkably, after a long delay, the U.S. Congress passed and President Obama signed in late 2015 a new version of the Elementary and Secondary Education Act, the law that since 1965 has staked out the federal government's commitment to education from kindergarten to 12th grade (K-12). This version, the Every Student Succeeds Act (ESSA), replaces the controversial version known as No Child Left Behind (NCLB) (*1*, *2*). With ESSA scheduled to take full effect in fall 2017, states are now in planning mode. What changes are in store for schooling in science, technology, engineering, and mathematics (STEM)? Although Congress sought advice on this topic, few changes in ESSA reflect new knowledge on STEM. Yet the law provides opportunities for improvement if states choose to act.

Despite sharing many parallel features, ESSA departs from NCLB in important ways. Whereas NCLB was fundamentally a federal accountability system that applied the same incentives and sanctions to schools across the nation, ESSA calls on each state to develop its own accountability system by setting standards and assessments, as in NCLB, but also to determine what counts as "success" and to establish its own incentives and supports for improvement. Whereas NCLB accountability focused solely on reading and math, ESSA is open to "multiple measures" of achievement, including those for science, if so desired by states.

During the NCLB era, Congress was often frustrated with academic achievement across U.S. schools. The House Committee on Appropriations, Subcommittee on Commerce, Justice, Science, and Related Agencies—which oversees the National Science Foundation (NSF) budget—requested that NSF provide a report identifying characteristics of effective schools and programs in K-12 STEM (*3*). The result was a report by the Board on Science Education of the National Research Council (NRC), now known as the National Academies of Sciences, Engineering, and Medicine (*4*). A positive reception from Congress led to a second report, a framework for monitoring whether recommendations from the first report were implemented (*5*). The reports offer a blueprint, based on best available evidence, for improving K-12 student learning and engagement in STEM, including broadening participation of women and members of minority groups (*6*). Given that Congress had embraced these reports, one might have expected ESSA to provide resources and incentives to implement strategies recommended for K-12 STEM education.

How has this turned out in practice? There is little in ESSA that picks up directly from the two NRC reports (*7*). However, by delegating accountability systems to states, Congress created potential for implementing effective STEM education reforms and supports. The science education reform context in which ESSA emerged is very different from that of NCLB, so it could set the stage for schools to improve in STEM fields.

WHAT ESSA OFFERS FOR STEM EDUCATION

The NRC reports provided recommendations in three domains: student access to quality STEM learning experiences, educators' capacity to deliver such experiences, and policy and funding initiatives to support such experiences (*8*). ESSA offers no mandates and little guidance in these areas. For example, in response to science being driven out of elementary school curricula by excessive pressure for reading and math, the NRC panels urged inclusion of science in federal and state accountability systems and use of assessments that measure core concepts of mathematics and science. ESSA permits, but does not require, states to include science in their accountability systems. Although ESSA allows states to use federal funds to develop assessments, as did NCLB, the new law prohibits the U.S. Department of Education from recommending or encouraging any specific set of standards or assessments (*9*).

For schools and districts, the NRC panels drew attention to the need for adequate instructional time and resources for elementary school science, yet this is not addressed in ESSA which, like NCLB, does not incentivize science instruction by requiring accountability in science. Where the NRC panels recommended rigorous STEM curricula focused on the most important topics in each discipline and organized in a sequence, ESSA merely targets the end point of standards to reach minimum requirements for public higher education and career and technical education in a state, to ensure the standards are "college and career ready."

In contrast, ESSA is responsive to needs identified in the NRC reports for teacher and leadership development. The NRC panels urged supports for STEM teachers coming from state and federal levels for professional learning at local levels, particularly in deepening their subject matter expertise and enriching their understanding of how students learn. ESSA has introduced a STEM Master Teacher Corps, which, if funds are appropri-

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Congress sought, and received, expert advice on the best approaches to improve and expand STEM education, but the influence of this advice on the Every Student Succeeds Act appears to be quite uneven.

ated, will provide support for state-led efforts to elevate the status and capacity of STEM teachers. ESSA permits schools to use federal funds to prepare teachers to use technology to improve STEM teaching and learning and to support professional development for teachers and leaders to advance high-quality instruction in STEM (see the second photo). ESSA allows school districts to use federal funds to establish STEM specialty schools, a provision that could respond to the Academies' recommendation to develop a variety of STEM-focused schools and programs.

WILL STATES RESPOND TO OPPORTUNITIES?

The shift from federally mandated to state-designed accountability systems means that the chances for improving K-12 STEM education rest entirely with states and local districts. At first glance, that may not give much grounds for optimism, as the Elementary and Secondary Education Act has a long history of states and localities using awarded federal dollars for their own purposes (*10*). Yet new resources are available that may lead states to use federal funds in research-tested ways that advance students' success.

The NRC panels confronted a dilemma. The absence of science from accountability systems had led to a decline in the amount of time devoted to science in elementary schools. Yet adding science to accountability systems with weak assessments might have undercut rather than supported learning, even if instructional time were increased. The panels thus recommended that "policy makers at the national, state, and local levels should elevate science to the same level of importance as reading and mathematics," but at the same time, "states and national organizations should develop effective systems of assessment that are aligned with the next generation of science standards and that emphasize science practices rather than mere factual recall" (*4*). With these twin provisions, states could both elevate the importance of science and improve how science is taught and assessed.

The Next Generation Science Standards (NGSS) are a new resource for states. Based on a framework developed by another NRC panel, the NGSS include core ideas of science disciplines, science and engineering practices, and cross-cutting concepts that connect disciplines (*11*). Eighteen states and the District of Columbia have adopted the NGSS (*12*) and efforts are under way to develop assessments aligned to the standards. When these assessments are ready, it will be possible to measure progress toward meeting academically rigorous standards with rich, meaningful assessments—if states choose to adopt them.

What are the chances that states will incorporate science into their accountability systems? Recent history offers a guide. From around 2011 to 2015, when NCLB was essentially inoperative owing to its unrealistic standards that led virtually all schools to be identified as failing, the Obama Administration granted waivers from key NCLB provisions to states that were willing to adopt their own accountability systems with features that accorded with the Administration's priorities. During this period, 29 states and the District of Columbia incorporated student science performance into their accountability systems, and 8 states included growth in science performance—the extent to which individual students made progress over time in science achievement—as part of their judgments about school performance (*13*). Many have adopted or are considering the NGSS as the basis for their standards, and are developing curricula aligned with the NGSS. This provides a foundation for states to incentivize science instruction in a way that was not present at the dawn of the NCLB era.

In mathematics, the Common Core State Standards (CCSS) also emerged during the NCLB waiver era in response to the Obama Administration's insistence on common, rigorous standards in reading and mathematics. Despite a backlash that largely responded to efforts to evaluate teachers based on student test performance (see the first photo), the CCSS have been widely implemented by states—whether or not they are actually called by that name (*14*). For most states, CCSS mathematics will form the basis of their standards under ESSA, more rigorous and coherent than were standards under NCLB (*15*).

Another resource available to states is the expansion of schools that specialize in STEM, including the possibility of using federal funds to establish such schools. The focus on specialized schools emerged from an interest in promoting highly selective STEM-focused schools that nurture top talent (*4*). However, research suggests that highly successful STEM teaching and learning occurs in a variety of settings, including nonselective STEM schools and career-oriented STEM schools. Recent research has also raised questions about the added value of highly selective STEM high schools (*16*). Interest has grown in "inclusive STEM schools" that focus on STEM but are nonselective and typically make special efforts to build diverse student populations (*4*). Establishing such schools has been a struggle and has yielded mixed success (*17*, *18*) with perhaps greater success in more supportive state contexts (*19*). Research may yield insights that help states achieve more consistent success with inclusive STEM schools.

CONCLUSIONS

So what does ESSA mean for school improvement in STEM? Not much, one might conclude, based on the language of the law itself. But if one examines opportunities for flexibility across states; new resources in standards, curriculum, assessments, teacher and leader development, and school structures; and federal funds to activate

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these resources, one may find grounds for cautious optimism. We should regard states as laboratories for education reform, making decisions based on their values and leadership but drawing on common resources. Astute researchers will compare across states and within states over time to examine which reforms are associated with improvements in mathematics and science, pursuit of postsecondary STEM training and careers, and broadening participation in STEM. We may then learn whether flexibility and resources afforded by ESSA, combined with state commitment to STEM education reform, result in better outcomes that can be applied across the nation. ESSA challenges states to take advantage of new opportunities to improve STEM participation and learning in all schools. ■

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BIOTECH REGULATION

A missed opportunity for U.S. biotechnology regulation

Policy options for change were not on the table

By **Jennifer Kuzma**

A rare policy window (*1*)—an opportunity for change that happens when problem, political, and policy streams come together—recently opened for biotechnology regulation in the United States: an explosion of genetic engineering (GE) techniques and products challenging federal oversight; intensifying public, media, and political attention; and a growing number of policy ideas for change. When the White House in summer 2015 called for a process to revisit the 1986 Coordinated Framework for the Regulation of Biotechnology (CFRB) (*2–4*), it was seen as an opportune moment to match biotechnology innovation with innovation in the design of a U.S. regulatory system. However, the White House's Office of Science and Technology Policy (OSTP) interagency process for reviewing the framework fell short in multiple ways. The process has prevented clarification and restructuring of the regulatory system, as well as opportunities to increase the legitimacy of that system in the eyes of the public and stakeholders.

The CFRB was predicated on the view that it is the final product of GE that bears the risk, not the process by which it is produced, and that there are no new categories of risk associated with GE compared with conventional genetic-exchange methods like breeding (*2*). No new biotechnology laws were enacted by Congress; instead, older laws covering product categories like pesticides, plant pests, toxic substances, and drugs were used to divide authorities among agencies to regulate products of biotechnology and GE organisms (GEOs).

New GE technologies, like synthetic biology, gene-editing, and gene-drive systems, as well as novel GE products, are challenging regulatory definitions, highlighting inadequacies in health and environmental assessments, and revealing gaps in agency jurisdiction (*5–7*). Confidence in the CFRB has been decreasing, as concerned parties question why ground-breaking technological products go through outdated and tangential regulatory processes (*4*).

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For example, release of the first GE mosquito in the United States was approved by the Food and Drug Administration (FDA) as an Investigational New Animal Drug (INAD). This GE line of *Aedes aegypti* contains sterility genes, designed to allow mating with wild members of the species but from which most of their offspring die in early development. By reducing the population of disease-carrying mosquitos, human illnesses from Dengue or Zika virus may decrease (*8*). However, typically, INADs submitted to FDA are for chemical substances injected, topically applied, or fed to animals to treat or prevent disease. INADs are primarily reviewed based on safety and efficacy to the target animal.

> ***"...opportunities [for] meaningful...change... were missed at this key juncture in the biotech revolution..."***

The ultimate goal of the sterile transgenic mosquito is the population's own demise; therefore, FDA's regulatory focus does not appear to fit the situation or address risks of greatest concern. FDA's statutory authority does not focus on environmental impacts, and although those impacts might be examined through the National Environmental Policy Act (NEPA), the agency is not known for its expertise in ecological risk analysis, a key area of concern about the release of transgenic mosquitos.

Uncertainty associated with review of existing and new GE products under the CFRB demanded attention and catalyzed the interagency review. As such, the White House called for a year-long process for "development of an updated CFRB to clarify the roles and responsibilities of the agencies that regulate the products of biotechnology" (*3*). During the past year, representatives from the three federal agencies of the CFRB (FDA, Environmental Protection Agency, and Department of Agriculture) and the White House (Interagency Working Group) have been meeting in closed session, while in parallel, three public meet-

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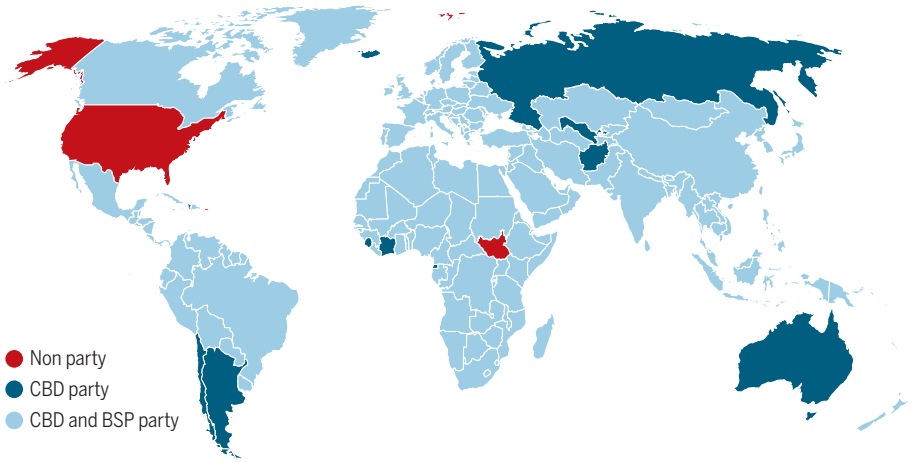
Parties to agreements on biodiversity

The countries that have ratified or accessioned the Convention on Biological Diversity (CBD) and its Cartagena Protocol on Biosafety (BSP) are shown, as well as nonparties.



MAP: (DATA) CONVENTION ON BIOLOGICAL DIVERSITY; C. SMITH/*SCIENCE*

ings were held. The memo also called for an external study, and for this, the National Academies of Sciences, Engineering, and Medicine (the Academies) was commissioned to identify the kinds of products that may be produced with biotechnology in the next 10 years and to provide advice on the scientific capabilities, tools, and expertise that may be needed for assessing risk in a report due December 2016 (*9*).

REGULATORY OPTIONS ARE OFF THE TABLE

During the first public meeting in October 2015, OSTP staff stated that the interagency process would likely not result in revision of authorities or creation of new ones. Rather it would clarify existing authorities (*4*). This was disappointing to many scholars and practitioners given that the CFRB had not been revisited in more than 30 years (*10*).

On 8 March 2016, just a day before the second public meeting, the OSTP published draft case studies of regulating GE products under the CFRB to illustrate these clarifications (*4*). However, the GE products chosen for the cases fell neatly into existing authorities under the CFRB, and their regulatory paths could have been predicted by attentive scholars and practitioners before the start of the OSTP process (e.g., plants engineered with plant-pest sequences or containing pesticidal proteins).

At the third and final public meeting, several participants questioned why the cases failed to represent a challenging set that could truly serve to clarify authorities for emerging GE products (*4*). Products that are already stretching regulatory definitions and agency boundaries, like gene-edited crops (*7*), or that are poised to be problematic in the near future, like GE insects with gene drives or GE animals for environmental release or as livestock, were absent from the OSTP case studies. The OSTP responded to this criticism in the third meeting, stating that "these case studies were derived in order to give an overview of the current roles and responsibilities for current products of biotechnology" (*4*). When pressed that there are current products for which agency jurisdictions are not clear or are absent (e.g., *5*–*7*, *11*), no further explanation was provided (*4*). Thus, the portion of the OSTP process that was in the public eye shifted from "development of an updated CFRB" in the White House memo to clarification of authorities for current products that clearly fall into existing authorities.

Perhaps the limitations in the case studies and scope of the OSTP process were due to challenges in the political stream, such as behind-the-scene influences of certain stakeholders or political appointees within agencies, or interagency differences of opinion. However, people outside these circles do not know the reasons for the shortcomings, as the interagency discussions have not been open to the public.

The interagency working group has also been tasked with developing a long-term plan for horizon scanning of new biotech products and mechanisms to ensure that the regulatory system is well prepared (*4*). A draft of this plan and the final cases are to be published by the summer of 2016 for public comment through the Federal Register. If this deadline is met, the timing will occur before release of the Academies' study, and it is difficult to see how this external expert process will inform the interagency long-term planning process slated for this political administration.

LIMITED FORMS OF ENGAGEMENT

Although the OSTP is to be commended for hosting three public meetings, the format did not meaningfully engage external scholars and practitioners, stakeholders, and interested and affected parties with local and specialized knowledge (*12*). Public meetings, with lengthy agency presentations and brief comments from various groups, are best for expressing views on controversial topics and are one of the least rigorous forms of public participation. Participants self-select, are generally wealthier, and have special interests (*13*). The OSTP public meetings took this form with some exception: The second meeting in Dallas, Texas, allowed for written questions, and the third at the University of California, Davis, involved a 1-hour breakout, discussion session (although results of the breakouts were not reported back to the full group for discussion). The least participatory meeting, the first one, for which the case studies were not available, took place in Washington, DC, yet this is where most interest groups with concerns about over- or underregulation reside (e.g., consumer and environmental groups or trade organizations).

Practitioners and scholars have been studying and implementing forms of public engagement that are more deliberative (i.e., that include back-and-forth exchange among diverse groups with bi-directional learning) and representatives (*11*–*14*). Meaningful public and stakeholder engagement is not easy. It takes planning and careful design, as well as time and resources. However, it is not expensive in comparison with biological research—it requires no high-tech equipment, materials, or facilities. There is a vast scholarly literature to guide when, how, and why to do it (e.g., *11*–*14*). Scholars have stressed the need for wider engagement in order to make decision-making for GEOs development and deployment more equitable, fair, and "strongly objective" (*15*).

The "public" political stream has magnified for biotechnology products, especially GEOs in the environment, with citizens demanding labeling of GE foods, and nongovernmental organizations promulgating lawsuits against federal agencies because of insufficient environmental assessments. In failing to conduct a rigorous mode of engagement, the OSTP process ignored the public part of the politics stream, constraining policy agenda setting to an inner circle of policy elites through the closed-door interagency process.

External experts do not seem to have been meaningfully engaged. Outside product developers and natural scientists are often a part of scientific or stakeholder advisory panels for federal decisions about biotechnology products and regulations. The current Academies' panel includes them, but its report is

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due after the OSTP draft long-term plan, and the scope does not include recommendations on revisions of authorities or regulations under the CFRB (*9*). Also, Academies' panels deliberate in private, and their composition is limited in expertise and experiences. Between the OSTP "clarification" scope and the Academies' study, this rare opportunity for changing biotech regulation is getting lost.

Another important expert group includes those who study policy evaluation, design, and implementation from the behavioral, economic, management, policy, political, and social sciences and fields of applied ethics, communication, democratic engagement, and risk perception. OSTP did not appear to make it a priority to engage them, even though arguably their expertise would be most important given that many have studied biotechnology regulation and governance. As a result, the OSTP process may be less responsive to the multitude of ideas for biotechnology regulation from the scholarly and think-tank literature [e.g., (*16*–*19*)].

The lack of transparency and few opportunities for meaningful, deliberative engagement stifled policy innovation during the OSTP process, which is almost sure to lead to further distrust, skepticism, and decreased public legitimacy in the CFRB.

MISSED OPPORTUNITY FOR GLOBAL ALIGNMENT

The possibility of harmonizing the CFRB with a major international approach was not explicitly considered in the OSTP public process (*3*). The issue arose in public comments, but responses were not given by the agencies because the public comment periods were designed only to listen (i.e., no back-and-forth dialogue) (*3*). The United States is not a party to the Cartagena Protocol on Biosafety (BSP) or the overarching United Nations (UN) Convention on Biological Diversity (CBD), which has been a key international mechanism for outlining countries' obligations to each other for safe transfer and use of living modified organisms (LMOs, also known as GEOs). With 196 parties to the CBD and 170 parties to the BSP (*19*), the protocol, although imperfect, is the closest mechanism possible for GEOs' oversight and international harmonization. It allows flexibility in design of national biosafety regulations but establishes rules and procedures for the safe transfer and use of LMOs and guidance for risk assessment.

Elements of the CBD-BSP are thought by many to run counter to U.S. federal policy, trade, and business interests [e.g., (*20*)]: In it, there are a form of the precautionary principle that troubles U.S. agencies and biotech developers and a call to consider socioeconomic impacts from LMOs, including impacts on indigenous and local communities (*19*). However, many parties to the CBD-BSP balance provisions in the protocol with producing and releasing LMOs. Several grow sizable acreages of GE crops (e.g., Brazil, second; India, fourth; and China, sixth in the world) (*21*). Other parties allowed release of transgenic insects before the United States (Panama, Malaysia, and Brazil) (*7*).

Although this article does not argue for U.S. participation in the CBD and BSP, it does lament the lack of discussion and analysis of pros and cons under the OSTP interagency process. For example, logical benefits of U.S. participation could be to have a voice in (i) drafting subprotocols under the BSP, (ii) interpreting the CBD-BSP statement on precaution less stringently in the formulation of risk assessment standards, and (iii) promoting the possibility (however remote) of harmonizing data packages to fit what U.S. companies do for risk assessment. U.S. participation could potentially increase global good will, perhaps inciting less controversy in trade disputes through the World Trade Organization.

"...this rare opportunity for changing biotech regulation is getting lost."

Another option that was not considered in the OSTP process, at least publicly, but was raised in the public comments (*4*) is the passing of a U.S. national law to consolidate biotechnology regulatory authorities under the CFRB. Authorities under the CFRB are diffuse, outdated, and confusing, especially for newer biotechnology products. This situation is bound to get worse. A new biotechnology regulatory law would require significant political will in Congress. If the OSTP process had considered this option and engaged scholars, practitioners, and stakeholders to do so, there could have been a higher probability of placing biotechnology regulation on the agenda for the upcoming presidential administration. If societal benefits were found to be greater than societal costs, Congress might have even mustered political will to pass new legislation.

A consolidated biotechnology regulatory law is not so preposterous. Many developing countries have passed specific biosafety laws for GEOs as a result of participation in the CBD-BSP, and some developed countries have specific laws for GEOs regardless of participation in the CBD-BSP (*23*). Some countries with specific biotechnology laws, such as Australia, are growing substantial acreages of GE crops (*22*). In the United States, a consolidated biotechnology law could create more certainty for GE product developers, especially important to small companies that have fewer resources to navigate complicated regulatory paths in the CFRB. A new biotechnology-focused law could provide an opportunity to harmonize with the CBD-BSP, if analyses showed benefits of doing so.

Open policy windows to improve biotechnology governance are rare, as they depend on the confluence of policy, political, and problem streams. Perhaps there is still time to alter the OSTP process before the next presidential administration takes over (a time when activities from the previous administration are often abandoned). Other policy windows may open in the future. But opportunities to effect meaningful and impactful change for safe, responsible, legitimate, and appropriate use of GEOs were missed at this key juncture in the biotech revolution, although it is poised to change nearly every sector and even our conceptions of nature. ■

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"[A] tree can be only as strong as the forest that surrounds it," writes Peter Wohlleben.

PHOTO: JONATHAN A. MEYERS/SCIENCE SOURCE

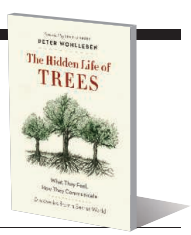
PLANT SCIENCE

The socially savvy tree

Anthropomorphisms abound, but science underlies a forester's reverent ode to woodland complexity

By **Gabriel Popkin**

The Hidden Life of Trees
What They Feel, How They Communicate
Peter Wohlleben
Greystone Books, 2016. 288 pp.



Who has not looked at a tree, outwardly static yet clearly full of activity, and wished for a translator to help us better understand its inner life? German forester Peter Wohlleben seeks to fill that role in his new book, which last year became a surprise best seller in Germany and is now being released in English.

It's a noble cause, but Wohlleben is venturing into fraught territory: Right from the start, the book's title evokes *The Secret Life of Plants*, the pseudoscience-ridden 1973 best seller that scared serious scientists away from plant intelligence and communication for a generation (*1*).

Wohlleben's main thesis is that forests are a far more complex and interconnected society than they appear. We see trunks, branches, and leaves, each belonging to what appear to be separate individuals, but beneath the surface, intertwined roots further linked by networks of fungi shuttle vital nutrients from soil to and among trees.

Trees, Wohlleben argues, are intelligent and community minded and—this being the digital age—socially networked. They share resources, they can selectively allocate sugar and nutrients to ensure equitable distribution, and they can keep alive stricken comrades that have lost the ability to photosynthesize.

Even distantly related conifers and deciduous trees collaborate belowground, according to research conducted by Suzanne Simard, an ecologist at the University of British Columbia. Simard is one of the leaders in a renewed effort to rescue plant intelligence and communication from the pseudoscience dustbin. (She also contributed an afterword to the English version of this book.)

Aboveground, trees communicate using chemical signals, which convey messages to other plants and to animals. Scientists have even found that trees can make sounds when they're parched, which Wohlleben suggests could be considered a cry of thirst, although he wisely notes that this is our interpretation, not the tree's intent.

Wohlleben largely avoids the kinds of claims that made *Secret Life* a hot potato for scientists (e.g., that plants respond to human emotions). But his anthropomorphizing may irritate those seeking to understand trees on their own terms. Chapter 1 is about tree "friendship"; chapter 8 about tree "school." On page 152, a tree feels "anxious"; on page 174, another experiences "disappointment."

Wohlleben's references to scientific research are more welcome, but I would have liked to learn more about areas of current scientific disagreements. His sourcing can also be thin, and he seems to regard peer-reviewed studies, university press releases, and media reports as equivalently authoritative.

When science has not yet elucidated something, Wohlleben often speculates freely, and while he usually alerts the reader, the back-and-forth can be confusing. Readers may also wish that Wohlleben, whose expertise is in relatively species-poor European forests, more than passingly mentioned tropical forests, which figure so prominently in modern carbon and biodiversity science.

Wohlleben is at his best when drawing from his 20-plus years of experience in production forestry and ecological woodland management. He is a keen observer of the strategies trees have evolved to cope with insects, deer, weather, and other threats—including other trees—despite being stuck in one place. And I appreciate that he resists the temptation to indulge in the all-too-easy "we could learn something from trees" brand of environmental moralizing.

Wohlleben also argues convincingly that growth-maximizing tree plantations, on which forestry science has long focused, do not allow trees to reach their full potential aesthetically, ecologically, or in any other way. He concludes with an eloquent appeal for a more holistic, long-term approach to forest management.

I expect that even the most knowledgeable reader will find their next trip to the forest or walk around town enhanced by Wohlleben's insights. But finding those insights can be a slog. The chapters frequently meander and seem ordered at random. And, at least in translation, the writing is functional but often inelegant—think rough-hewn log rather than fine woodwork.

The Hidden Life of Trees has captured a zeitgeist—perhaps the message of arboreal harmony comforts during a time of societal disharmony—but I suspect that over time, it will get crowded out by stronger competitors.

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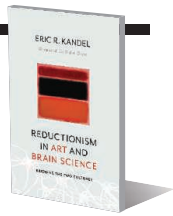
AESTHETICS

Scientist's guide to modern art

Scientific inquiry and abstract artistic expression may have more in common than you think

By **Alva Noë**

Reductionism in Art and Brain Science
Bridging the Two Cultures
Eric R. Kandel
Columbia University Press, 2016. 236 pp.



In *Reductionism in Art and Brain Science: Bridging the Two Cultures*, Eric R. Kandel, the noted brain scientist, states that painting—from the Renaissance until fairly recently—sought to create the illusion of the three-dimensional world on a two-dimensional canvas. What makes this sort of painting convincing is not its realism or its naturalism. What is distinctive, rather, about what Kandel calls figurative art is that it affects the visual system in much the way that the depicted events and figures do or would. Painting in this tradition moves us, he argues, for the same reason that the world moves us.

It is abstract art that is Kandel's real concern in this provocative short book. Paintings of the sort made by Wassily Kandinsky, Piet Mondrian, Willem de Kooning, Jackson Pollock, and Mark Rothko are, for Kandel, stimuli of an entirely different sort. They are not pictures; they do not trigger low-level processes whereby the brain can parse an image into objects standing in spatial relations. Instead, he argues, they provide an opportunity for "top-down" processing to occur.

We have no choice, when confronted with the nonfigurative, but to free ourselves from normal seeing. Memory, emotion, and past experience become our guide. Abstract painting, in this way, frees us from the ties that bind us to reality and allows for more powerful feeling and for emotional, even spiritual, response.

Immanuel Kant, the great German philosopher, proposed that by affording opportunities for experiences that are not subservient to the usual task of extracting information about the world around us, art allows for what he called the free play of the imagination. Kandel's view is a bit like this.

Like Kant, and like many neuroscientists today, Kandel holds that art and life are continuous. Works of art affect us because they tap into the very perceptual, cognitive, and also emotional and associative mechanisms that enable us, or our brains, to have any experience at all, he argues.

One problem with any theory that emphasizes art-life continuity is that it may explain too much. After all, it is only in the most exceptional circumstances that we cannot distinguish a flesh-and-blood encounter from a corresponding artistic rendering. Must not an account of art explain why and how it is distinct, or special, or at least different from ordinary experience?

Guggenheim visitors contemplate a painting by Mark Rothko in Bilbao, Spain.

PHOTO: VINCENT WEST/REUTERS IMAGES

Kandel offers an answer to this question, which is both interesting and illuminating, related to the ungainly word "reductionism" that figures so prominently in the book's title. Works of abstract art, he argues, reduce our experience of the natural world to its fundamental elements of form, line, movement, color, and light.

In some paintings (those by Rothko, for example), color is freed from form and line. In others, like the works of Mondrian, line is freed from figure and ground, from body and shape. Still other artists, such as de Kooning and Pollock, afford us unfettered encounters with movement, with pattern.

This kind of painting is reductionist, as Kandel means it, because it affords the viewer the opportunity to have experiences that are simpler and, in a way, more perspicuous. The resulting art experiences are exemplary; they are models, and they are, as such, comparable to model organisms like the *Aplysia* sea slug that figure in Kandel's own research into the neural basis of memory and learning.

Even figurative painting, which uses color to enhance shape and line and the production of a three-dimensional depiction, is reductionist, in this sense. It serves as a model of the way in which our experience of a dynamic, moving, animate visual world is grounded in our biologically inherited capacity to make sense of line, color, perspective, and other basic elements of consciousness.

The assumption that works of art are first and foremost triggers producing static emotional or perceptual responses, accompanied by a reliable set of neurological effects, is implausible. Like a judgment or opinion, an aesthetic response is continuously changing and getting revised in light of not only ongoing perceptual and emotional reactions but also the attitudes and judgments of others and changing cultural circumstances. We learn to be moved by art.

Although Kandel sometimes suggests that he endorses this overly simplified perspective, the view he delivers is much more subtle. For him, works of art are windows into ourselves or into what he refers to, following the art historian Ernst Gombrich, as "the beholder's share." Works of art do not merely cause experience, he argues; they also figure into art's ongoing effort, like that of science, to understand ourselves.

In *Reductionism in Art and Brain Science*, Kandel proposes that what is needed to bridge what the English author and physical chemist C. P. Snow called the "two cultures" of science, on the one hand, and art and humanities, on the other, is genuine collaboration. This is a proposal that deserves full support.

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LETTERS

Edited by **Jennifer Sills**

Passport power: Entrenching inequality

IN 2015, GLOBAL media fixated on the plight of migrants crossing the Mediterranean Sea in search of better lives in Europe. While thousands of lives were lost at sea (*1*), many European citizens called for tighter immigration restrictions ("Tensions of refugee politics in Europe," R. Hansen and S. Randeria, Policy Forum, 2 September, p. 994; published online 25 August). The Brexit referendum result and the popularity of Donald Trump's anti-immigrant rhetoric (*2*) exemplify how anti-immigrant sentiments continue to grow across the developed world.

Protectionist attitudes not only negatively affect scientific progress in rich nations (*3*) but also impede much-needed socioeconomic progress in developing nations. Despite sending substantial resources for development aid to poor nations, rich nations simultaneously impose harsher hurdles for citizens of those poor nations who wish to enter rich nations (*4*), thereby impeding capacity-building opportunities for those they profess to help. Because scientists from poor countries are increasingly subjected to expensive visa application fees, visa delays, and denials, they may reconsider short-term visits to developed countries. In addition, students fear attending international conferences; given that a visa does not guarantee entry to a country [e.g., (*5*)], they could be denied re-entry into their developed host countries at the discretion of a customs agent. Anti-immigrant policies thereby exacerbate already strong inequalities between rich and poor nations.

Streamlined immigration policies could better facilitate travel by scientists. For example, developed nations could adopt programs to allow third-country access for holders of visas issued by countries with stringent vetting standards, similar to developing countries that accept U.S., U.K., Canadian, Australian, and Schengen visas as substitute visas for short-term visitors that would otherwise require travel authorization [e.g., (*6*)]. Alternatively, European countries could follow the example of the U.S. visitor visa, valid for 10 years, alleviating the burden of current Schengen visas, which seldom extend beyond 6 months.

Science is enriched when people with different cultures come together to solve challenging problems. Developing countries strengthen their scientific capacity, and developed countries receive an injection of ideas. Immigration policies that impede scientist travel weaken innovation and progress, leaving both developed and developing countries short-changed.

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Precision medicine: Fantasy meets reality

IN THEIR POLICY Forum "Countering imprecision in precision medicine" (29 July, p. 448), S. P. Hey and A. S. Kesselheim discuss the many combinations of biomarkers and treatments that drive precision medicine research. They propose that "Funding agencies could award responsibility for specific regions of the [biomarker x treatment] parameter space through their grants." However, this is an extremely inefficient way to search such a large number of combinations. Researchers should be able to rapidly and adaptively refocus their research efforts as results become available. Bureaucratic funding agencies cannot react quickly enough to facilitate this dynamic process. Electronically connected networks of collaborating scientists can.

Several researchers have proposed, and some have piloted, a Global Cumulative Treatment Analysis (*1*). In this giant prospective trial, all patients with a given condition who enroll in the trial are tracked and treated based on the best available knowledge, and when there is uncertainty about whether a treatment will be beneficial, the decision algorithm (be it computational, human, or more likely a combination) randomly assigns treatments to patients in real time, integrating response information as soon as it becomes available and updating the

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decision algorithm immediately.

This strategy would allow biomedicine to operate as a gigantic distributed robotic discovery system (*2*) or air traffic control system: New hypotheses injected into the system by pharmaceutical companies or researchers would be validated or refuted by being pushed through a huge distributed and rapidly intercommunicating network, which would integrate information nearly instantly.

This may seem like a fantasy that would require a giant connected computer system over many sites, but the Veterans Administration has just such a system and is in the process of piloting exactly this idea, in what they call a "Point of Care" trial (*3*). If Apple, or some other organization, has its way in centralizing medical records for the rest of us (*4*), that system could operate the same way. Interdisciplinary doctors already meet to discuss the best precision treatment options for patients on panels referred to as "molecular tumor boards." A centralized system could network the tumor boards of the world.

There are, of course, many complex issues that need to be worked out in creating such a model for treatment, and treatment discovery (*5*), but something like a Global Cumulative Treatment Analysis is the only way that the increasingly enormous combinations of biomedical parameters can possibly be searched with any efficiency.

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Public feedback at risk in Brazil

IN HIS POLICY Forum "Brazilian politics threaten environmental policies" (19 August, p. 746), P. M. Fearnside discusses the risks that recent environmental policy reforms pose to the conservation of Amazonian biodiversity. He calls for continued input by the scientific community but fails to point out that PEC 65/2012, the proposed constitutional amendment, threatens public participation in environmental decision-making.

If passed, PEC 65 would violate human rights of participation in environmental decision-making by bypassing public hearings (*1*). In the current licensing system for large construction projects, public hearings are a mandatory step. Before the project is approved for a license, environmental impact assessments are conducted and presented to the public. Based on the results of the assessment and public feedback, procedures for compensation and mitigation of impacts may be assessed, adjusted, and monitored. Society has the right to be consulted about actions that might affect the country's environmental well-being. Public participation in environmental matters is also granted by international policies of which Brazil is a signatory, including International Labour Organization Convention 169, which requires free, prior, and informed consent by indigenous peoples for projects that affect their well-being (*2*).

Without local peoples' input and participation, not only are ecological systems threatened by authorizations of mining, hydroelectric dams, and other projects, but so too are interlinked social-ecological processes that have protected Amazonian biomes for millennia (*3*). Scientists' input is essential to inform environmental decisions, but the complexities of social-environmental licensing in Brazil need to be addressed by enhanced dialogue and coproduction of knowledge across diverse groups such as local actors, scientists, governmental and nongovernmental institutions, private sector, and public prosecutors.

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Can seeds from sustainably grown *Camelina sativa* replace fish such as the Arctic char as a source of heart-healthy omega-3 fatty acids?

THE NEW HARVEST

Translational plant science yields sustainable oils, pharmaceuticals, and proteins

By **Pamela J. Hines** *and* **John Travis**

PHOTO: SASHA GITIN

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The Dust Bowl in the U.S. Great Plains in the 1930s resulted from a toxic combination of drought and inappropriate farming methods. Even today, yields are limited by poor fit between the land available and the farming technology used, particularly in developing countries, exacerbating the problem of feeding an ever-increasing population. Meanwhile, interest in healthy foods has put increasing pressure on existing sources such as fisheries.

As much as we are already dependent on agriculture for food, feed, and fuel, we can now ask even more of the plants grown for human uses.

The plant metabolic pathways that make oils and fatty acids can be retuned to make products such as adhesives and lubricants that we might otherwise derive from nonrenewable petrochemicals. Small metabolites generated by plants, ranging from aspirin to the anticancer agent etoposide, offer a universe of chemical diversity remaining to be fully explored. Trichomes, tiny hairs on the surface of leaves where certain plant products accumulate, can serve as ready-made factories. Medicinal or other special-purpose proteins can be produced in bulk in plants: Early successes include antibodies for treatment of Ebola virus infection and therapeutic enzymes for treatment of certain human metabolic disorders. Engineering plants to improve uptake of nutrients such as phosphorus and nitrogen can reduce the need for those inputs from fertilizers. Beyond agricultural production, plants can be used for bioremediation of damaged land, such as removing toxic byproducts of explosives.

Despite new challenges for regulatory bodies, there is no doubt that translational plant science is poised to produce a promising new harvest.

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THE PLANT ENGINEER

Dan Voytas has worked tirelessly to make targeted genome editing of plants a reality

By **Elizabeth Pennisi**

At age 5, with some gentle guidance from his parents, Dan Voytas grew more tomato seedlings than could fit in the household garden. So his dad suggested the young gardener put the extras in his wagon and market them to neighbors. The small boy was hooked—the next year, he grew even more plants. A few years later, his dad built a greenhouse off the family's laundry room so the dryer vent would warm the plants, giving his son a head start on the growing season. Voytas added petunias and other bedding plants to his product mix; by high school, he netted $1000 a summer, impressive for a teenager in the late 1970s.

A budding entrepreneur, Dan Voytas grew and sold plants to neighbors.

Now, Voytas's green thumb and entrepreneurial spirit are poised to reshape 21st century agriculture. Over the past 20 years, he has pioneered novel ways of precisely editing a crop's DNA to give it new traits or delete undesirable ones. It's an approach that is potentially more powerful than the traditional way of making genetically modified (GM) crops: using bacteria to smuggle in genes from other species. And because it can leave no foreign DNA behind, it could free these products from the stigma and regulatory burden of being labeled as GM organisms (see sidebar, p. 1222).

Voytas, now 54 and director of the Center for Genome Engineering at the University of Minnesota (UM), Twin Cities, has had to overcome recalcitrant technologies, navigate intellectual property fights, and endure commercial failures. But he has emerged from it all as a recognized world leader in plant engineering. "When you think of gene editing in plants, he's one of the first names that comes to mind," says Michelle Christian, a molecular biologist at the Seattle Children's Research Institute in Washington. Voytas helped invent a key genome-editing platform, transcription activatorlike effector nucleases (TALENs), and a company he's partnered with already has new modified soybean, potato, and wheat varieties in the works, including a reduced-gluten wheat.

Voytas has also made a name for himself by rallying the field, forging collaborations, and disseminating new tools including CRISPR, the genome-editing system that is taking biology by storm (*Science*, 18 December 2015, p. 1456). Voytas is excited by CRISPR's ease of use and versatility, but he is not abandoning TALENs. It's like cellphone technology, he quips: "The iPhone 6 comes out and everyone wants it, but the iPhone 5 can still do everything you need."

"What makes Dan stand out is he's not wed to one tool or approach," says Adam Bogdanove of Cornell University, a co-inventor of TALENs. "It's about adopting whatever tools you need to gain control of [editing] crops." Indeed, Voytas is committed to sparking a revolution in plant science by any means necessary. In a world with a burgeoning population and limited land and resources, he says, it's a matter of survival. And one of the few things that can shake his habitually mild manner is what he believes is misguided suspicion of GM organisms: "At some point, it will come down to: Are we going to use genome-edited plants or are people going to starve?" he says.

VOYTAS'S LOVE OF PLANTS was a constant in a childhood that took him from rural Minnesota, where his dad was a forester for the U.S. Department of Agriculture (USDA), to northern New Hampshire, and finally to Harvard University. When he arrived at the Cambridge, Massachusetts, campus to start his freshman year, he immediately looked for the horticulture department—he planned to major in plant science—but dis-

PHOTOS: (LEFT TO RIGHT) FRANK VOYTAS; DAVID BOWMAN

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Genome-edited plants flourish in the growth room of Dan Voytas's new company.

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covered to his chagrin there was none. The best he could do was take a course in plant taxonomy. But he loved Cambridge, despite his mom's concerns about there not being enough green grass and too many cars, and after he found a plant lab at Harvard that would take him, he stayed on to get a Ph.D.

Working for Harvard geneticist Frederick Ausubel, "I immediately felt at home," Voytas recalls. The lab was pioneering the use of a then–little-known mustard plant, *Arabidopsis thaliana*, as a model for plant biology. Voytas was willing to look into whether it had so-called transposable elements: pieces of mobile DNA that can jump from one chromosome spot to another. Watching what happened to maize and insects when transposable elements disrupted a DNA sequence had proven useful in determining the function of many genes, but it was unclear whether they even existed in *Arabidopsis*.

"He was willing to take somewhat of a risk and start working at the dawn of a new field," Ausubel says. Papers in *Nature*, the *Proceedings of the National Academy of Sciences*, and *Genetics* attested to the lab's success. "Serious and earnest but also a lot of fun," is how Ausubel remembers Voytas, who once ordered baseball hats with an *Arabidopsis* plant on the front and the logo "Today's weed, tomorrow's paradigm."

Ph.D. in hand, Voytas postponed a move to Iowa State University (ISU) in Ames as an assistant professor because he was under the spell of transposable elements and their potential for genome manipulation. Instead, he made a detour to Johns Hopkins University in Baltimore, Maryland, joining yeast biologist Jef Boeke for a brief postdoc to study transposable elements in yeast, which is even easier to work with than *Arabidopsis*. With bioinformatics savvy unusual for biologists in the early 1990s, Voytas scoured the newly sequenced chromosome 3 of baker's yeast, *Saccharomyces cerevisiae*, for unknown transposable elements.

Even at that early stage of his career, when he and Henry Levin, a fellow postdoc in Boeke's lab at the time, regularly swam laps at a local Baltimore pool, Voytas would talk with enormous enthusiasm about his dream of precisely engineering plant genomes as a better way to improve

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When is a GM plant not a GM plant?

By **Elizabeth Pennisi**

The recently developed genome-editing methods, from zinc finger nucleases to transcription activator-like effector nucleases (TALENs) to CRISPR, are shaking up the debate over how to regulate genetically modified (GM) plants. Canada, for example, stuck to its rule that a plant should be regulated as GM if a novel trait has been introduced to it, regardless of the technology used. But the U.S. Department of Agriculture (USDA) has so far exempted plants altered by TALENs and CRISPR from its GM regulations.

Developed decades ago, when "gene guns" and bacteria were used to insert new genes, USDA's rules focus on whether a plant contains "foreign" DNA from a plant pest or pathogen. Advocates of the latest technologies say they leave no such DNA behind, so the plants that result should be indistinguishable from ones that could be naturally bred with enough time. In accepting this reasoning, "USDA has been very progressive [even though] they are working with statutes that were put in place before anyone could imagine you could edit a genome," says Dan Voytas of the University of Minnesota, Twin Cities, a genome-editing pioneer who is also chief scientific officer at the New Brighton, Minnesota, company Calyxt (see main story, p. 1220).

Developing, testing, and getting regulatory approval for a plant modified with earlier techniques could take decades and cost more than $100 million, limiting commercial use of GM plants primarily to large companies trying to improve commodity crops. But USDA exemptions for CRISPR and TALENs-edited crops favor smaller firms such as Calyxt, allowing them to work with food companies and respond quickly to consumer preferences, Calyxt's CEO, Federico Tripodi, argues. The development of Calyxt's first TALENs-modified soybean strain, which produces oil with fewer polyunsaturated fats, has taken just 6 years and cost $6 million, he notes.

USDA, however, has only ruled so far on crops in which the new genome-editing methods knocked out DNA bases, not those in which they've introduced, replaced, or added genes. And the United States may still alter its existing policy on GM organisms—the National Academies of Sciences, Engineering, and Medicine and a White House group involving USDA, the Food and Drug Administration, and the Environmental Protection Agency are examining the issue and will release recommendations soon (see Policy Forum, p. 1211).

Many other countries haven't taken a clear stance yet. China has encouraged research into genome-edited crops, but public opposition to GM organisms in general has kept the nation from clarifying its approval process. The European Union has postponed its own decision on CRISPR- and TALENs-edited crops for almost a year now, and some suspect it will leave the issue to individual members—a compromise it has made in the past for GM plants. ■

crops than the then-current methods. Levin would shake his head. "It was a shout out into the desert," says Levin, now a molecular biologist at the Eunice Kennedy Shriver National Institute of Child Health and Human Development in Bethesda, Maryland.

The DNA-editing techniques of 20 years ago were crude. They relied either on creating random mutations with radiation or chemicals or on injecting a gene for a desired trait and hoping it would land at a spot in the genome where it could be active. But in his yeast studies, Voytas unearthed something that promised more precise genome surgery. He found a set of transposable elements, called Ty5, that seems to target specific DNA, jumping only into inactive chromosome regions. In 1995, Voytas and a colleague filed for a patent on using Ty5 to manipulate genomes, and in 1999 he left ISU to found a company called Phytodyne to develop targeted plant gene-editing technologies.

Voytas and Phytodyne never got very far with transposable elements, but around the same time a few researchers were playing around with another way to target distinct places in a genome: harnessing pieces of transcription factors, the proteins that home in on specific DNA sequences to regulate a gene's activity. In a few cases, molecular biologists had linked the targeting portions of the proteins, called zinc fingers, to DNA-cutting enzymes called nucleases. At Phytodyne, Voytas and colleagues began to make these molecular scissors for plants, but they struggled to make progress. "The science was too hard and it was at too early a stage," Voytas says.

"It was frustrating," recalls co-founder David Wright, an agronomist at ISU. "We ran out of cash and we ran out of time." Voytas took the failure hard: "I vowed I would never do a company again."

WHEN PHYTODYNE CLOSED its doors in 2004, Voytas went back to ISU. One day, he got a call from Keith Joung, then a new pathologist at Massachusetts General Hospital in Boston, who was eager to develop zinc finger technology so that he could try it in mammalian cells. Their collaboration produced a 2009 *Nature* paper reporting that zinc finger nucleases (ZFNs) efficiently modified a tobacco gene to make the plant resistant to herbicides. They also founded a consortium that developed a set of ZFNs everyone could use, a tricky experience because a company was vigorously protecting its patent rights to the hard-to-make molecules and because of the inherent difficulties of teamwork. "I didn't quite realize it at the time, but he was a good mentor about how to handle the complex politics," Joung says. "I always felt that I could completely trust Dan."

Voytas's collaborative nature and willingness to try new things is legendary. Despite running a plant lab, he once took in an undergraduate who wanted to explore using ZFNs to cure AIDS—and later helped the student get a technician's job in Boston. Others liken him to a big brother "that knows everything and knows what help you need," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences's Institute of Genetics and Developmental Biology in Beijing.

A new chapter began in 2009 when Bogdanove, then at ISU, approached Voytas with a proposal that could render ZFNs obsolete. Bogdanove and, independently, a team in Germany had figured out that plant pathogen proteins nicknamed TALEs home in on specific DNA sequences based on the arrangement of their amino acids (*Science*, 14 December 2012, p. 1408). Rearranging the amino acids in TALEs to target different stretches of DNA was much easier than re-engineering zinc fingers, Bogdanove argued.

Voytas hesitated for a while, but after moving from ISU to UM, he teamed up with Bogdanove to link specific TALEs to nucleases—voilà, TALENs were born. "It worked as good as we had hoped," Bogdanove says. "Before CRISPR came out, it was the best tool out there."

And as he had done for ZFNs, Voytas became an evangelist for the new techno-

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A bumper crop of genome-edited plants

Challenging older forms of genetic modification, four recently developed genome-editing methods—meganucleases, zinc finger nucleases (ZFNs), TALENs, and CRISPR—have produced many plant varieties with useful new traits, some highlighted below. A few are in field trials already.

Genome-editing methods

- CRISPR
- TALENs
- ZFNs
- Meganucleases

- Drought resistant
- Disease resistant
- Consumer benefits
- Herbicide resistant
- Yield, storage, or processing improvement
- Altered growth

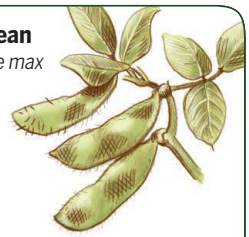
Corn
Zea mays

DuPont Pioneer plans a CRISPR-altered version of its "waxy corn" variety; Dow AgroSciences is using ZFN technology to make corn simultaneously healthier and herbicide resistant.

Tobacco
Nicotiana spp

In the 1990s, researchers used enzymes called meganucleases to edit tobacco's genome; TALENs have now helped make a tobacco relative that can synthesize human therapeutic proteins.

Soybean
Glycine max



Calyxt's TALEN®-edited soybeans may help food companies reduce trans fats in their products by 2018, as required by the United States for health reasons.

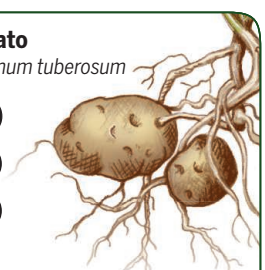
Wheat
Triticum aestivum

Chinese plant geneticists used TALENs to knock out all six copies of a gene that confers susceptibility to powdery mildew. Calyxt is readying field trials of the disease-resistant strain.

Rice
Oryza sativa

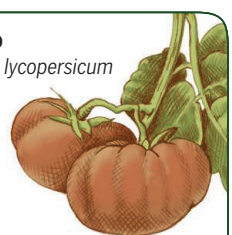
USDA gave a green light to Iowa State University's bacterial blight–resistant rice made by TALENs, but there are no plans for commercialization yet.

Potato
Solanum tuberosum



Calyxt's TALEN®-altered potatoes store longer in the cold, and yield less of a potential carcinogen when cooked; CRISPR'ed spuds are resistant to herbicide.

Tomato
Solanum lycopersicum



Genetic engineers have been trying to improve the shelf life of tomatoes since the 1990s, and now new genome editors are making the fruit resistant to bacterial and viral infections.

Poplar
Populus sp

At least two groups have used CRISPR to alter the lignin and tannin content of poplar seedlings, paving the way for trees better suited for biofuel and other uses.

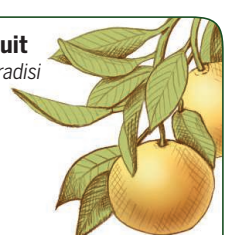
Lettuce
Lactuca sativa

Using a version of CRISPR that involved no introduced DNA—just RNA and proteins—a South Korean group altered lettuce to be more stress resistant.

Grape
Vitis vinifera

A Chinese group knocked out a gene promoting the production of tartaric acid, possibly paving the way to wine richer in vitamin C; others hope to engineer grapes to resist powdery mildew.

Grapefruit
Citrus paradisi



After demonstrating that CRISPR works in oranges, a team knocked out a grapefruit gene that confers susceptibility to citrus canker, a widespread bacterial disease.

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logy. "Anyone who works with TALENs got help from him," says Gao, who has become a leader in plant genome editing in China. Using these new DNA scissors, she recently developed a wheat that's resistant to powdery mildew, a serious fungal disease—a feat that entailed simultaneously knocking out all six copies of a gene that represses the plant's antifungal defenses. Gao and Voytas now have an ongoing text message conversation about their technologies, their results, and other activity in the field. "We are collaborators but we are also competitors," she says.

TALENs, which UM patented with Voytas, Bogdanove, and Feng Zhang (not the same as Feng Zhang of CRISPR fame), have made their mark outside the plant world as well. A company called Recombinetics, for example, licensed the method to improve livestock and develop better animal models of disease. More dramatically, the French biotech company Cellectis recently helped save the life of two infants with leukemia by using TALENs to transform immune cells so that they would target the malignant cells. "The babies just brought tears to his eyes," says Helen Voytas, Dan's mom.

CELLECTIS HAD EMBRACED TALENs for its medical interests after recruiting Voytas to start a plant science division. Voytas was willing to break his anticompany vow for a new chance to get TALENs-edited crops into farmers' fields. The effort started small, with Voytas and a skeleton crew setting up shop in a university "incubator" space, where the only place to grow plants was the loading dock. But it expanded fast, and the plant division, spun off as a subsidiary now called Calyxt Inc. in New Brighton, Minnesota, has just finished state-of-the-art greenhouses about 6 kilometers from the campus.

Already, the company has harvested 27 tons of a new genome-engineered soybean variety from Argentina and has a summer crop in several U.S. states to see how it fares in different environments and to build up the seed supply. This soybean was edited to produce no trans fats and high concentrations of oleic acid—a "healthier" fatty acid—and so could help food companies meet the Food and Drug Administration requirement of getting rid of trans fats in the food supply by 2018. Three other products have the green light from USDA, including a spoilage-resisting potato that also produces less of a potential carcinogen when fried. It is in fields now and could be marketed by 2019, according to the company; USDA has already said it won't treat the soybean or the potato as GM, which has sped their development.

Recently, the new CEO of Calyxt came to Voytas and asked whether TALENs were dead given the emergence of CRISPR. In response, Voytas showed him data on TALENs activity in diverse crop species. The company can make 1000 novel TALENs a week, more than enough to develop new products at a competitive rate, he argues. And whereas CRISPR technology is mired in intellectual property disputes, ownership of TALENs is clearly defined. TALENs can also be better than CRISPR at hitting an exact location in the genome and making clean cuts in DNA, Bogdanove says.

This tomato's purple hue signaled that Voytas's team had harnessed a virus to more efficiently deliver the CRISPR genome editor to plants.

Voytas acknowledges his group was slow to embrace CRISPR. The first CRISPR reports "sort of seemed too good to be true," says Colby Starker, Voytas's longtime lab manager at UM. "We got burned a little bit by not jumping on that bandwagon." But by now, 80% of their work is CRISPR-based, and, true to form, Voytas is developing tools for all to use. "Dan has a very strong sense that the technology we develop should be shared," Starker says.

One challenge for CRISPR and other editing platforms is the difficulty of getting the DNA-editing molecules past plants' rigid cell walls. To solve that problem, Nicholas Baltes, now at Calyxt, and others in Voytas's group have pioneered the use of plant viruses called geminiviruses, which naturally deliver genetic material into plant cells. The researchers have engineered the viruses to carry the genes encoding CRISPR's components—a nuclease and a so-called guide RNA that directs the nuclease to a target DNA sequence—into plant cells and then get the cell to churn out the gene editor. So far, these modified viruses have done the job in tobacco and tomato, increasing CRISPR's gene-editing activity 10- to 100-fold, Voytas says.

Already requests are pouring in for an extensive set of CRISPR tools that Voytas's lab soon plans to release. They've crafted 150 different CRISPR constructs—some encoding nucleases that make double- or single-stranded breaks; others that simply ferry molecules, such as fluorescent tags, to specific spots in the genome; still others that can go after up to 12 targets at once.

Now that genome engineering is such a hot field, Voytas is almost in as much demand as the inventors of CRISPR—in June he was traveling so much for talks and conferences that he spent only 6 nights in his own bed. That's particularly tough on him because his house is one of his few nonwork passions. The midcentury modern building, designed by one of Minnesota's most famous architects, had fallen into disrepair before Voytas restored it to almost its original state, adding an orchid garden that he tended for several years in the basement. He is also torn between his work and his dedication to his mother and handicapped brother, whom he visits weekly, bringing flowers from his community garden plot. Lately he has been so distracted by his many projects that his mom sometimes sends him home. "I'm talking and he's not hearing," she says.

It's all worth it to Voytas, who believes crops created with TALENs or CRISPR will be vital to the world's food security. Giving a talk about Calyxt's soybean project one day, he recalls, "I got all choked up and had to stop and take a long, hard breath." And last year, when he visited a big field of the company's soybeans, he snuck a few pods home, and kept them on the kitchen counter, picking them up every so often. A proud thought went through his mind: "My TALENs did that." ■

PHOTO: KIT LEFFLER

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Just a touch of nitrogen hikes yields in depleted soil, common in Africa. But fertilizer is scarce and costly for small farmers.

PHOTO: PETER ESSICK/AURORA PHOTOS/ALAMY STOCK PHOTO

THE NITROGEN FIX

Few projects in plant biotechnology are harder, or promise a greater payoff, than enabling crops to make their own nitrogen fertilizer

By **Erik Stokstad**

Plants already perform one life-sustaining feat of biochemistry, transforming carbon dioxide and water into sugars through photosynthesis. Perhaps it's too much to ask for a second. But a handful of biologists hopes to endow major crops with the ability to "fix" nitrogen from the air into a biochemically usable form, a talent that is currently limited to certain microbes—and is essential to life. Fixed nitrogen is a key ingredient in important biomolecules, including amino acids, the building blocks of proteins. And, for now, farmers have to laboriously supply it by applying fertilizer or planting legumes, which host nitrogen-fixing bacteria in their roots.

If wheat, rice, maize, and other cereals made their own nitrogen fertilizer, they might help solve two big problems. They could cut down the overuse of artificial fertilizer, which in many places leaches from the soil and pollutes aquifers or surface water. And they could make up for the shortage of fertilizer that plagues poor farmers in the developing world, particularly sub-Saharan Africa. "It's a grand challenge for humanity," says Philip Poole, a plant microbiologist at the University of Oxford in the United Kingdom.

Almost half a century ago, microbiologist Ray Dixon of the U.K.'s University of Sussex managed to engineer the bacterium *Escherichia coli* to fix nitrogen, inspiring a generation of scientists to try to match the feat in crops. But the tools for transforming plants, with their complex genetics, lagged those of microbiologists. "We were too early in thinking we could achieve it," says Dixon, who continues to work on nitrogen fixation at the John Innes Centre (JIC) in Norwich, U.K.

Now, a new generation of researchers has taken up the challenge. One contingent aims to turn cereals themselves into fertilizer factories by inserting the genes for nitrogenase, the key enzyme. Another hopes to engineer cereals so that they can mimic the symbiotic relationships with nitrogen-fixing bacteria found in beans and other legumes. Although little has been published, the research has received major funding from the Bill & Melinda Gates Foundation and government sources. "In the next year or so, you'll see some amazing breakthroughs in the ability of plants

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to fix nitrogen," predicts Jane Silverthorne, deputy assistant director of biological sciences at the National Science Foundation in Arlington, Virginia.

Many technical hurdles remain. "It's a long shot and it will take a long time still," says Ken Giller of Wageningen University and Research Centre in the Netherlands, an agronomist who is not involved in plant engineering. Even if the work is a technical success, the crops may struggle for public acceptance, because they will be genetically engineered. A quicker payoff may come from focusing on nitrogen-fixing bacteria themselves, tweaking them to release more fertilizer. Nevertheless, Giller and other observers say the potential of self-fertilizing cereals is so great that investment in engineering them is worth the gamble. "If you never try it," he says, "you'll never know if it's possible."

FARMERS REMOVE NITROGEN from soil whenever they harvest grain or other plant matter; in time, the soil gets depleted and yields shrink. Traditionally, they replaced the missing nitrogen by adding manure or compost, or by planting legumes. In the 19th century, scientists figured out that cereal yields could be greatly boosted with new sources of nitrogen: bird guano or nitrate minerals, both mined in South America. The deposits became so valuable that Chile and Peru even fought a war over them in the 1880s.

The Haber-Bosch process changed everything. Invented in 1909, it uses heat, pressure, and iron catalysts to crack the triple bond between nitrogen atoms and create ammonia, which is converted into nitrogen fertilizer. Yields of wheat, corn, rice, and other cereals skyrocketed. Starting in the 1960s, synthetic nitrogen fertilizer was a crucial factor in the green revolution in Latin America and Asia, which prevented major famines. About 450 million tons of nitrogen fertilizer are made each year; the Haber-Bosch process now accounts for about a third of all biologically available nitrogen.

Yet, on average, only half the nitrogen fertilizer applied in the developed world makes it into plants. That's not just inefficient; the excess can contaminate drinking water and feed noxious algal blooms. Then there's the climate impact, from the potent greenhouse gases released when soil microbes break down unused fertilizer, and the energy needed to run fertilizer factories, which consume 1% to 2% of all fossil fuels.

Much of Africa faces the problem of too little nitrogen, not too much. The soils are ancient, and their nitrogen quickly runs out. Legumes add it back, but crop rotation with legumes isn't enough to support adequate yields of cereals, the crops in greatest demand, Giller says. He is familiar with the challenge: He runs a nearly $50 million program supported by the Gates Foundation, N2Africa, to improve nitrogen fixation by legumes. Decidedly low-tech, it has provided several hundred thousand farmers with seeds, small amounts of fertilizer, and packets of nitrogen-fixing bacteria.

THE GATES FOUNDATION is also supporting the high-tech approaches to nitrogen fixation with major grants to two projects. One is led by Luis Rubio, a biochemist at the Technical University of Madrid, and the other by geneticist Giles Oldroyd of JIC. The motivation: Even minor increases in the amount of nitrogen available to cereals in poor soils can boost yields severalfold. "That's why we're willing to invest in higher risk science," says Katherine Kahn, a program officer in agricultural development with the Gates Foundation in Seattle, Washington. "The reward is potentially so high."

The hurdles are just as high. Rubio's project, to put nitrogenase in plants, is daunting for two reasons. First, the enzyme has an intricate structure, and its assembly, assisted by several proteins, is one of the most complicated known. Compared with swapping the genes among bacteria, it is no simple matter to move all the genes into plants and to get their relative expression levels correct. This task might be slightly easier to accomplish in organelles, such as chloroplasts, which have their own genomes. Second, the enzyme is degraded by oxygen, as are most of the proteins that put it together, and plant cells—chloroplasts in particular—are awash in oxygen, thanks to photosynthesis.

One possible solution is to design the enzyme so that it is active only in the dark; oxygen levels inside plants dip at night when they stop photosynthesizing. Another is to insert the genes for nitrogenase into the genomes of mitochondria, the rice-shaped organelles that crank out the cellular fuel adenosine triphosphate (ATP). Making ATP consumes oxygen, so mitochondria can be relatively anaerobic in places.

That's the approach Rubio's group is taking. Working in yeasts, in part because they have simpler genetics than plants, they engineered one major part of nitrogenase, encoded in a gene called *NifH*, into mitochondria, along with three genes for proteins that help put it together. Results of a recent proof-of-concept experiment were encouraging. In the April issue of *Nature Communications*, Rubio's team showed that the *NifH* subunit functions inside the yeast mitochondria. A different group, led by Natalia Ivleva at Monsanto in Chesterfield, Missouri, reported last month in *PLOS ONE* that they had added the *NifH* subunit into chloroplasts of tobacco plants, although they saw much lower activity.

Adding the other part of nitrogenase to yeast or plants will be harder, Rubio says. This component, called *NifDK*, requires many accessory proteins. He's optimistic about putting the pieces together, but is not holding his breath. "In a few years, we may have a pathway," he says. "If we can get a rudimentary pathway to work, we will have to improve performance."

The task won't end with getting nitrogenase itself to work inside plant cells. Researchers must ensure that the electrons zipping through the enzyme don't cause other aspects of cellular metabolism to go haywire. Another important task is to get the nitrogen out of the mitochondria and into the chloroplasts and other places where proteins are synthesized. Regulating the nitrogen levels is also a challenge, as an excess of ammonium (the final product, created when another proton attaches to ammonia) can cause stunted roots, yellowed leaves, and lower yield.

WHILE RUBIO TRIES to get crops to synthesize their nitrogen directly, Oldroyd and others want to make cereal crops more like legumes. Beans, peas, and other legumes allow nitrogen-fixing rhizobia to invade their roots, where these bacteria release ammonia in exchange for carbohydrates made by their

Legumes host nitrogen-fixing bacteria in root nodules, a trait that might be engineered into cereals.

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host. The symbiosis begins with an exchange of signals between the plant roots and soil-dwelling microbes. This chemical handshake establishes a friendly relationship that continues with the roots creating nodules to house the microbes.

Efforts by many scientists have unraveled the chemical and genetic basis of the signaling pathway. The core of the pathway already exists in cereals and most other plants. It probably dates back 400 million years, to when plants first colonized the land, and it establishes a relationship between roots and soil fungi, which help supply the plant with water, phosphate, and other nutrients in return for carbohydrates. Co-opting this pathway to allow cereals to host nitrogen-fixing bacteria has only recently seemed within researchers' grasp. "Although it sounds like a terrifying feat, it's not as bad as it sounds, because you're not starting from scratch," says Ottoline Leyser, a plant biologist at the U.K.'s University of Cambridge, who does not work on the project.

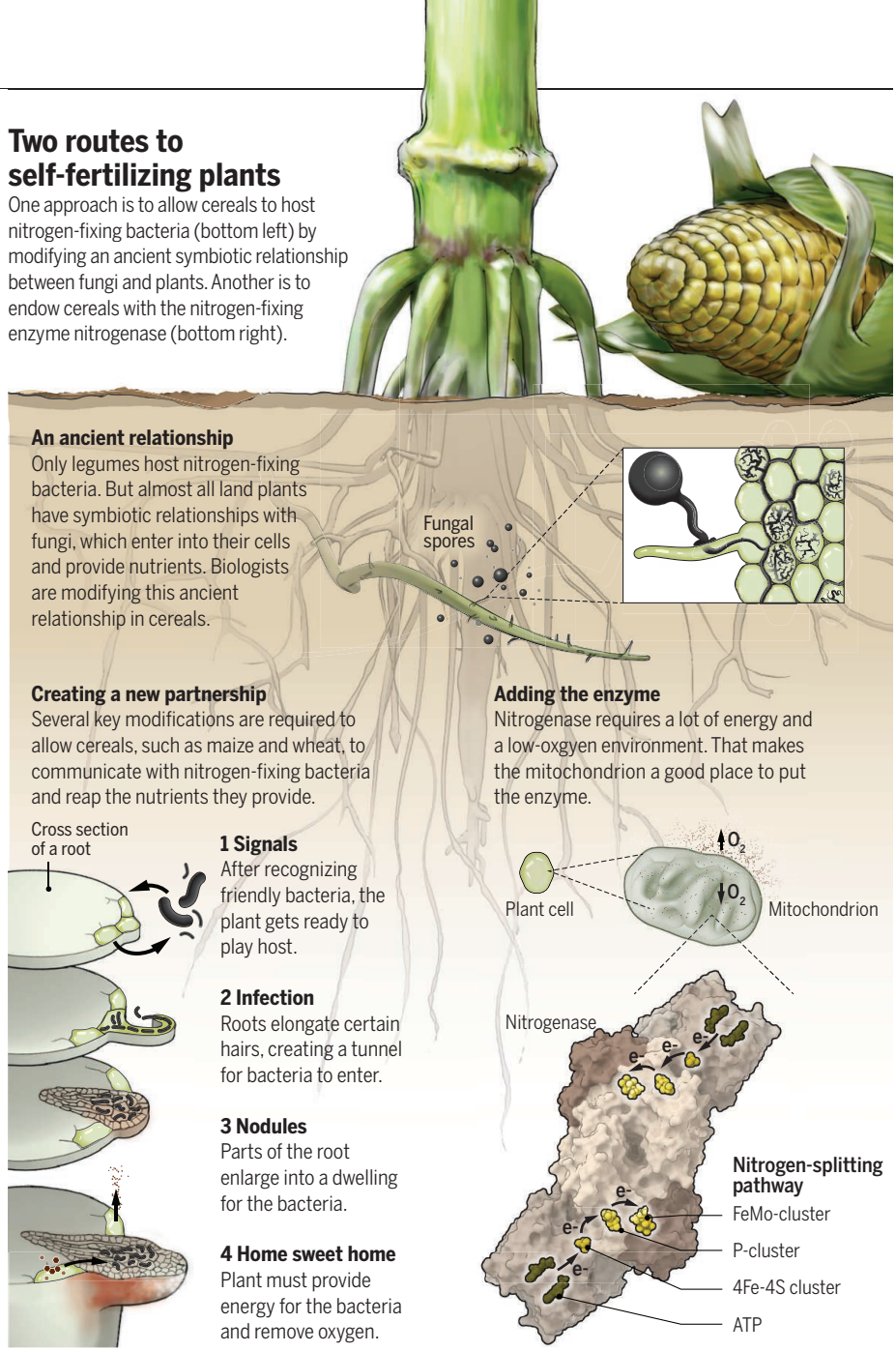
The first step is to get cereal plants to exchange signals with nitrogen-fixing bacteria (see graphic, right). Although the Gates Foundation has its eyes on nitrogen-fixing corn, Oldroyd's team is focusing on barley plants, which are easier to modify. The group is already testing plants engineered to detect the rhizobial signals, Oldroyd says. They have yet to publish results. "When you engineer cereals, you learn to be patient," he says.

The second, and much harder, step is to convince cereals to let themselves be invaded by the bacteria. Legumes beckon their bacteria into structures known as infection threads, but the process is mysterious. Researchers hope to adapt the preexisting mechanisms that allow symbiotic fungi to invade roots.

Then the plants need to be tweaked to form root nodules and to make their bacterial guests comfortable and productive. Like legumes, the host cereal must deliver carbohydrates and leghemoglobin, a molecule that binds to oxygen to prevent it from interfering with nitrogenase. "We know how to do it, but I don't know how hard it will be," Oldroyd says. Like Rubio, he expects that initial stages of the work will create low levels of ammonia, which could then be enhanced.

Two routes to self-fertilizing plants

One approach is to allow cereals to host nitrogen-fixing bacteria (bottom left) by modifying an ancient symbiotic relationship between fungi and plants. Another is to endow cereals with the nitrogen-fixing enzyme nitrogenase (bottom right).



GRAPHIC: V. ALTOUNIAN/*SCIENCE*

IN THE MEANTIME, some researchers are concentrating not on plants, but on soil bacteria, which can have a range of positive effects on crops (*Science*, 14 August 2015, p. 680). Many companies already sell nitrogen-fixing bacteria, which can increase growth of soybeans and other legumes when added to the soil. Microbes are also used on cereals and can raise yields by 10% to 20%—but by secreting growth-promoting hormones rather than fixing nitrogen.

Microbiologist Nicolás Ayub is one of several researchers building nitrogen-fixing bacteria for cereals. In 2013, he and colleagues at the National Institute of Agricultural Technology's Institute of Genetics in Buenos Aires and elsewhere took a stretch of bacterial DNA with 52 genes related to nitrogenase and put it into bacteria that glom onto crop roots but don't fix nitrogen. The modified bacteria, dubbed *Pseudomonas protegens* Pf-5 X940, fixed nitrogen and released it to the roots at a rate 1000 times higher than other nitrogen-fixing bacteria. Now, the team has shown in greenhouse tests that maize and wheat treated with the microbes grow as well as those given nitrogen fertilizer, as they reported 27 June in *Environmental Microbiology*.

Ayub and his colleagues have recently begun field trials. The bacteria survive only while growing on roots, and they do not enter the plant or its edible products—important facts for biosafety approval, Ayub says. Treating a field with microbes costs just 1% as much as adding nitrogen fertilizer, he adds. "The availability of this recombinant inoculant in the market can significantly increase the profit of farmers."

Rubio, Oldroyd, and others hope their labors could lead to an even brighter future, in which farmers will plant their crop and its fertilizer in a single, exquisitely engineered package. "It's such an attractive idea," Giller says. "At the end of the day, you can't not want it to happen." ■

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REVIEW

The plant lipidome in human and environmental health

Patrick J. Horn[1] and Christoph Benning[1,2]*

Lipids and oils derived from plant and algal photosynthesis constitute much of human daily caloric intake and provide the basis for high-energy bioproducts, chemical feedstocks for countless applications, and even fossil fuels over geological time scales. Sustainable production of high-energy compounds from plants is essential to preserving fossil fuel sources and ensuring the well-being of future generations. As a result of progress in basic research on plant and algal lipid metabolism, in combination with advances in synthetic biology, we can now tailor plant lipids for desirable biological, physical, and chemical properties. We highlight recent advances in plant lipid translational biology and discuss untapped areas of research that might expand the application of plant lipids.

Glycerolipids consist of a glycerol backbone with various combinations of fatty acids and head groups, thus generating a vast array of molecular species (Fig. 1). Glycerolipids constitute the largest fraction of the plant lipidome, or the total collection of plant lipids, and their chemical diversity is associated with many cellular functions. Plants build photosynthetic and cell membranes from polar lipids (Fig. 1). During the evolution of land plants, developmental adaptations led to the sequestration of carbon fixed by photosynthesis into high-energy compounds in the form of neutral lipids (Fig. 1), such as triacylglycerols in seeds. Algae accumulate triacylglycerols to survive adverse conditions—for instance, in response to nutrient deprivation. Glycerolipids also serve as mobile signals in cellular regulation and communication, and they function as components in photosynthetic and other enzymatic complexes. Basic research in model plants such as *Arabidopsis thaliana* and algae such as *Chlamydomonas reinhardtii* has generated insights into the regulation, synthesis, assembly, storage, and turnover of the plant and algal lipidomes. This intellectual framework leads to a design toolbox that enables translational research, construction of novel biotechnological processes, and generation of bioproducts.

Although most plants share a common set of reactions in lipid metabolism, the unique, specialized metabolism of select plants draws interest as a starting point for novel bio-based industrial processes and for improving human nutrition. Rapid technological advances in synthetic biology aided by complementary "omics" approaches have enabled the translation of these basic design principles through genetic modification (GM) of crops to synthesize valuable lipids. Examples of successful translational plant lipid research abound. Plant oils can be produced with tailored compositions for downstream production of desirable varnishes, soaps, or specialized lubricants (*1*–*6*); vegetable oil in nonseed plant tissues, such as leaves, to increase the biomass energy content (*7*, *8*); healthy fish oil–like vegetable oils in field-grown GM crops without depleting the oceans (*9*, *10*); and a potentially leaner seed oil with lower caloric content that may aid in combating obesity (*11*). In more futuristic efforts, basic research on the formation of lipid droplets, which store neutral lipids in plants and algae, may lead to practical synthetic biology platforms for the safe sequestering and easy harvesting of bioactive and hydrophobic lipid-based molecules. Finally, uncoupling the intertwined nutritional state and regulation of cell division of algal cells governing oil accumulation looks promising as a model system for potentially addressing instances when this analogous relationship goes awry in human cells, which may lead to cancer.

"Unusual fatty acids provide chemical feedstocks serving as precursors for lubricants, nutraceuticals, plastics, paints, natural insecticides, biodiesel, and jet fuels."

Plants as chemical feedstock factories

A number of nondomesticated plant species (Table 1) produce less common fatty acids that are of interest for industrial or human health applications. These include fatty acids with short (<8 carbons), medium (8 to 14 carbons), or very long chain lengths (>20 carbons), with distinct patterns of unsaturation such as conjugated (separated by 1 carbon bond) ω3 or ω7 double bonds (positioned 3 or 7 carbons from the methyl end of the fatty acid, respectively), and with addi2tional chemical modifications such as hydroxyl, epoxy, or cyclic groups (Fig. 1). Unusual fatty acids provide chemical feedstocks serving as precursors for lubricants, nutraceuticals, plastics, paints, natural insecticides, biodiesel, and jet fuels. In most cases, considerable constraint remains on cultivating nondomesticated plant species because of their poor agronomic performance or their adaptation to climates or ecological niches not permissive for widespread cultivation. Nevertheless, exploring the synthetic capacity of these nondomesticated plants has been essential for identifying specialized fatty acid synthesis and modification enzymes. These enzymes can be introduced into model plants for proof-of-concept demonstrations and ultimately into high-performing GM crops (Table 1).

The introduction of a diacylglycerol acyltransferase from the ornamental burning bush (*Euonymus alatus*) into the promising niche crop relative of canola, *Camelina sativa*, resulted in seeds with altered oil composition in which up to 85% of all triacylglycerols occur in the form of acetyl-triacylglycerols (*11*). *Camelina* is not a high-volume commodity crop, which should enable GM varieties to be more readily commercialized because of its easier identity preservation and stewardship. Acetyl-triacylglycerols contain a two-carbon acetyl chain at the *sn*-3 position of the glycerol backbone (Fig. 1). Development of this GM crop would address concerns about sustainable energy sources and the environment because acetyl-rich oils have reduced viscosity and freezing points and are therefore useful as replacements for fossil fuel–derived diesel #4 in heavy train and ship engines. More relevant to the average consumer, acetyl-triacylglycerols have a lower caloric value, providing a potentially leaner and healthier vegetable oil for human consumption.

Medium-chain fatty acids are also key ingredients for personal health care products such as lotions, shampoos, and soaps (*1*, *6*) and are often sourced from plants with a limited global distribution, such as coconut. For the purpose of generating GM crops for the production of medium-chain fatty acids, cDNAs encoding specialized fatty acyl–acyl carrier protein thioesterases with altered substrate specificities have been overexpressed in canola (*6*), tobacco (*1*), or *Camelina* (*12*); this prevents the conventional medium-chain fatty acid elongation to 16- or 18-carbon fatty acids in the plastid, resulting in altered acyl compositions of lipids. For example, after co-producing these thioesterases and medium-chain coenzyme A (CoA)–specific lysophosphatidate acyltransferases, which promote *sn*-2 incorporation into glycerolipids, from *Cuphea* sp. in *Camelina*, 14:0 (carbons:double bonds) accumulated up to 37% of total fatty acids in seeds (*12*). In vegetative tissues that are often even less accommodating to unusual fatty acids, medium-chain fatty acids were generated in tobacco leaves by transient co-production

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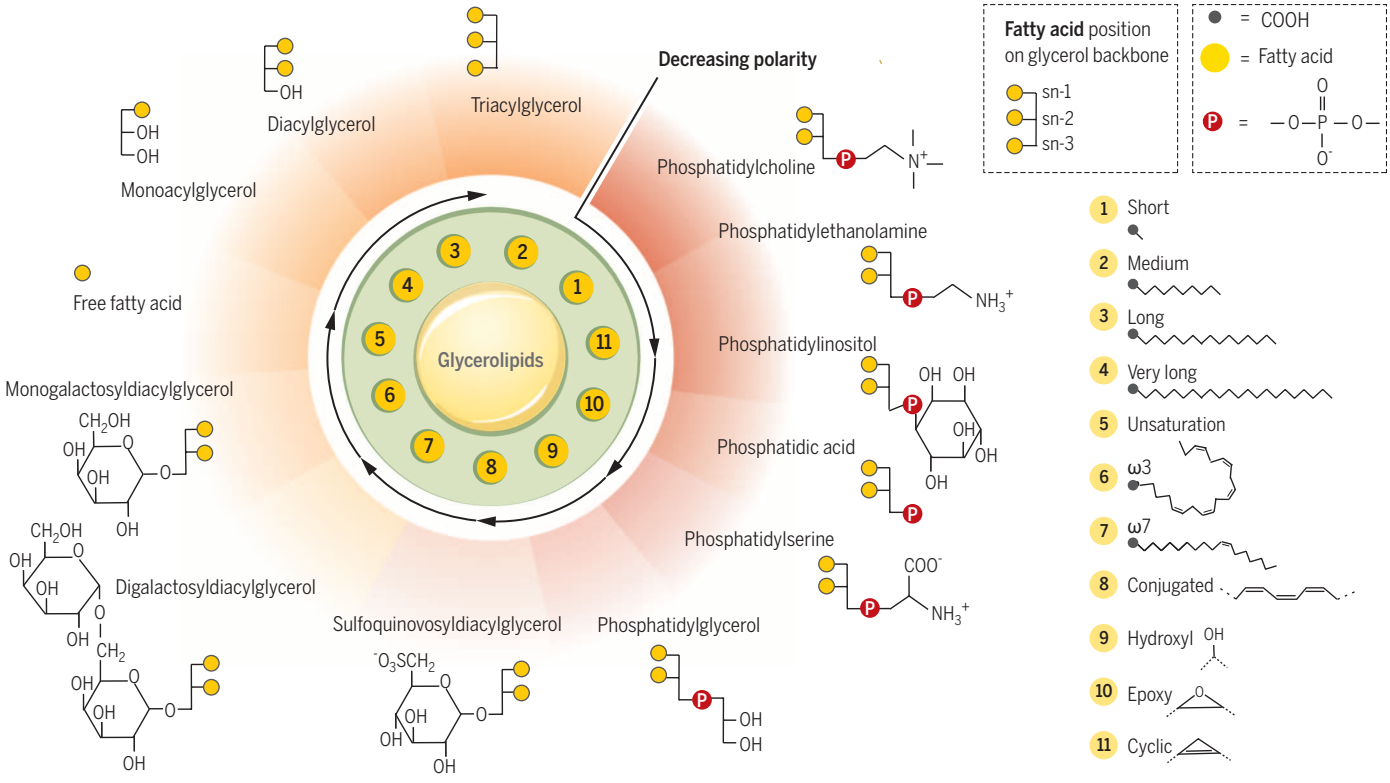

Fig. 1. The plant glycerolipidome. Plants are capable of combinatorial lipid chemistry. Glycerolipids consist of a combination of one to three fatty acids (inner dial) connected to a glycerol backbone. Positions on the glycerol backbone are designated as *sn*-1, *sn*-2, or *sn*-3. The glycerolipid subclass (outer dial, arranged in order of decreasing polarity) is specified by different polar head groups at the *sn*-3 position, or a hydroxyl (–OH) or acyl group in the case of neutral lipids.

of selected thioesterases with the coconut lysophosphatidate acyltransferase and the *Arabidopsis* WRINKLED1 transcription factor, which governs fatty acid biosynthetic gene expression in all oil-accumulating tissues tested, such that 12:0, 14:0, and 16:0 accumulated up to 10%, 19%, and 38%, respectively (*1*). Incorporation of unusual fatty acids at the *sn*-2 glycerol position is limited by endogenous fatty acid–editing mechanisms (*13*) that still need to be overcome to achieve higher productivity.

Engineering of very-long-chain fatty acids in two distinct but related species showed that selection of the engineered host plant is absolutely crucial. The expression of a cDNA encoding β-ketoacyl–CoA synthetase, which elongates long-chain fatty acids from oilseeds enriched in nervonic acid (24:1), produced only 13% 24:1 in *Camelina* versus up to 30% of total fatty acids in the close relative *Brassica carinata* (*4*, *14*). Presumably, the higher erucic (22:1) content in some *Brassica* varieties presents a more readily available substrate for 24:1. *Crambe abyssinica*, naturally high in erucic fatty acid, was further engineered to enhance the production of 22:1 and to reduce competing lipid pathways, such that levels were increased from 60% to 73% of all fatty acids (*3*). These very-long-chain fatty acids find uses as lubricants, synthetic rubber, and cosmetics additives; as precursors for nylon and plasticizers; in the treatment of neurological diseases such as multiple sclerosis; and in infant nutrient supplementation (*4*).

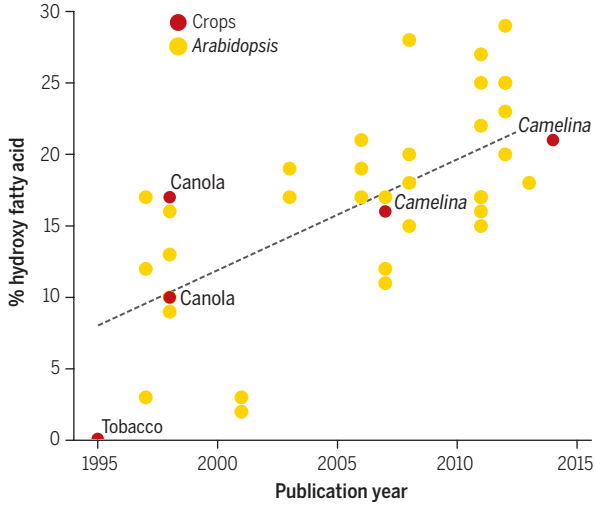

Fig. 2. Twenty years of engineering hydroxy fatty acids in oilseeds. Data points represent individual reports of GM crops or *Arabidopsis* accumulating hydroxy fatty acids. Data are adapted from table S1 of Horn *et al*. (*17*).

Many of the useful chemical modifications in unusual fatty acids arise from catalysis by specialized, evolutionarily diverged fatty acid desaturases. Introduction of hydroxyl groups into fatty acids allows these molecules to be directly bonded as lubricants to surfaces of high-performance engine parts. Castor bean, the main source of hydroxy fatty acids, produces more than 90% hydroxy fatty acid (relative to total fatty acid content) but is also a natural source of the infamous poison ricin. Introducing the hydroxy fatty acid trait into GM crops has frequently led to deleterious side effects—for instance, a 20% reduction in total seed oil and poor seed germination (*15*). Combinatorial studies of hydroxylases introduced into *Arabidopsis* and GM plants have led to steady increases in hydroxy fatty acid production over time (Fig. 2). The highest levels of hydroxy fatty acids in *Arabidopsis* and *Camelina* were reported at 29% and 21% of total fatty acid content, respectively (*5*, *16*). A comparison of global gene expression profiles from closely related *Camelina* to hydroxy fatty acid–accumulating developing seeds of *Physaria fendleri* suggested coevolution of many lipid biosynthetic genes and others associated with the production of hydroxy fatty acids (*17*). Furthermore,

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multiple differences in developmental timing of gene expression, substrate promiscuity by enzymes, allelic variation, ploidy, etc., likely contribute to differences in lipid composition among host systems. Consequently, a strategy for further increasing unusual fatty acid levels might require engineered plants with coexpression of a larger number of genes with targeted precision than previously assumed. In synthetic biology terms, the appropriate chassis makes a difference.

Up to 65% of the total fatty acids in *Camelina* seeds have been converted to ω7 monounsaturated fatty acids, a sustainable precursor for 1-octene used in the production of polyethylene (*2*). Punicic acid, an 18:3 conjugated fatty acid from pomegranate (*Punica granatum*) thought to contribute to its antiatherogenic health benefits, was produced in amounts up to 21% of total fatty acids when introduced into a high linoleic (18:2) *Arabidopsis* background (*18*). By co-producing a cyclopropane fatty acid synthase and a cyclopropane-specific lysophosphatidate acyltransferase, up to 35% cyclopropane fatty acids, which serve as natural insecticides, of total fatty acids were produced in *Arabidopsis* seeds, although they showed reduced germination rates (*19*).

Increasing energy density and nutritional value

Diversity among land plants is also evident in the wide range of lipid content in storage and specialized tissues. Many domesticated crops such as palm, soy, and canola produce lipid-rich seeds with extractable seed oils, the primary source of commercial vegetable oils. Even modest increases in the total seed oil content will increase the crop's value. Because yield increases by conventional breeding of high oil-producing natural varieties are expected to reach a limit, attempts have been made to improve performance of these crops through targeted engineering. For example, a 16% relative increase in soybean seed oil was achieved by introducing specialized amino acid residues into the main soybean diacylglycerol acyltransferase, DGAT1 (*20*). In canola, constitutive overexpression of the *Arabidopsis* transcription factor gene *LEAFY COTYLEDON1* acting upstream of the WRINKLED1 transcription factor produced a range of relative seed oil increases up to 16% (*21*). Alternatively to targeting anabolism, the suppression of *SUGAR-DEPENDENT1*, a triacylglycerol lipase, by RNA interference (RNAi) increased relative canola seed oil content up to 8% (*22*).

Energy density and nutritional value of vegetative plant tissues can also be increased by diverting photosynthetically fixed carbon into triacylglycerols. An integrated strategy for increasing triacylglycerol levels in vegetative leaves has been applied through simultaneous alterations that increase the production of fatty acids by overexpressing *WRINKLED1*, in addition to driving the incorporation of fatty acids through increasing diacylglycerol acyltransferase abundance, and by protecting accumulated triacylglycerols by reducing lipase-mediated breakdown, as well as by directly altering carbon partitioning (*7*, *8*, *23*). Applying this approach to *Arabidopsis* roots, stems, and leaves resulted in accumulated triacylglycerol levels from 5 to 8% dry weight (*23*). In crop systems, tobacco leaf triacylglycerol levels increased up to 15% of dry weight (*7*), whereas triacylglycerol levels in sugarcane leaves and stems increased up to 1.9% of dry weight (*8*). Ectopic expression of a green algal diacylglycerol acyltransferase type II encoding cDNA in *Arabidopsis* increased leaf triacylglycerol content by a factor of 10, such that caterpillar larvae feeding on transgenic tissue gained 45% more weight, thereby demonstrating the nutritional enhancement of this GM plant (*24*). However, there remain challenges for further increasing oil in some vegetative tissues, as demonstrated by overexpressing *WRINKLED1* in the grass *Brachypodium distachyon*, where local cell death is likely due to a doubling of free fatty acid levels (*25*).

One of the most striking successes for the nutritional enhancement of seed oil, while at the same time providing a solution for the protection of natural resources, is the production of fish oil–like vegetable oils in GM crops. The daily intake of ω3 long-chain polyunsaturated fatty acids by most humans—especially consumers of the Western diet, rich in saturated animal fats—falls short of recommendations despite well-documented health benefits, in particular those of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (*26*, *27*). The current global supply of naturally produced EPA and DHA in the form of marine fish oils is insufficient to meet increasing demand. Although many plants accumulate another ω3 fatty acid that is readily available in the human diet, α-linolenic acid (18:3), its conversion to EPA and DHA in humans is inefficient. Therefore, introducing these fish oil–like vegetable oils into GM crops provides a feasible, sustainable avenue for the production of ω3 long-chain polyunsaturated fatty acids for human consumption (*9*, *10*). This synthetic biology–based engineering feat was achieved in *Camelina* by redirecting the endogenous production of 18:3 through a series

Table 1. Examples of engineering GM crops and model plants to accumulate desirable fatty acids.

Fatty acid (FA)		Natural high-accumulating source			GM plant			Reference
Type	Structure	Species	Tissue	% FA	Species	Tissue	% FA	
Short chain	2:0	*Euonymus alatus*	Seed	90	*Camelina sativa*	Seed	85*	(*11*)
					Soybean	Seed	70*	(*11*)
Medium chain	8:0	*Cuphea pulcherrima*	Seed	95	*Arabidopsis thaliana*	Leaf	12	(*39*)
	10:0	*Cuphea viscosissima*	Seed	64	*Camelina sativa*	Seed	10	(*12*)
	12:0	Oil palm	Seed	50	*Brassica napus*	Seed	40	(*6*)
					Camelina sativa	Seed	18	(*12*)
					Tobacco	Leaf	10	(*1*)
	14:0	Coconut	Seed	19	*Camelina sativa*	Seed	37	(*12*)
					Tobacco	Leaf	19	(*1*)
Very long chain	22:1Δ13	*Crambe abyssinica*	Seed	60	*Crambe abyssinica*	Seed	73	(*3*)
	24:1Δ15	*Lunaria annua*	Seed	20	*Brassica carinata*	Seed	30	(*14*)
Hydroxylated		Castor bean	Seed	90	*Camelina sativa*	Seed	21	(*5*)
					Arabidopsis thaliana	Seed	29	(*16*)
ω7		*Dolichandra unguis-cati*	Seed	64	*Camelina sativa*	Seed	65	(*2*)
ω3	DHA	Bulk fish oil	Fish	13	*Camelina sativa*	Seed	14	(*28*)
	EPA	Bulk fish oil	Fish	13	*Camelina sativa*	Seed	31	(*28*)
Conjugated	Punicic	Pomegranate	Seed	80	*Arabidopsis thaliana*	Seed	21	(*18*)
Cyclic		*Litchi chinensis*	Seed	40	*Arabidopsis thaliana*	Seed	35	(*19*)

*Percent acetyl-triacylglycerols of total triacylglycerols.

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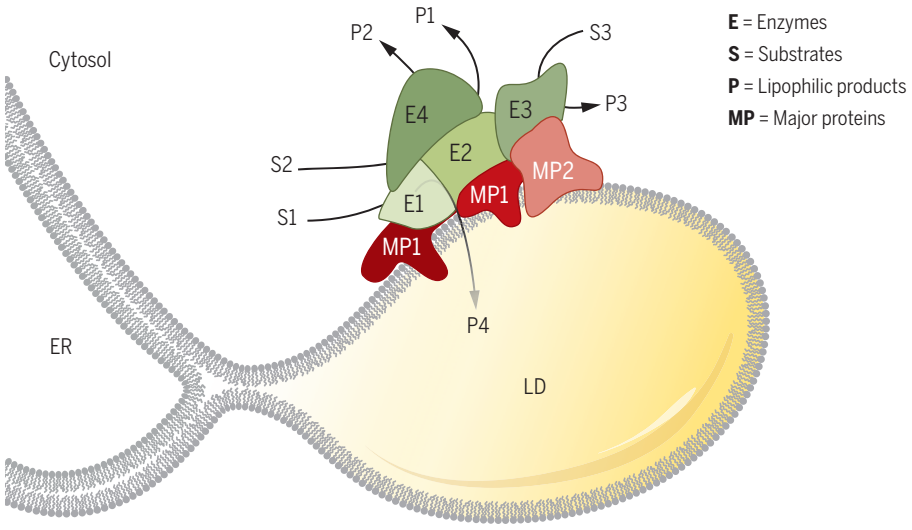

Fig. 3. Redesigning lipid droplets (LDs) as synthetic biology platform. The proteome of plant and algal LDs is dominated by a set of "major proteins," MP1 or MP2. These contain specific LD-targeting information and are inserted into the monolayer membrane at the periphery of the LD. Enzymes of interest (E) may be targeted to the LD using protein fusions (E1 fused to MP1) or through scaffold associations with other already LD-anchored proteins (E4). These enzymes would then catalyze available or directed substrates (S) on the LD surface, sequestering their lipophilic products (P4) within the LD or releasing hydrophilic products (P1, P2, P3) into cytosol. The LD shown is forming at the endoplasmic reticulum (ER) but could also be associated with chloroplast envelopes.

of unusual desaturases and elongases derived from different microalgae and fungi. In seeds, up to 12% EPA and 14% DHA of total fatty acids accumulated when targeted for cosynthesis, or up to 31% when only EPA was targeted (*28*). In tests of the bioavailability of plant EPA and DHA, *Camelina* ω3s fed as supplements to mice or fish in aquaculture led to the improvement of key health markers, such as 36 to 38% lower blood glucose concentrations, indicating that these GM crop–derived vegetable oils could effectively replace marine fish oils (*29*, *30*) used in aquaculture and the human diet.

Lipid droplets as synthetic biology platforms

The accumulation of high amounts of engineered plant oils is enabled by a plant's innate capacity to dynamically generate lipid droplets capable of safely sequestering excess fatty acids as triacylglycerols. Lipid droplets are spherical organelles with a neutral lipid triacylglycerol-rich core enveloped by a polar lipid monolayer into which proteins are embedded. Plant lipid droplets associate with other compartments such as the endoplasmic reticulum and, particularly in microalgae, plastid envelope membranes. These membranes assemble triacylglycerols destined for lipid droplets. More than merely storage organelles, lipid droplets participate in maintaining cellular energy homeostasis, membrane remodeling during stress responses, and postgerminative growth (*31*, *32*). The complex multicompartment nature of fatty acid synthesis, fatty acid modification, and triacylglycerol assembly in plants and algae complicates strategies to engineer total oil and unusual fatty acid accumulation. Synthesis and trafficking of lipids destined for lipid droplets currently represent a major knowledge gap and an opportunity for discoveries.

Being able to manipulate the formation, composition, and turnover of lipid droplets to broaden the engineering capacity of plants should enable us to reengineer lipid droplets, such that multiple enzymatic reactions constituting orthogonal pathways could be directly assembled on the lipid droplet surface (Fig. 3). One approach might be to use proteins known to reside at the lipid droplet surface, such as oleosins (*33*, *34*), or algal lipid droplet proteins that recruit various proteins to the lipid droplet surface (*32*, *35*). Protein(s) of interest could be fused to these lipid droplet surface proteins for targeting or scaffold engineering such that they associate at the surface through protein-protein interactions. For example, if one could produce hydroxy fatty acids at the lipid droplet surface through fatty acid hydroxylase supplied with its requisite cofactor provided by cytochrome b5 reductase and then channel these fatty acids to triacylglycerols by a lipid droplet–localized acyltransferase, one might be able to bypass many of the deleterious side effects these chemical modifications exert on membranes. As an alternative approach, existing divergent fatty acid desaturases targeted to lipid droplets could be engineered to directly act on triacylglycerol. This lipid droplet catalysis approach could also extend beyond lipid metabolism to generate and sequester bioactive compounds, such as drugs, or to introduce enzymes for non-plant purposes. As an example, human fibroblast growth factor 9 fused to oleosin and produced in *Arabidopsis* cells has been targeted to lipid droplets (*36*). Challenges include the short half-life of some lipid droplets, substrate availability and competition within competing pathways, and incompatibility of proteins targeted to the same membrane.

Unlinking triacylglycerol biosynthesis and cell division

Interest in microalgae has surged because of their high capacity for high-energy compound production in culture systems not competing with food crops. However, one of the biggest conundrums has been that microalgae accumulate triacylglycerols in lipid droplets when they are nutrient-deprived and cease growth. That is, triacylglycerol accumulation and biomass production are inversely correlated. Conceptually, cells must progress from the cell division cycle to the quiescent state, when cells are metabolically active but do not divide, to produce high amounts of triacylglycerol. How this transition is controlled is not yet known, but one possible regulatory factor, COMPROMISED IN THE HYDROLYSIS OF TAG 7 (CHT7), has been identified in *Chlamydomonas* (*37*). This protein is a component of a presumed large nuclear transcriptional complex not unlike the retinoblastoma tumor suppressor protein complex governing the cell division cycle in *Chlamydomonas* (*38*). Understanding how the nutritional state of cells affects quiescence and cell division will provide a solution to the conundrum hampering algal feedstock engineering. Although many studies suggest that nutrition determines the metabolic state of human cells and can thereby affect the outlook for the development of cancer, mechanistic insights are scarce. *Chlamydomonas* provides a single-celled, easily manipulated model system that has the same basic machinery governing cell division and cellular quiescence as found in human cells. Hence, basic research in algae on cell cycle regulation and triacylglycerol formation during nutrient deprivation–induced quiescence, and research into mechanisms by which the nutritional state of human cells affects cell division for better or worse, may converge in the near future. As such, fundamental insights gained by studying the regulation of algal lipid metabolism promise to allow us to address health and environmental issues of concern to current and future generations.

Conclusions

Measurable progress in engineering the plant lipidome has been made, but additional advances will require a holistic approach. Versatile "omics" approaches aided by bioinformatics will be needed to address several areas of lipid metabolism, including complexities within acyl exchange and lipid remodeling, characterization of orthologous enzymes for lipid modification, availability and localization of multiple lipid substrate pools within subcellular compartments, and trafficking of these lipid constituents between

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organelles. Addressing these areas of basic research, a renewed focus on characterizing the subtleties of lipid metabolism among natural and GM plants, and improved gene stacking and gene editing technologies should enable engineers of the next generation of GM plants to make better use of the lipidome for the benefit of all.

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REVIEW

Plant metabolism, the diverse chemistry set of the future

Eleanore T. Wurtzel[1,2]* and Toni M. Kutchan[3]*

New technologies are redefining how plant biology will meet societal challenges in health, nutrition, agriculture, and energy. Rapid and inexpensive genome and transcriptome sequencing is being exploited to discover biochemical pathways that provide tools needed for synthetic biology in both plant and microbial systems. Metabolite detection at the cellular and subcellular levels is complementing gene sequencing for pathway discovery and metabolic engineering. The crafting of plant and microbial metabolism for the synthetic biology platforms of tomorrow will require precise gene editing and delivery of entire complex pathways. Plants sustain life and are key to discovery and development of new medicines and agricultural resources; increased research and training in plant science will accelerate efforts to harness the chemical wealth of the plant kingdom.

From the ancient valleys of Mesopotamia to the Amazonian rainforests, mankind has sought plants for food and nutrition, biomaterials for living, and treatment of pain and disease. Traditional medicine worldwide has relied on the medicinal properties of plants, such as the opium poppy, *Papaver somniferum*, first cultivated by the Sumerians in 3400 B.C.E. Plant extracts provided ceremonial dyes, such as henna from *Lawsonia inermis*, still in use today, and the essential oils such as rose oil have provided sensual pleasure since antiquity. Cotton fibers from *Gossypium* plant species were first woven into clothing thousands of years ago, and papyrus used for writing in ancient Egypt evolved into the paper industry of today. Selected examples of this structural diversity and for which use humanity has exploited it are given in Fig. 1.

The chemical diversity of plants is enormous. Plants evolved the biosynthesis of a cornucopia of novel chemicals to survive and communicate in a complex ecological environment. Although some plant chemicals are sharp or bitter tasting (glucosinolates and pyrrolizidine alkaloids) to deter herbivory, others such as anthocyanins and carotenoids are brightly colored flower pigments that attract pollinators. Chemicals that are cytotoxic or otherwise physiologically active in mammals are used, for example, as pain-killers, chemotherapeutics, and other drugs. All of these plant chemicals are made through species-specific, specialized biochemical pathways that modify metabolites of primary metabolism. A plethora of new chemicals and metabolic pathways are likely hidden in plant genomes awaiting discovery. Although structures for 200,000 natural products are known, only 15% of the estimated 350,000 plant species have been investigated for their chemical constituents (*1*). This estimate suggests that a relatively small percentage of the chemical space present in the plant kingdom has been discovered. With every new enzyme and underlying gene discovered, there is potential for a biochemical reaction that can improve modern medicine, human and animal health, bioenergy, and agriculture.

In this review, we present recent examples of selected exciting technologies that are transforming how we study plant metabolism and how we implement these discoveries to develop the plants and microbes of tomorrow. Although the pace of progress during the past several years has accelerated, there are still challenges to be met in order to fully harness the chemical wealth of the plant kingdom.

Accelerating pathway discovery

Inexpensive DNA sequencing, together with computational tools for genome assembly, have revolutionized pathway discovery in plants. The relatively small number of sequenced genomes revealed a surprising distribution of genes encoding specialized biochemical pathway enzymes (*2*). For a growing number of pathways, enzymes are encoded in clustered genomic regions (*2*) having shared chromatin signatures to coordinate expression (*3*). Pathways are also encoded by unlinked genes or a combination of linked and unlinked genes. Specialized plant chemicals function to communicate with the ecological environment, and environmental cues are likely necessary to regulate expression of certain pathways, whether organized in clusters or randomly throughout a genome. The breadth of the chemical space of plants is not known, in part because of reliance on environmental signals for the expression of some pathways. This clustered genome feature can be exploited to identify silent pathways or pathways expressed at levels so low that the metabolites that they produce accumulate below our current levels of chemical detection and have remained undiscovered. Development of computational methods that link chromatin signatures with transcript and metabolite profiles will accelerate discovery of unknown pathways encoded by gene clusters. Development of facile heterologous expression platforms will enable

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functional expression of the large number of genes (20 to 30) required to validate and exploit the biosynthetic pathways to complex plant chemicals.

Recent examples of discovery of clustered biochemical pathway genes illustrate that, given a high-quality genome sequence assembly, biochemical pathways that have long been studied but not completely elucidated at the enzyme and gene levels can be efficiently solved and exploited. Bioinformatic interrogation of genomes of the Solanaceae led to identification of genes encoding enzymes for biosynthesis of steroid alkaloids in two clusters and coexpressed on the genomes of tomato (α-tomatine) and potato (α-solanine). One gene, *GAME4*, encoding a cytochrome P450 along the common steroid alkaloid pathway, was then used to engineer potato tubers and tomato fruit with reduced steroid alkaloids (*4*). When a high-quality genome is not yet available, insight into specialized metabolism can be gained from the combined use of a draft genome together with transcriptome and metabolite data, as illustrated with indole alkaloids in Madagascar periwinkle, *Catharanthus roseus*. This medicinal plant produces a suite of biologically active monoterpene indole alkaloids, including the anticancer compounds vincristine and vinblastine (*5*). Genes encoding known precursors and intermediates for biosynthesis of monoterpene indole alkaloids were found coexpressed and clustered on the genome. Genic sequences interspersed among and flanking the known genes encode enzymes and transporters that may be candidates for the yet-unknown enzymes of vincristine biosynthesis and transporters that are required to move intermediates along the multicellular pathway to monoterpenoid indole alkaloids.

Genetic and biochemical variation within a plant species can be used to identify genes associated with phytochemicals. This approach was elegantly demonstrated early on with limited transcript and metabolite data sets with the identification of flavonoid biosynthetic genes and transcription factors in red and green *Perilla frutescens* and, more recently, using large omic data sets in perilla, *Arabidopsis*, and *Oryza sativa* (*6*, *7*). Transcriptome and carotenoid analysis of a maize germplasm collection helped pinpoint key enzymes for optimizing provitamin A carotenoids. Additional genome association analysis led to development of molecular markers for breeding high provitamin A crops needed to address global vitamin A deficiency (*8*). Genome-wide association study of a germplasm diversity collection led to discovery of nine clustered genes that encode enzymes that catalyze formation of the bitter triterpenoid cucurbitacin of wild cucumber. Further analysis revealed another genome location encoding two transcription factors that regulate the pathway gene cluster (*9*). Insights into the domestication process and the observation that certain varieties of cucumber become bitter when exposed to cold can now be exploited in breeding. In the search for genes related to scent production of roses, *Rosa* × *hybrida* rose varieties differing in scent profiles were examined for differentially expressed genes. A candidate gene was linked to a scent-related terpenoid that is synthesized by a novel pathway not found in other plants producing the identical compound (*10*). This gene can be used in breeding scent back into modern rose cultivars that have largely lost the aromatic properties of their predecessors. Having a repertoire of enzymes that are structurally unique

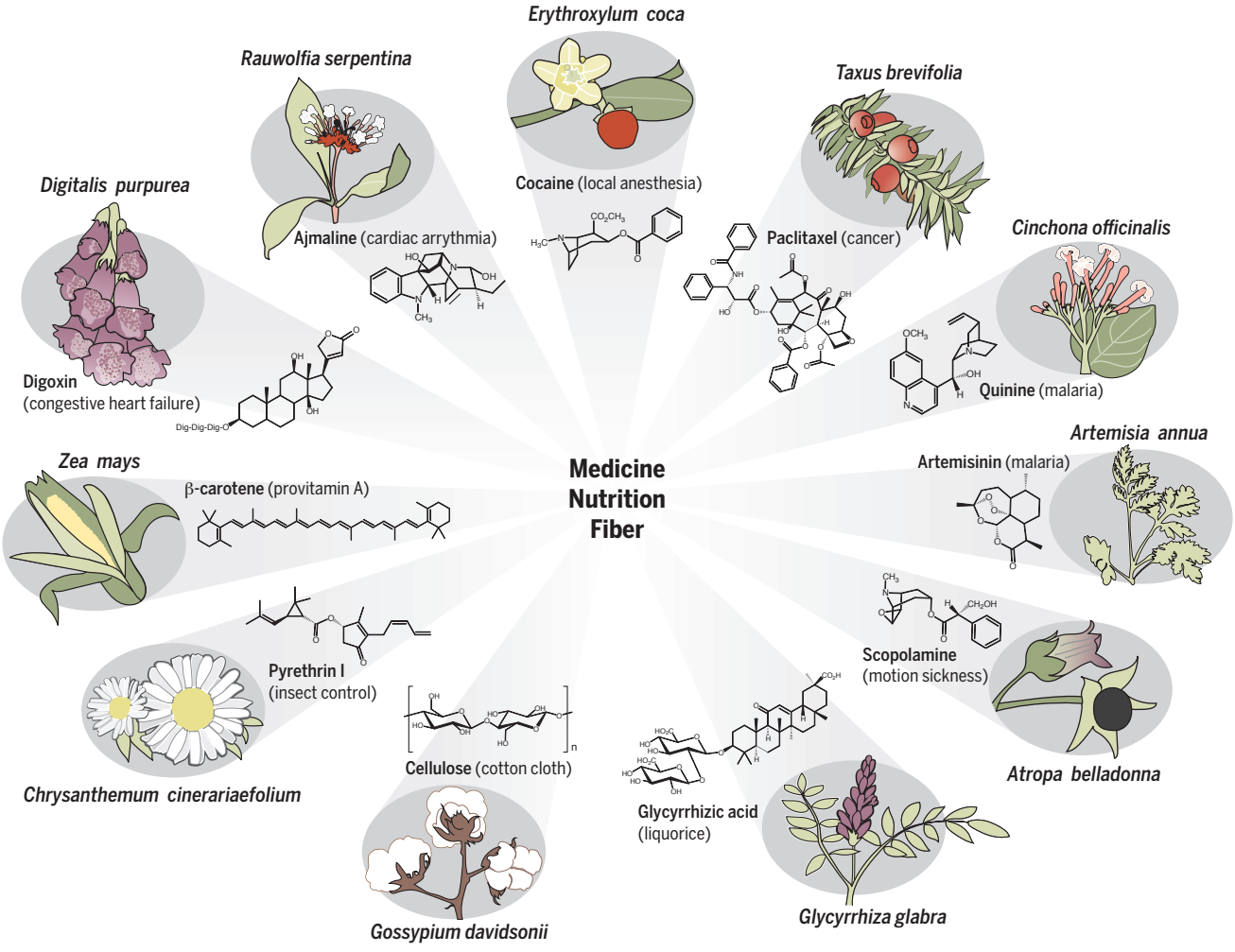

Fig. 1. Selected plants and their uses. Throughout history, plants have served as sources of a plethora of chemicals that provide humankind with medicine, fiber, and nutrition.

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but perform the same biochemical transformation also expands the palette for metabolic engineering. The more enzymes that are discovered and characterized that have novel catalytic mechanisms and steady-state kinetic properties, the greater the potential for developing synthetic biology systems for known and novel chemicals.

Although high-quality genome sequencing and assembly are still a challenge with the large genomes of many plants, metabolite and transcriptome data are now easily obtained for any plant, changing the paradigm for biosynthetic pathway discovery. Computational interrogation of large-scale transcriptomes and metabolomes of plant tissues has revealed that many genes encoding pathway enzymes are coexpressed with each other and with accumulation of pathway metabolites. Pathway elucidation is no longer limited to a handful of model plants or to simple biochemical pathways but is a reality for the most complex pathways and for the entire plant kingdom, including rare or endangered species. As an example, the medicinal plant, *Podophyllum hexandrum* (mayapple) produces the chemotherapeutic etoposide. Four of the etoposide biosynthetic steps were known, and six others were predicted, on the basis of putative chemical transformations. Investigators mined mayapple transcriptome data using bioinformatic tools for coexpression analysis, and selected candidate genes to fill pathway gaps for predicted biochemical transformations (*11*). Elucidating pathways to commercially relevant complex plant chemicals can now be accelerated by high-throughput nucleotide sequencing and coexpression analysis, but the challenges associated with complex genomes, low expression levels of genes, and low accumulation levels of metabolites limit the number of metabolites that can be investigated using these technologies. In addition, algorithms used to interrogate these data sets still require human curation.

Peering into the single cell

Biosynthesis of some metabolites, such as medicinal alkaloids, can be compartmentalized across various subcellular locations in multiple, specialized cell types and can require a dedicated trafficking mechanism (*12*). Moreover, the induction of alkaloid biosynthesis may be linked to developmental changes signaled through interactions between plants and other organisms. A better understanding of how development and metabolism interconnect and are regulated could have far-reaching impact on the fields of chemical ecology, agriculture, and metabolic engineering. For example, association between rhizobial bacteria and legume hosts elicits formation of nitrogen-fixing nodules in roots, which in the plant *Crotalaria* is accompanied by induction of plant enzymes that catalyze formation of antiherbivore defense compounds, the pyrrolizidine alkaloids. The alkaloids synthesized in the root nodules are subsequently transported to the aboveground parts of the plant (*13*). Whole-plant or whole-organ metabolite analysis is insufficient to capture such compartmentalization. To optimally match genes with metabolites, instrumentation sensitivity and spatial resolution must discern compartment-specific metabolites. Recent advances in single-cell metabolite detection and quantification have greatly improved the opportunity to dissect pathway metabolites with temporal and spatial resolution. Metabolite analysis is conducted using an array of complementary analytical instrumentation, each of which offers certain advantages [reviewed in (*14*, *15*)]. Compounds can be identified within complex mixtures (*16*), and chemical structures can be elucidated from relatively small amounts of extracted samples (*17*). To obtain a spatial map of metabolites in situ, the current method of choice for nonvolatile compounds is matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) (Fig. 2A). Using MALDI-MSI, together with a time-of-flight analyzer, flax specialized metabolites could be analyzed at 20-μm resolution (*18*), which affords cellular resolution, as a typical plant cell has a diameter of 50 μm. Using this same technology allowed analysis of various lipid structures in a single cotton seed and revealed an unexpected cell-type compositional variation (*19*). Improved beam-delivery optics have increased spatial resolution to 5 μm (*20*), which approaches subcellular resolution and would facilitate an analysis of subcellular metabolite trafficking that is a component of most metabolic pathways. The promising method of laser-ablation electrospray ionization mass spectrometry utilizes fresh tissue without pretreatment, as required by MALDI-MSI, but offers only 200-μm resolution (*21*), which currently suffices only for tissue-level analyses. Another nondestructive method for three-dimensional metabolite

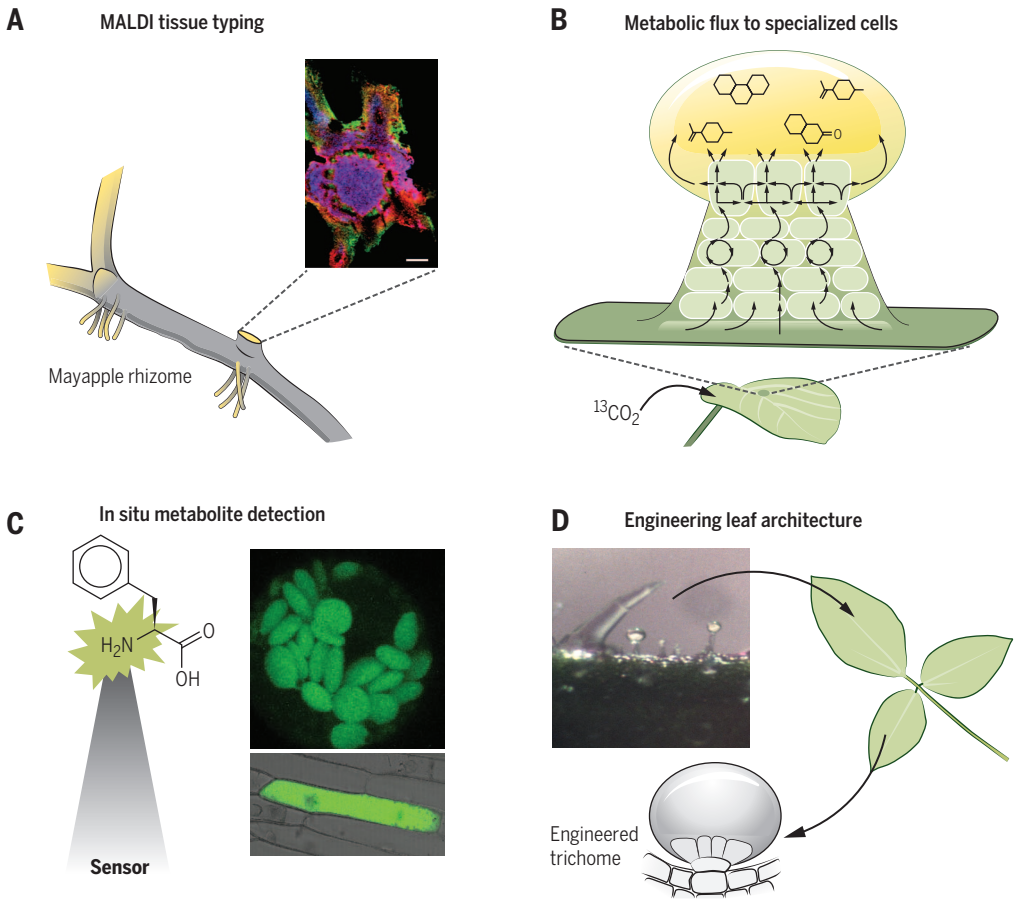

Fig. 2. Game-changing technologies and concepts under development. (**A**) Merged MALDI MSI of podophyllotoxin [mass/charge ratio (*m/z*) 453.0969 of the molecular ion plus potassium, $[M+K]^+$; the difference between the calculated and the measured mass (Δ ppm) = 3.8, red]; podophyllotoxin-β-D-glucopyranoside (*m/z* 615.1496 $[M+K]^+$, Δ ppm = 2.6, blue); and kaempferol-3-*O*-(6″-*O*-malonyl)-glucoside (*m/z* 573.0660 $[M+K]^+$, Δ ppm = 2.3, green) in *Podophyllum hexandrum* rhizome. Scale, 2 mm. (**B**) Schematic of ^{13}C metabolic flux to specialized metabolites in trichomes. (**C**) Schematic of in vivo sensors. Development for a large catalog of plant metabolites would greatly facilitate in situ quantitation of metabolites for metabolic engineering. (**D**) *Salvia sclarea* trichomes. Engineering trichomes to produce specialty chemicals can be achieved today with plants such as mint; the challenge of the future is to engineer trichomes into species that do not normally develop these specialized surface structures.

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analysis combines confocal microscopy with Raman spectroscopy to link subcellular architecture with chemical composition at the submicron level (*22*, *23*). To build a more complete cellular and subcellular model of biochemical pathway components, large-scale mass spectrometry–based proteomic efforts are cataloging enzyme locations at the cellular and subcellular level (*24*), which complements that achieved for metabolites. For rational engineering, it is important to elucidate dynamics of pathway metabolon assembly, including how scaffold proteins affect enzyme allostery in complexes and how protein modification controls enzyme function in vivo. Proteomic surveys, however, do not always detect all pathway components or distinguish among enzyme variants. Alternatively, the effect of sequence variation can be examined *in planta* by confocal microscopy using transient expression of plant genes fused to fluorescent tags to track subcellular localization (*25*). Together with large-scale protein and metabolite profiling, the complementary use of cell biological tools contributes to elucidating the dynamic structural organization of pathway components. Coupled with single-cell transcriptomics, single-cell metabolite analysis aids in linking metabolites to the genes that underlie biosynthesis and pathway regulation by reducing the size of omic data sets and, thereby, simplifying computational analysis.

Building biochemical factories

The speed with which we can elucidate biosynthetic pathways to plant natural products is ever increasing, especially with implementation of the exciting new technologies just described. Synthetic biology platforms in microbes and plants are now driving production of plant natural products. With elucidation of complete biosynthetic pathways to selected commercially valuable plant natural products, production platforms in *Escherichia coli*, *Saccharomyces cerevisiae*, *Nicotiana benthamiana*, *Camelina sativa*, and *Populus* hybrids are being developed (Fig. 3). Synthetic metabolic networks are designed with biosynthetic enzymes that originate from plants and other organisms, such as microbes and mammals. Endogenous host pathways can be constructed on scaffolds to improve flux into new pathways that produce useful chemicals. For example, artemisinin, a potent antimalarial from the plant *Artemisia annua* L, is costly and difficult to obtain by chemical synthesis. Plant genes encoding key enzymes for biosynthesis of artemisinic acid, the immediate precursor for artemisin had been previously identified. To create yeast cells that secrete artemisinic acid that can be chemically converted to the antimalarial drug, a synthetic pathway was constructed by tethering plant metabolic enzymes to a yeast scaffold (*26*). The artemisinic acid thus formed was secreted and retained outside the yeast cells. Secretion of metabolic products not only facilitates end-product purification but also reduces toxicity to the host cell, a feature that is certain to be further exploited by use of efflux transporters that could be endogenous (*27*), synthetic, or an existing metabolite transporter from plants.

The most recent synthetic biology successes are reported for the benzylisoquinoline alkaloids and lignans, a consequence of the extensive knowledge attained on the enzymes and genes and to the pharmacological importance of selected members of these classes of natural products. Although not yet optimized, thebaine and related opiate alkaloids have been produced in yeast (6.4 μg/l thebaine; 0.3 μg/l hydrocodone) (*28*) and bacteria (2.1 mg/l thebaine) (*29*) with the melding of more than 20 genes from plants, mammals, bacteria, and yeast. These examples are a first demonstration of engineering pathways to complex alkaloids from glucose or glycerol;

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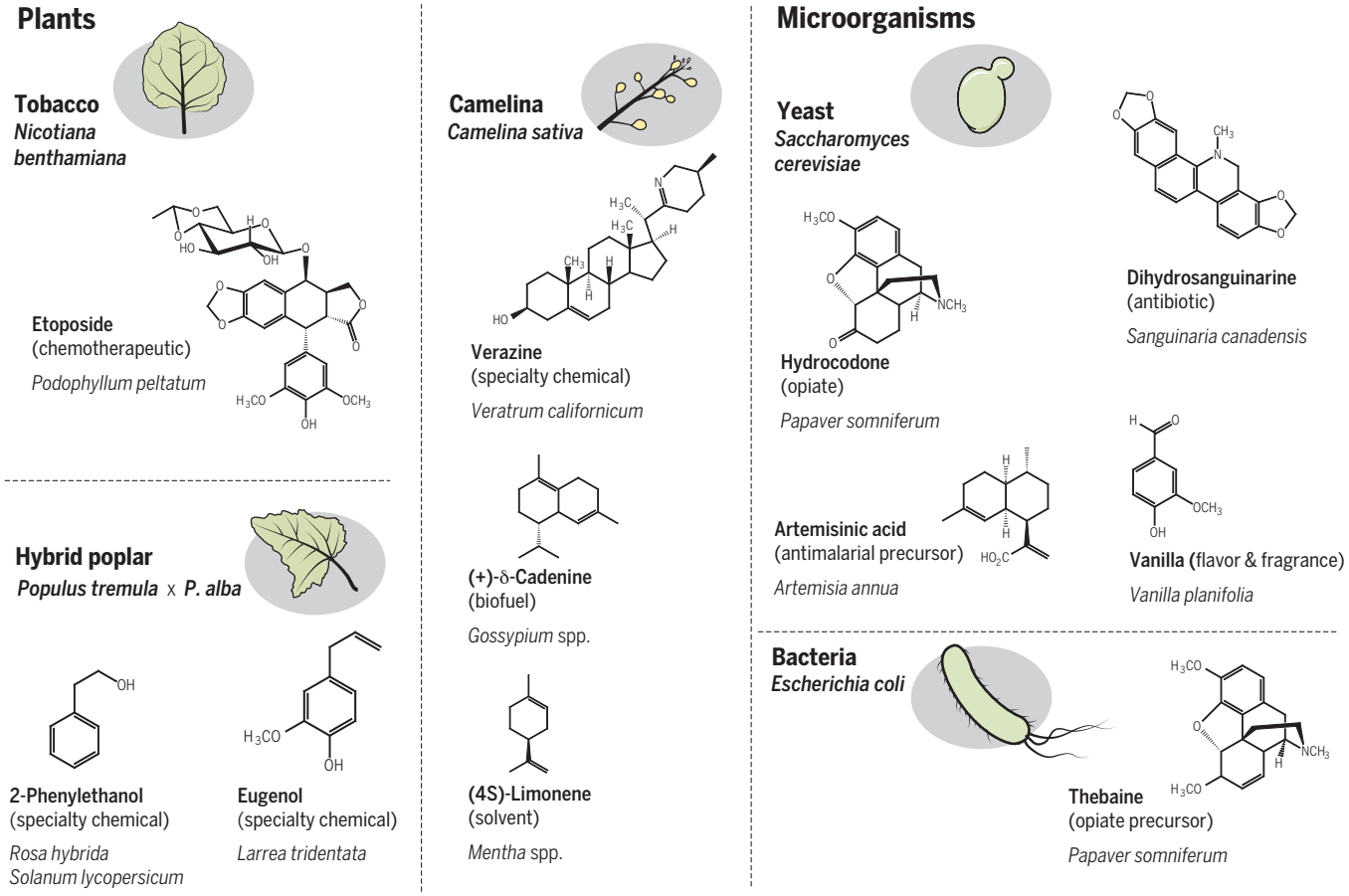

Fig. 3. Natural products produced from plants. Plant natural products currently produced in heterologous systems that range from microbial fermentation to cultivated trees.

commercial feasibility requires that at least 5 g/l be achieved. The entire pathway of 10 genes to the chemotherapeutic etoposide aglycone was successfully reconstructed and validated in tobacco (< 1 ng/mg dry weight) (*11*). As for the opiates, the levels of drug achieved are far from commercially viable levels, but demonstrate proof of concept. Vanillin, the most important flavor and fragrance component of vanilla extracted from cured pods of the vanilla orchid, is commercially biotechnologically produced using bacteria, fungi, and yeast (*30*). The synthetic pathway to yet another flavor and fragrance chemical 2-phenylethanol has been introduced into hybrid poplar with ~4% accumulation in leaf and stem of 4-month-old plants (*31*). Woody plants can accumulate up to 60% phenylpropanoid-derived chemicals in heartwood and this example suggests that trees may serve as platforms for production of commodity chemicals. (4*S*)-Limonene and (+)-δ-cadinene have been produced in seed of *C. sativa* (~1.4% dry weight), thereby demonstrating the utility of an oilseed as a host for chemical production (*32*).

Biochemical engineering of the future

For rational engineering of biosynthesis of plant chemicals, pathway flux reflective of cellular and subcellular metabolism within a metabolic network needs assessment. Now, we mostly work with an enzyme "parts list." Even if the concentration of an enzyme in situ is known, the level of that enzyme is not indicative of enzymatic activity and does not directly inform on a rate-limiting biosynthetic step or on biochemical regulation. To better understand how carbon progresses through metabolism, ^{13}C-labeling–based metabolic flux analysis has been developed to comprehensively quantify flow in metabolic networks (Fig. 2B). Mass spectrometry or nuclear magnetic resonance spectroscopy is used to measure isotope distribution in steady-state metabolite pools. In plants, steady state–labeling studies have quantitatively assessed developing seed metabolism and recently isotopically nonstationary metabolic flux analysis (INST-MFA) has been applied to leaves (*33*). INST-MFA can be used to study short-lived metabolic steady states and pathways that are linear or use a single carbon precursor and, therefore, require the monitoring of transient labeling to be informative concerning fluxes (e.g., autotrophic or specialized metabolism). If cellular or subcellular attributes are to be defined in a computational model, experimental measurements that serve as inputs to the model must adequately inform about spatial complexity so as to resolve the fluxes in different locations.

To achieve cellular and even subcellular resolution, genetically encoded nanosensors hold great promise as in vivo metabolite beacons for real-time quantification of metabolites without sample destruction. Förster resonance energy transfer (FRET) biosensors consist of two distal fluorescent proteins that sandwich a metabolite ligand-sensing protein domain that alternates in conformation depending on ligand binding and is visualized as a FRET signal (Fig. 2C). For any metabolite for which there is a known receptor, the ligand-binding domain could be used to develop a FRET sensor (*34*). For other metabolites, structure-guided protein engineering based on a collection of scaffolds could be used to design artificial ligand-binding domains. Limitations here remain the small number of chemicals for which binding proteins are known. An alternative in vivo quantitation of metabolites is the use of RNA sensors, riboswitches. Natural riboswitches are RNA sensors that bind specific metabolites within a small aptamer region and transduce the binding signal to control gene expression through a regulatory domain (*35*). Artificial riboswitches that recognize a given metabolite can be created by incorporating synthetic aptamers that are identified through in vitro selection strategies to bind to an immobilized metabolite (*36*). Aptamers have been created for a few plant alkaloids, such as cocaine (*37*) and the caffeine biosynthetic intermediate, theophylline (*36*). Metabolite sensors developed for a large catalog of plant metabolites would facilitate the speed with which we can design metabolic pathways in native and recombinant systems.

Alongside metabolite detection and carbon flux determination, biosynthetic pathway elucidation is also limited by computational methodology. Gene annotation is based on primary sequence homology and many genes are annotated as "unknown" or are misannotated, such as a symporter concealing a new class of heme enzymes (*38*). More accurate annotation based on tertiary structure necessitates development of high-throughput tools for homology modeling together with expansion of available protein structure templates, the latter now closer to realization with recent advances in structure determination using high-resolution single-particle cryo–electron microscopy (*39*). Many gene sequences could be more efficiently linked to biological processes with computational tools that simultaneously query multiple large data sets and associate genes with pathways. Algorithms are needed that incorporate machine learning to correlate data from an expanding horizon of data sets—such as RNA, protein, cell-specific metabolites, enzyme localization, protein-protein interactions, plant-microbe interaction, chromatin signatures—for the purpose of linking genes, proteins, and pathways without a priori knowledge.

Metabolic engineering of tomorrow will target a suite of biosynthetically unrelated compounds, such as a multivitamin crop augmented with a nutraceutical, for which metabolic tailoring will also optimize plant growth in a changing climate. Engineering multiple chemical traits requires a fundamental understanding of pathway regulation including flux control, regulatory interactions between specialized and primary metabolism, and coordination with plant development, abiotic signaling, and signaling between plants and other organisms in shared ecological niches. Crafting plant metabolism for crops of tomorrow will be achieved by precise gene editing and delivery of whole pathways on synthetic chromosomes (*40*). Genome editing has not yet been applied to synthetic chromosomes; however, genome-editing techniques have the potential to advance meaningfully the applications of synthetic chromosomes. Specialized plant structures, such as trichomes (Fig. 2D), could be exploited to channel compounds for alternate and novel biosynthetic transformations. Advanced tools, as those mentioned herein, are the gateway to accelerating metabolic engineering and stacking of complex traits. These technologies are the future of plant engineering and synthetic biology production of the plethora of plant chemicals useful to humanity.

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REVIEW

Plant-produced biopharmaceuticals: A case of technical developments driving clinical deployment

George P. Lomonossoff[1]* and Marc-André D'Aoust[2]

The ability to express heterologous proteins in plants has led to the concept of using plants as "bioreactors" or "biofactories" for the production of pharmaceutical proteins. Although initial studies were promising, the pathway to commercialization and deployment in a clinical setting has proven to be a somewhat rocky road. This Review examines the technical developments that have led to the current increase in interest in the use of plants for the production of pharmaceutical proteins, particularly in the context of clinical trials.

The development in the early 1980s of effective means of expressing heterologous (nonplant) proteins in plants offered the prospect of using plants as bioreactors for the production of pharmaceuticals at reduced cost for both human and animal health. In addition, plant cell cultures are not susceptible to mammalian viral pathogens and, conversely, plant viruses do not infect human cells (*1*). An early indication that mammalian proteins produced in plants would retain their desired pharmaceutical properties was provided by the expression, in transgenic tobacco plants, of a correctly assembled and functional human antibody (*2*). This initial success was quickly followed by the successful expression, also in transgenic plants, of hepatitis B virus-like particles (VLPs) (Fig. 1) (*3*), more complex antibody derivatives such as secretory immunoglobulin A (sIgA) (*4*), and orally immunogenic proteins (*5*). Simultaneously, vectors based on plant viruses were used to express antigenic peptides, usually as fusions to the viral coat protein. In several instances, assembled particles were able to protect animals from challenge with the pathogen from which the epitope was derived (*6*, *7*). This article will review how these initially promising studies have progressed during the past 25 to 30 years toward their ultimate goal of producing practical pharmaceuticals, what technical advances have helped toward this goal, and what still remains to be achieved.

Advances in expression technologies

Stable transformation technologies that lead to regenerated plants play a seminal role in the development of plants as bioreactors, have the potential for large-scale production, and are applicable to a wide range of plant species. Still, these technologies have the disadvantage of being labor-intensive and time-consuming. Thus, much of the early work in this area focused on the production of molecules that had previously been made successfully in other systems. Additionally, the long production timelines mean that these technologies are not suitable for rapidly producing pharmaceuticals to combat emerging diseases.

"The major shift in gene delivery technology has involved the use of **Agrobacterium tumefaciens** *to deliver genes to somatic tissue via infiltration..."*

The major shift in gene delivery technology has involved the use of *Agrobacterium tumefaciens* to deliver genes to somatic tissue via infiltration, as well as reliance on transient expression to produce the desired proteins in a matter of days. Because the introduced sequence is not heritable, this is essentially a batch process; scaling up production involves infiltrating more tissue. Thus, it is essential to maximize expression, either by using replicating viral RNAs to increase mRNA levels and/or ensuring that mRNA is maximally stabilized and translated (*8*, *9*). Additionally, it was important to develop technologies that could be applied on a large scale. The plant species routinely used for transient expression is *Nicotiana benthamiana*—a wild relative of tobacco originally from Australia—which has a fast growth rate, is particularly amenable to infiltration, and appears to have a defective RNA silencing system (*10*). Yields using this approach can reach 1 g of product per kilogram of leaves within 5 to 7 days, though the levels are protein-dependent.

As an alternative to using whole plants, there has been considerable progress recently in the use of plant cell cultures such as tobacco BY-2 cells and carrot suspension cultures (*11*). Such suspension approaches offer several potential advantages in terms of the control of growth conditions and a greater similarity to more conventional methods of pharmaceutical production, such as mammalian, insect, and yeast cell cultures. Conversely, the cost of these approaches may obviate the financial advantages of plant-based

Fig. 1. The green vaccine machine. This image shows an artistic representation of the use of plants to produce vaccines; in this case, virus-like particles (VLPs). Here, the VLPs and the leaf are not on the same scale. [Credit: © Eva Thuenemann]

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production. To reduce this disadvantage, a fully disposable system for the large-scale production of plant cell suspension cultures (*11*) has been developed. Methods have also been developed for the production of cell packs from suspension cultures (*12*). In addition to using suspension cultures from higher plants, the possibility of using cultures of simpler or unicellular plants has been explored.

The above may give the impression that, in terms of the methods used, the field of plant-based expression is quite fragmented. Whereas this has certainly been the case in the past, there has been a convergence within the past few years, and though the details of the various technologies may differ, many of the tools can be used interchangeably. Thus, many *Agrobacterium* binary vectors can be used for both stable and transient expression as well as to introduce sequences into either whole plants or suspension cultures. Small-scale transient expression in leaves is often used to express a new molecule before embarking on the production of transgenic lines (Fig. 2).

Major categories of pharmaceutical proteins successfully produced in plants

Until now, the main classes of pharmaceutical proteins produced in plants have been antibodies, subunit vaccines and VLPs, and therapeutic enzymes. However, other types of molecules, such as toxins, have also been expressed. A wide range of antibodies and antibody derivatives—including sIgA and IgM, as well as numerous IgG molecules (*13*–*15*)—has been successfully expressed. Likewise, numerous VLPs derived from a variety of mammalian viruses have been produced. These include VLPs consisting of only a single type of capsid protein, more complex VLPs consisting of several types of capsid proteins, VLPs that require a maturation step for their formation, and VLPs from enveloped viruses (*16*). Though there are few examples in which VLPs expressed in different systems have been directly compared, work with hepatitis B VLPs indicates that plants are particularly adept at assembling high-quality homogeneous particles (*17*). Several therapeutic enzymes aimed at treating metabolic disorders such as Gaucher disease and Fabry disease have also been successfully expressed (*18*, *19*). In 2012, taliglucerase alfa for the treatment of Gaucher disease became the first plant-produced biopharmaceutical to obtain approval for human administration by the Food and Drug Administration (*20*).

Clinical development of plant-produced biopharmaceuticals

Vaccines intended for oral administration were the first generation of plant-produced biopharmaceutical proteins to reach clinical development. The concept of edible vaccines was proposed at a time when production systems were dominated by transgenic plants, the idea being that antigens could be expressed in transgenic fruits, tubers, or seeds and administered orally without purification, alleviating the need for parenteral administration, formulation, and (possibly) refrigeration. Edible vaccines thus had the potential to be affordable, easily administered, and accessible to the developing world.

The first human study of a plant-produced pharmaceutical evaluated the immune responses to ingestion of transgenic potato tubers containing *Escherichia coli* heat-labile enterotoxin B subunit (*21*). This study demonstrated, for the first time, that oral administration of antigens in edible plants induces mucosal and systemic immune responses. However, the results highlighted the short duration of the immune response. Similar modest and short-lived responses were also observed in subsequent studies [for reviews of this early work, see (*6*–*8*)]. Despite the early challenges faced by edible vaccines, the idea of delivering biotherapeutics in edible plant tissues remains alive. The most recent proposal, passive oral immunotherapy, builds on the capacity of plants to produce sIgA, a primary component of mucosal immunity (*22*).

Parenteral administration in humans of a plant-produced biotherapeutic was first performed at the turn of the millennium by Large Scale Biology Corporation (*23*), who used transient expression via a replicating vector. This phase 1 clinical study, conducted over several years, evaluated purified idiotypic vaccines [in the form of a ~30-kDa single-chain variable fragment (scFv) of the idiotype] for the treatment of non-Hodgkin's lymphoma. Purification of the antigens allowed the group to control the dosage of antigen administered. It also enabled the evaluation of adjuvanted vaccine doses, using granulocyte-macrophage colony-stimulating factor as an adjuvant.

Protalix Biotherapeutics has invested in the alternative strategy of using plant cell cultures as a means of producing biopharmaceuticals, including taliglucerase alfa (β-glucocerebrosidase). Optimal bioactivity of therapeutic glucocerebrosidase requires that the enzyme is taken up by macrophages via binding to their mannose receptors. Hence, the glycosylated enzyme needs to carry N-glycans with terminal mannose residues (*24*). Shaaltiel *et al.* achieved in planta maturation of N-glycans to a paucimannose structure (a short form with two terminal mannoses) by targeting the enzyme to storage vacuoles. This approach was efficient and eliminated the need for postpurification maturation of N-glycan (*18*), thus improving the cost-efficiency of the process compared with that of imiglucerase (Cerezyme), the competitor CHO-produced β-glucocerebrosidase. Pivotal clinical trials of taliglucerase alfa showed no serious adverse events and demonstrated efficacy results similar to those of other enzyme replacement therapies for Gaucher disease (*25*).

The swine (H1N1) influenza outbreak that highlighted the limitations of traditional influenza vaccine–manufacturing technologies led Medicago to use the speed of transient expression to produce a pandemic H5N1 vaccine based on influenza VLPs (Fig. 3). The adjuvanted vaccine was well tolerated at all doses, and immunogenicity studies revealed a definite dose response (*26*). Subsequent clinical studies with monovalent and quadrivalent influenza vaccines have confirmed the strong antibody responses to influenza and have shown that plant-derived influenza VLPs elicit pronounced, long-lasting, and cross-reactive polyfunctional $CD4^+$ T cell responses, even in absence of an adjuvant (*27*, *28*). Of the 280 subjects receiving either the H1 or H5 VLP vaccine in phase 1 and 2 studies, none

Fig. 2. The process of vacuum infiltration on a small scale. In this case, a single *N. benthamiana* plant is inverted and the aerial parts immersed in a suspension of *A. tumefaciens* in a vacuum desiccator. The pressure above the suspension is reduced using a vacuum pump (seen in the background), which results in the loss of air from the intercellular spaces. When the vacuum is released, this air is replaced by the *A. tumefaciens* suspension.

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Fig. 3. Transient expression for the large-scale manufacturing of vaccines. Medicago's facility in North Carolina is scaled to produce 10 million doses of pandemic influenza vaccine per month. (**Left**) Plants are grown in a greenhouse before infiltration. (**Right**) An automated device handles tables of 128 plants for infiltration.

have developed an allergic or hypersensitive response, despite the plant-specific N-glycosylation profile on the viral surface glycoprotein, hemagglutinin, used as an antigen (*29*). An ongoing phase 2 clinical trial of the quadrivalent seasonal influenza vaccine in healthy adults and elderly populations is aiming to define the optimal dose, establish potential competitive advantages over current influenza vaccines in terms of protection against heterologous strains, and support the design of future studies (*30*).

Another important clinical development came with the study of transiently expressed idiotypic vaccines (*31*). Building on the previous demonstrations of the capacity of plants to produce personalized vaccines against B cell follicular lymphoma, and learning from the challenges faced with the first-generation products (*23*), Icon Genetics studied a second generation of idiotypic vaccines, in the form of full-size human IgG1 coupled to keyhole limpet hemocyanin, produced with an improved transient expression platform (*8*). For this specific product, the platform provided the robustness, yields, speed, cost-effectiveness, and quality needed for production and human administration of individualized tumor-targeted idiotypic vaccines. The study demonstrated that the idiotypic vaccine was well tolerated, as no severe adverse events related to vaccination were observed. Of the patients who could be evaluated at the end of the study, 82% displayed a vaccine-induced, idiotype-specific cellular and/or humoral immune response (*31*).

Although several plant-produced antibodies have been assessed in animal studies, only a few have made it to clinical development. The field of plant- and algae-produced antibodies was recently reviewed by Yusibov *et al.* (*32*). Early clinical developments of plant-made antibodies include a hybrid secretory IgA-IgG antibody targeting *Streptococcus mutans* for the treatment of dental caries (*33*) and Avicidin, a full-length IgG specific for epithelial cell adhesion molecule (EpCAM) (a marker of colorectal cancer), produced in maize (*34*). Both reached phase 2 trials. In addition, the plant-expressed IgG, 2G12, which is intended for topical application to prevent the transmission of HIV infections, has also undergone phase 1 clinical testing (*35*).

Whereas the first clinical studies were performed with antibodies produced in transgenic plants—the only available technology at the time—transient expression is now generally used, not only because of the advantages described above but also because glycoengineered *N. benthamiana* plants have been developed that enable the production of antibodies with improved effector functions. The ZMapp cocktail of antibodies developed for the treatment of Ebola virus infection and administered to 15 patients under compassionate-use protocols during the recent Ebola virus outbreak in West Africa represents a good example of plant-produced antibodies with improved potency via glycoengineering. The cocktail, resulting from a collaboration involving Mapp Biopharmaceutical and LeafBio (San Diego, California), Defyrus (Toronto, Canada), the U.S. government, and the Public Health Agency of Canada, is produced in *N. benthamiana* plants with knocked-down plant-specific glycosyltransferases (β1,2-xylosyltransferase and α1,3-fucosyltransferase) (*36*, *37*), as studies with previous generations of anti-Ebola antibodies and antibody cocktails have indicated that nonfucosylated antibodies show increased potency, most likely via improved effector functions (*38*, *39*). Earlier this year, LeafBio, the commercial arm of Mapp Biopharmaceutical, released the results of the first human study with the ZMapp treatment. The results obtained showed that the product was well tolerated and that the mortality rate in the treated group appeared to be 40% lower than in the nontreated participants, although the difference did not reach statistical significance in such a small sample size.

Future prospects

To paraphrase the 17th-century English poet John Donne, no branch of science is an island entire of itself. Thus, the direction that the field of plant-based pharmaceutical production takes in the future is likely to be governed not just by developments within the field but also by advances in other areas of bioscience. This has been illustrated recently by genome editing technologies, particularly the CRISPR-Cas9 system, which was originally developed in nonplant organisms but is now being increasingly deployed to modify the host plants used for expression (*40*). Developments in entirely different branches of science are also likely to influence the attractiveness of plants as an expression system (e.g., the recent developments in light-emitting diode technology that have reduced the cost of providing the lighting necessary for efficient plant growth). The clinical developments mentioned in this Review (see Table 1 for a summary) clearly demonstrate the capacity of plant-based production systems to deliver effective biopharmaceuticals of high quality for a wide diversity of

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Table 1. Clinical development of plant-produced biopharmaceuticals mentioned in this article.

Product	Application	Developer	Plant	Transformation technology	Stage of development	Reference
E. coli heat-labile enterotoxin B subunit	Oral vaccine against enterotoxic *E. coli*	University of Maryland, Boyce Thompson Institute for Plant Research, and Tulane University School of Medicine	Potato	Stable transformation	Phase 1	(*21*)
Tumor-derived idiotype scFv	Therapeutic vaccine for non-Hodgkin's lymphoma	Large Scale Biology Corporation	Tobacco	Transient transformation using replicating viral vector (tobacco mosaic virus)	Phase 1	(*23*)
Taliglucerase alfa	Replacement therapy for Gaucher disease	Protalix Biotherapeutics	Carrot (cultured cells)	Stable transformation	Approved in the United States and other countries	(*20*, *25*)
H5N1 influenza virus-like particle	Pandemic influenza vaccine	Medicago	*N. benthamiana*	Transient expression using *Agrobacterium* infiltration	Phase 2	(*26*)
Quadrivalent influenza virus-like particle	Seasonal influenza vaccine	Medicago	*N. benthamiana*	Transient expression using *Agrobacterium* infiltration	Phase 2	(*30*)
Tumor-derived idiotype IgG	Therapeutic vaccine for non-Hodgkin's lymphoma	Icon Genetics	*N. benthamiana*	Transient expression using *Agrobacterium* infiltration	Phase 1	(*31*)
Hybrid secretory IgA-IgG	Treatment of dental caries	Planet Biotechnology	Tobacco	Stable transformation	Phase 2	(*33*)
Radiolabeled anti-EpCAM murine IgG	Treatment of colorectal, lung, and prostate cancer	NeoRx Corporation	Maize	Stable transformation	Phase 2	(*34*)
Human anti-HIV IgG	Prevention of HIV transmission	Pharma-Planta Consortium	Tobacco	Stable transformation	Phase 1	(*35*)
Chimeric anti-Ebola IgG cocktail	Treatment of Ebola virus infection	Mapp Biopharmaceutical	*N. benthamiana*	Transient expression using *Agrobacterium* infiltration	Phase 1/2	(*37*)

products, ranging from prophylactic vaccines to therapeutic human enzyme replacement therapies. Today, the speed of deployment enabled by transient expression technologies, combined with the application of the above transformational technologies, is further accelerating platform and product developments to meet the growing need for novel and innovative therapeutics.

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REVIEW

The next green movement: Plant biology for the environment and sustainability

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From domestication and breeding to the genetic engineering of crops, plants provide food, fuel, fibers, and feedstocks for our civilization. New research and discoveries aim to reduce the inputs needed to grow crops and to develop plants for environmental and sustainability applications. Faced with population growth and changing climate, the next wave of innovation in plant biology integrates technologies and approaches that span from molecular to ecosystem scales. Recent efforts to engineer plants for better nitrogen and phosphorus use, enhanced carbon fixation, and environmental remediation and to understand plant-microbiome interactions showcase exciting future directions for translational plant biology. These advances promise new strategies for the reduction of inputs to limit environmental impacts and improve agricultural sustainability.

Our reliance on plants as resources is ancient and only promises to expand in the future. Faced with a growing population, estimates suggest the need for a 70% increase in agricultural production by 2050 and highlight the parallel challenges of maintaining food security, minimizing environmental damage, and managing water and resource use under shifting climate conditions (*1*). Along with efforts to reduce post-harvest losses and waste in the food chain, the intensification of agriculture will be essential to increase productivity (*2*). Precision agriculture, the breeding and genetic engineering of new crop varieties, and better management of energy, fertilizer, field systems, and irrigation inputs will also help to meet this challenge (*2*, *3*). This growth in global production will come in regions where land and water assets are either overused or constrained and in developing countries where smallholder farmers dominate (*4*). Any intensification of agriculture also risks increased fertilizer use, degradation of soil quality, water availability issues, salinization, potential contamination from chemicals, and loss of genetic diversity.

The path to increased production requires a variety of approaches that minimize inputs, maximize efficiencies, and limit ecological impact. These goals drive efforts to translate advances in fundamental plant biology toward the application of plants for sustainability. Current agricultural technologies require large energy inputs for soil preparation, irrigation, and the synthesis and application of fertilizers and pesticides; these processes also produce greenhouse gases. One route to reducing those inputs is the development of plants that more efficiently use fertilizers, remove more carbon from the environment, and maintain soil and water quality. Are these challenges new? Not really. The friction between efficient production and use of inputs that drive agriculture to feed the world has always been there; that friction drove the Green Revolution of the 1960s and continues to motivate innovation and the integration of new technologies to tackle the problem (*5*, *6*). What has changed is the increased rate of population growth and climate change that will push agriculture to the limits in the coming decades, but the tools available to meet these problems have also expanded. Building from a deeper understanding of plant genetics, biochemistry, microbiology, chemistry, and systems biology, plant scientists are working toward the next generation of crops that more efficiently use inputs for growth and mitigate environmental damages.

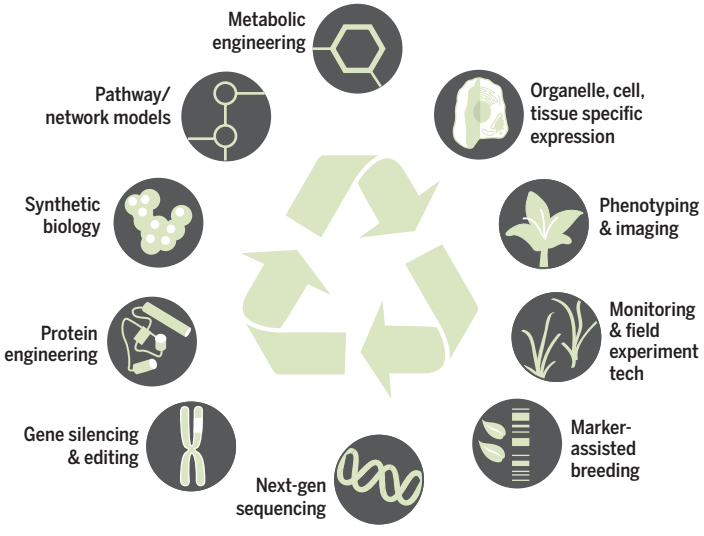

Fig. 1. Modern research tools support a cycle of discovery spanning atoms to ecosystems.

The modern plant engineering toolkit

Since completion of sequencing the first plant genome in 2000, the increasing amount of data and the development of new technologies have accelerated the discovery and evaluation cycle for plant science (Fig. 1). Cheaper next-generation sequencing and improved computational power for data analysis are key for marker-assisted selection and quantitative trait locus (QTL)–guided breeding (*7*). The selection of plant traits by using these tools accelerates market entry of new varieties. These same tools are also essential for studies of plant-associated microbiomes that are unraveling the interplay between plants, bacteria, and fungi (*8*). In addition to genomic data, imaging technologies now allow large-scale phenotyping of plant growth and development. Noninvasive sensors and high-resolution spectroscopy enable image reconstruction of root and leaf architectures and real-time capture of mineral and nutrient flow (*9*). Similarly, monitoring technologies, including drones and satellites, and experimental systems, such as free-air concentration enrichment facilities that simulate altered climate under field conditions, expand experimental trials beyond growth chambers and greenhouses. Precision agriculture is also bringing soil, water, and atmospheric chemists together with plant scientists to simulate and anticipate changes across geographic regions.

Along with "big data" that connect genomes, phenotypes, and the environment, molecular technologies have evolved since the first generation of transgenic crops. A variety of new gene silencing and genome editing methods offers accuracy not possible with earlier methods (*10*, *11*). Comparative genomics and computational modeling of gene and protein networks are invaluable for mapping control points and the fine-tuning of biochemical, signaling, and transcriptional pathways (*12*, *13*). At the molecular level, protein engineering can improve activity and generate new functionality that extends metabolism. Better techniques for the introduction of multiple genes and for the targeting of gene expression to specific tissues, cells, and organelles are also bringing metabolic engineering to new levels of sophistication (*14*). The pallet of tools with which to understand how plants work and interact with their environments now spans from genomes to proteins and metabolism to agro-ecosystems (Fig. 1). Recent progress highlights how new technologies allow research to move from the molecular to ecosystem

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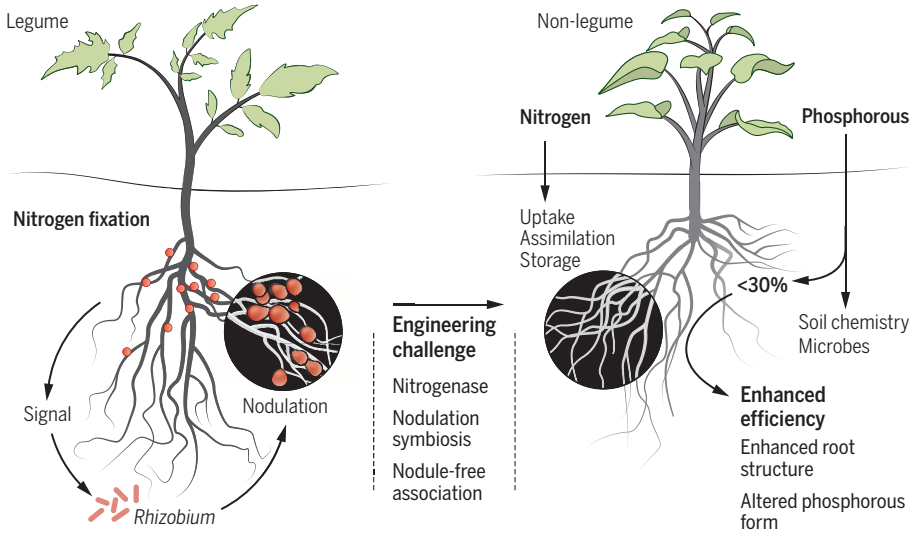

Fig. 2. Improving nitrogen and phosphorus use. (**Left**) Legume-Rhizobium symbiosis leads to nodule formation and nitrogen fixation. (**Right**) In nonlegume plants, nitrogen-related pathways are targets for breeding and genetic engineering. Different strategies for engineering nitrogen fixation are indicated. Most phosphorus is lost, but alterations of root structure and engineered pathways that select for phosphorus forms can enhance efficiency.

scales to tackle environmental and sustainability problems.

Improving nitrogen use

The success of modern agriculture is largely due to the availability of fertilizers that provide essential nutrients, such as nitrogen and phosphorus, to plants (Fig. 2). Without nitrogen fertilization, agriculture would support less than half our current population (*15*). Annually, chemical synthesis by means of the Haber-Bosch process provides ~118 million metric tons of nitrogen valued at $100 billion, with nearly half of this tonnage applied to wheat, rice, and maize worldwide (*15*). The Haber-Bosch process, which burns 3% of the world's natural gas and contributes the same percentage to global carbon emissions, mixes nitrogen and hydrogen gas under high pressure (270 atm) and temperature (450°C) with an iron catalyst to generate ammonia, which is then used in fertilizers. Overuse of nitrogen fertilizers can also disrupt terrestrial and aquatic ecosystems. Compared with chemical synthesis, the symbiosis between nitrogen-fixing rhizobial bacteria and legumes, such as soybean, accounts for <25% of global nitrogen fixation (*16*). The development of plants with improved nitrogen use would help reduce fertilizer applications, lower energy costs and greenhouse gas emissions associated with its synthesis, and help mitigate the consequences of nitrogen loss into soil and water sources.

Plant nitrogen uptake, assimilation, and storage pathways offer targets for breeding and genetic engineering of enhanced efficiency (*16*). For example, transgenic canola overexpressing alanine aminotransferase required 40% less fertilizer compared with wild-type plants under low-nitrogen conditions (*16*). Substantial efforts also aim to improve rhizobial colonization of legume roots (*17*). This symbiosis provides an oxygen-free environment for nitrogenase, a multisubunit bacterial metalloenzyme that converts nitrogen into ammonia for the plant (Fig. 2); however, engineering nitrogen fixation into nonlegume plants remains a challenge (*18*). Introducing nitrogenase into plants requires the transfer of the *nif* gene cluster, which encodes multiple nitrogenase subunits and accessory proteins required for metal-cofactor and subunit assembly and is under highly coordinated transcriptional control (*18*). Moreover, the oxygen-free environment needed by nitrogenase requires targeted expression in plants. These hurdles have led to investigations of other plant-microbial interactions that could enhance nitrogen use, such as nonlegume recognition by Rhizobium, altering plant-mycorrhizal interactions, and engineering nitrogen-fixing bacteria that associate with crops.

The signaling pathway required for rhizobial nodulation in legumes is also involved in mycorrhizal fungi associations (*19*). Because mycorrhizal interaction is common across plants, dissecting and rewiring the system for nonlegume-Rhizobium symbiosis may be possible (*19*). Likewise, ectomycorrhizal and arbuscular mycorrhizal associations—in which the extensive fungal hyphae allow for efficient acquisition and delivery of soil nutrients, including nitrogen, in exchange for carbon from the plant—are also the focus of intensive research. Ectomycorrhizal fungi associated with plant roots are critical for overcoming nitrogen limitation and maintaining plant growth (*20*). Alternatively, nitrogen-fixing endophytic bacteria that form nodule-independent associations with crops and free-living nitrogen-fixing bacteria, such as Azotobacter and Azospirillum, are routinely used as biofertilizers and could be engineered for enhanced nitrogen fixation (*19*). Nitrogen tracer studies that have used the model grass *Setaria viridis* and bacterial inoculants suggest that this plant uptakes bacterial nitrogen, but the efficiency and universality of the process is unclear (*21*). If widespread, engineering of the *nif* cluster into bacteria that associate with nonlegume crops is possible. Experiments demonstrating the transfer of *nif* clusters between bacterial species and synthetic biology optimization for nitrogenase expression have been successful (*22*). Given the complexity of plant-rhizosphere interactions, the application of plant-microbe niche engineering needs further investigation but could provide ways for improving nitrogen use efficiency.

There is also the exciting possibility of hybrid biological-chemical approaches for nitrogen fixation. Synthetic nanoparticles as generators of fixed nitrogen may be feasible; a cadmium sulfide nanocrystal/nitrogenase molybdenum-iron protein hybrid driven by light, instead of adenosine triphosphate hydrolysis, was recently described (*23*). Whether such nanoparticles can be stable enough to compete with the Haber-Bosch process or work under field conditions with plants remains to be explored, but synthesis of ammonia by using a light-driven catalyst is an intriguing future direction that showcases the value of multidisciplinary approaches to sustainability.

Improving phosphorus use

Phosphorus, like nitrogen, is an essential nutrient and limits crop growth in nearly 70% of agricultural land (*24*). Orthophosphate is the only bioavailable form for plants and is a nonrenewable resource obtained from mining. Because of its reactivity with soils rich in iron and aluminum oxides and conversion by soil microbes to forms inaccessible to plants, crops capture <30% of phosphorus applied as fertilizer. Moreover, phosphate excesses are a primary contributor to eutrophication. Recent progress toward the development of plants with improved phosphorus use will help maintain supplies of this resource and could limit overuse (Fig. 2).

Traditional breeding and QTL analysis identified a rice variety with a protein kinase [*phosphorus-starvation tolerance 1* (PSTOL1)] that acts as an enhancer of early root growth and provides tolerance to phosphorus deficiency (*25*). PSTOL1 overexpression enhanced grain yield in phosphorus-deficient soil through altered root architecture that allows for better nutrient uptake. Variations in root structure that enhance phosphorus mobilization could also be combined with improved knowledge of key plant-fungal interactions. For example, the endophytic fungus *Colletotrichum tofieldiae*, which associates with *Arabidopsis thaliana* in the wild, transfers phosphorus to shoots to promote plant growth and increases plant fertility under phosphorus-limited conditions (*26*).

Complementing these efforts, our molecular understanding of how phosphorus is sensed was advanced by the identification of the SPX inositol polyphosphate-binding domain shared by phosphate transporters, signal transduction proteins, and inorganic polyphosphate polymerase (*27*). This work suggests that association of

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SPX domain proteins with transcription factors modulates phosphorus starvation responses. There are also efforts to alter the form of phosphorus fertilizer used by plants, with the added benefit of weed control (*24*). Transgenic plants expressing a bacterial protein that oxidizes phosphite to orthophosphate can grow with 30 to 50% less phosphorus input. Moreover, because orthophosphate does not discriminate between crops and weeds, use of phosphite fertilizer allowed transgenic plants to grow with up to 10-fold greater biomass in competition with weeds than when provided with orthophosphate. Deciphering the phosphorus response network in plants promises to reveal new targets for improving nutrient use.

Photosynthesis and carbon fixation

As part of terrestrial ecosystems, which sequester ~25% of anthropogenic CO_2, plants use clean and renewable processes for removal of this greenhouse gas: photosynthesis and carbon fixation. The application of modern plant biology approaches toward improving photosynthesis and carbon fixation may provide solutions for carbon capture and sequestration. Because photosynthesis links sunlight to carbon fixation for the production of seeds and grains, this will also affect agriculture. Each step—from efficient light capture by leaves to the conversion of sunlight into high-energy compounds to the partitioning of carbon into plant growth and seed production—is a target for improvements through breeding and genetic engineering (Fig. 3) (*28*).

Advances in genome data and phenotyping contribute to understanding how plants adapt to light environment and control leaf anatomy and morphology in sunlight versus shade (*29*). Such insights can guide the breeding of plants with improved photosynthesis. Light-capture research ranges from the macroscopic to the molecular scales. Altering developmental programs that affect leaf area and geometry would allow light to penetrate to lower-canopy leaves for maximizing light-harvesting efficiency (*30*). Similarly, the discovery of new chlorophylls and strategies for altering the structure of light-harvesting antenna complexes to match photosynthetic efficiency may extend the spectrum of energy available for photosynthesis and carbon fixation (*31*).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the entry point for CO_2 into the Calvin cycle, is an obstacle for improving carbon fixation. Plant RuBisCO has a slow activation response and catalyzes an oxygenation reaction that competes with CO_2 fixation. Recent work describes the first success in replacing a plant RuBisCO with a more rapid cyanobacterial homolog (*32*). Although the cyanobacterial RuBisCO is faster at CO_2 fixation, it is also more reactive with oxygen. Expression of a subunit from the cyanobacterial carboxysome, a microcompartment that excludes oxygen and concentrates CO_2, was required for higher fixation rates in transgenic plants (*32*). Future improvements of photosynthetic efficiency by using protein engineering to reduce RuBisCO oxygenation and to express carboxysomes and other parts of the cyanobacterial CO_2-concentration machinery are logical next steps (*32*).

A more technically demanding approach is the conversion of plants that use C_3 photosynthesis to C_4 photosynthesis (*33*). Most plants use C_3 photosynthesis with RuBisCO in leaf mesophyll cells to directly fix CO_2. In contrast, C_4 plants separate CO_2 fixation from the Calvin cycle. Making up only 5% of plant species, C_4 plants fix ~30% of global CO_2. Fixation of CO_2 into the C_4 acid oxaloacetate occurs in mesophyll cells, followed by diffusion of oxaloacetate to bundle sheath cells. There, decarboxylation of oxaloacetate provides CO_2 to RuBisCO and the Calvin cycle. The oxygen-free environment enhances the efficiency of RuBisCO. Evolutionary history suggests that such radical engineering is possible; C_4 plants independently evolved from C_3 plants in 60 different taxa (*33*). All of the genes and proteins for C_4 photosynthesis are known; however, generation of transgenic plants would require the introduction of more than 20 genes—a technical problem similar to that of engineering nitrogenase into plants. The more formidable challenge is how to alter C_3 plants for the compartmentalization of carbon fixation and the Calvin cycle in distinct cell types. As yet, the control of leaf cell development and identification of promoters that target expression to mesophyll versus bundle sheath cells are not well understood.

Water and salinity

Irrigation of agricultural crops accounts for 70% of global fresh water consumption (*34*). Sustainable agriculture requires improved plant survival during short-term periods without water and more efficient water use. Although breeding has altered flowering time, height, and other traits that affect water use, the multigenic nature of plant drought responses and a lack of natural variability for crop drought tolerance have limited progress (*34*). Engineering plants for drought tolerance emphasizes the need for systems-level approaches that center on molecular control points with broad effects across multiple downstream targets (Fig. 3).

Development of the first commercialized drought-tolerant plants used a bacterial RNA/cold stress chaperone protein to minimize drought stress effects on photosynthesis, carbon fixation, and water loss (stomata closure) in early development of maize (*35*). Since then, genes and proteins linked to osmoprotectants, the detoxification of reactive oxygen species produced under drought stress, and plant hormone systems that regulate stomata closure and protein degradation have been engineering targets. For example, expression of trehalose-6-phosphate phosphatase—an enzyme that alters levels of the signaling molecule trehalose—in maize improved crop yields by 49 and 123% under mild and severe drought conditions, respectively (*36*). Although efforts to improve crop drought tolerance are promising, the variability of crop cultivars, field conditions, and regional weather necessitate continued field studies.

In some regions of the world, such as the tropics, increasingly wetter climates with prolonged rainfall and severe floods affect farming with losses comparable with those from drought (*37*). In Asia, flooding affects rice with annual losses of $1 billion in areas dominated by smallholder farms (*37*). Waterlogged roots and submergence of aerial parts typically kill plants within a week; however, adaptations in certain rice cultivars allow for survival up to twice as long. QTL analysis identified *submergence 1* (*Sub1*) as a mediator of stress-induced expression of ethylene-responsive transcriptional regulators in rice. As with drought, the search for submergence tolerance traits is identifying key control points in low-oxygen-sensing and -signaling pathways and regulators of pathways linked to flooding survival (*37*); however, the extension of such knowledge from rice to other crops is just beginning.

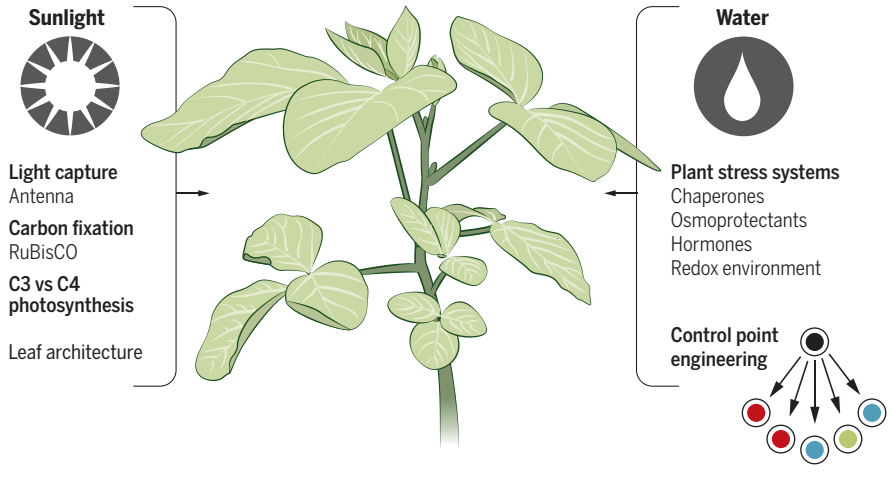

Fig. 3. Improving carbon fixation and water use. Breeding efforts to alter leaves and engineering of photosynthesis aim to improve the efficiency of carbon fixation. The multigenic nature of water-stress–related pathways requires researchers to target key control points that alter multiple other steps in various response/protective networks.

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Directly related to crop irrigation and coastal flooding is the problem of soil salinity or sodium toxicity. Crop irrigation increases salinity because of trace salts in water that accumulate over time. Saline soil conditions affect 7% of land, and nearly 30% of irrigated crops suffer from sodium contamination (*38*). The identification of the high-affinity potassium transporters (HKTs), which are selective for sodium and prevent accumulation of this toxic ion in leaves, provides a molecular mechanism for salt tolerance. Natural variation in HKTs is a resource for introducing salt tolerance into crops. Breeding of a wheat relative with a HKT gene into durum wheat produces a variety with reduced sodium in leaves and 25% higher grain yield on saline soils (*38*). Other pathways involved in salinity tolerance—including osmoprotectant synthesis, degradation of reactive oxygen species, and altered signaling systems—are also being explored for the breeding and engineering of plants that tolerate growth on saline soils (*39*). In addition to excluding contaminants such as sodium, plants can serve as environmental cleanup tools.

Environmental engineering for remediation

All human activity has the potential to create environmental contaminants, including organic molecules from fuel and chemical spills, military activities, agriculture, industry, and forestry and naturally occurring inorganic contaminants that are mobilized and concentrated by mining, irrigation, and geochemical cycles. The use of microbes and plants for remediation dates back to the Romans and offers noninvasive, cost-effective, and environmentally friendly options to clean up contamination sites (*40*). Plants can stabilize, extract, degrade, and/or volatilize organic and inorganic contaminants, and extensive metabolic engineering efforts can improve the remediation efficiency of various pathways (*40*).

Better understanding of plant metabolism is leading to new approaches for using plants as environmental remediation tools. Although many explosives, including 2,4,6-trinitrotoluene (TNT), are recalcitrant to degradation, plants can detoxify TNT to a limited extent (*41*). Metabolism of TNT in plants generates a nitro-radical that reacts with oxygen to form toxic superoxide. Plants with a knockout of the gene encoding monodehydroascorbate reductase, which generates the nitro-radical, display enhanced TNT tolerance and may be useful for degradation of explosives on military sites (*41*). In addition to knockout engineering, fine-tuning enzymatic activity can also tailor plant responses to toxic metals. Directed evolution of phytochelatin synthase (PCS), an enzyme that synthesizes heavy metal–binding peptides, revealed an unexpected way of improving heavy metal tolerance through the attenuation of enzymatic activity (*42*). A catalytically inferior PCS resulted in phenotypic superiority because it maintained cellular redox homeostasis while synthesizing peptides for detoxification of cadmium (*42*). In addition to remediation applications, plants can also serve as biosensors for monitoring contamination sites. Introduction of a fluorescent zinc biosensor into Arabidopsis and poplar trees demonstrates a useful strategy for the development of sentinel plants (*43*). Given the physical dimensions of many waste sites and the expense of soil sampling to monitor and define contamination, improvements in plant-based remediation and the development of biosensors can provide cost-effective tools for environmental cleanup.

Conclusions

During the past century, agriculture moved beyond the knowledge of local farmers to become a global endeavor. Investments in fundamental plant biology and agriculture revolutionized food production, but larger problems loom ahead. To meet the challenges of this century, plant biologists are working on many fronts, some highlighted here, to make sustainability a reality. In this next green movement, new technologies and multidisciplinary approaches are enabling the translation of fundamental plant biology knowledge toward the reduction of inputs and ecological footprints.

Aside from technical hurdles, agro-economics, the evolutionary constraints of plants, and the public perception of plant biotechnology remain issues. Basic science opens many routes toward a sustainable future; however, crops are commodities with a narrow profit margin, which can temper the implementation of new varieties and practices. Breakthroughs must be useful, make economic sense, and be adaptable to multiple regions and agro-ecosystems worldwide. More frequently, the combination of classic breeding and genetic engineering supported by new technologies provides greater options. Natural variation remains essential for plant improvement, but biotechnology can target complicated traits not amenable to traditional breeding. This is especially noticeable as efforts shift away from a one-gene-one-trait model toward broader systems-level targets, in which key control points that regulate downstream steps are exploited or combined to regulate multiple pathways. This also highlights the potential limits of plant genetics. Modern crops evolved under "normal" conditions, and the diversity needed for breeding new traits to meet changing climate is not always available. Moreover, efforts to improve particular traits often ignore combinations of stresses, diseases, and altered inputs. Additional work needs to consider multiple simultaneous challenges to plant growth and can be aided by the integration of large data sets and modeling studies. There is also substantial progress toward a mechanistic understanding of the plant microbiome and how bacteria and fungi work with plants for nutrient mobilization. In particular, recent discoveries on the relationships between plants and fungi in nitrogen (*20*) and phosphorus (*26*) may lead to enhancing plant-microbe interactions to minimize inputs and environmental impacts. Last, the public perception of plant biotechnology necessitates the continued education of people about the potential, and the limitations, of both breeding and genetic engineering.

The coming decades will be an exciting time for plant biologists with an eye for how to use plants for environmental and sustainability applications. A single look at teosinte and maize and one sees how adaptable plants can be for meeting our needs. What remains to be seen is whether we can harness our understanding of plants to innovate fast enough to meet the challenges poised by 9 billion people in 2050.

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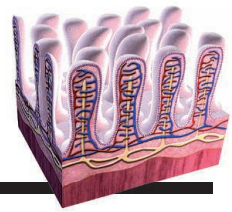
RESEARCH

Pathogen induces oxygenation of the gut

Lopez et al., *p. 1249*



IN *SCIENCE* JOURNALS

Edited by **Caroline Ash**

SENSORY BIOLOGY

Swapping senses to avoid the din

Species that use sound for foraging or movement are at a disadvantage in our increasingly noisy world. However, some species have the capacity to operate despite background din. Fringe-lipped bats typically hunt frogs by listening for their breeding calls. In noisy environments, Gomes *et al.* found that the bats shift from passive acoustic reception of the frogs' sounds to using echolocation to detect the calling frogs' expanding throat sacs. —SNV

Science, this issue p. 1277

Fringe-lipped bats can swap senses to hunt frogs.

BRAIN MICROCIRCUITS

Neuronal assembly dynamics in the brain

Sharp-wave ripples are a form of neuronal activity pattern observed in the hippocampus that may play a role in memory consolidation. To investigate this phenomenon, Malvache *et al*. simultaneously imaged calcium dynamics in multiple hippocampal neurons of live mice. A category of neurons called place cells became active when mice ran on a treadmill. When the mice paused, fast replay events occurred that recalled the sequence of cell activations while the mice were running, and these coincided with the sharp-wave ripples. Reactivation periods in which different assemblies of cells took part were also observed. Imaging of the mice indicated that patches of neurons might cooperate to produce fragments of the ripple events. —PRS

Science, this issue p. 1280

EXTINCTION

Like in scale, different in pattern

Before now, there have been five major extinction events in Earth's history. Understanding the patterns and processes that characterized the previous extinctions are important for understanding, and perhaps mitigating, the levels of extinction occurring now. Payne *et al.* looked at marine taxa, including vertebrates and mollusks, and found several important differences. Most notably, current extinctions are centered on large-bodied species, irrespective of their life-history strategy. This makes sense considering that the main drivers of marine extinctions today are human hunters and fishers, rather than climate—at least so far. —SNV

Science, this issue p. 1284

Using fossils can help to understand present-day extinction patterns.

QUANTUM SIMULATION

Staring antiferromagnetism in the face

Quantum gas microscopy affords a direct, site-resolved view of atoms in an optical lattice. The possibilities for studying quantum phases appear endless, with a major goal being the achievement of d-wave superfluidity akin to the high-temperature superconductivity of cuprate compounds. Three groups have made important progress toward this goal by directly imaging antiferromagnetic correlations, which also appear in the cuprate phase diagram. Parsons *et al.* did so in a two-dimensional (2D) lattice of fermionic atoms near half-filling, whereas Boll *et al*. investigated 1D chains. Additionally, Cheuk *et al*. revealed the influence of doping on the charge correlations in a 2D lattice. Reducing the temperature further should bring these experiments closer to the superfluid regime. —JS

Science, this issue pp. 1253, 1257, and 1260

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POLYMER SCIENCE

Stretching the connections

Measuring the elastic behavior of polymers is complicated by their long chain architecture, which can lead to transient entanglements and time-dependent behavior. A further complication arises in the case of covalently linked polymers, such as hydrogels, that contain fixed loop defects. By controlling defect structure during polymer synthesis, Zhong *et al*. can count the defects in a polymer gel and correlate them to elastic and rheological behavior. Current models are not able to capture their observations, but a real elastic network theory, based on a modified phantom network theory, can account for the impact of topological molecular defects. —MSL

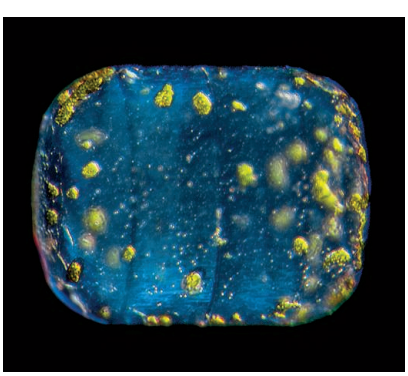
Science, this issue p. 1264

MARINE MICROBIOME

Functional microbial distribution in the ocean

Bucketsful of genome data for marine microorganisms are available. Although taxonomic patterns in the data are carefully sifted for meaning, Louca *et al*. show that taxonomy alone does not represent community structure. Meta-analysis of the data gathered during the Tara Oceans project classified over 30,000 bacteria and archaea into functional metabolic groups ranging from photoautotrophy and nitrogen fixation to sulfate respiration. Disentangling functional variation from taxonomic variation indicates that environmental conditions select from a global marine "seedbank" for function, not taxonomy. —CA

Science, this issue p. 1272



Functional and taxonomic variation can be distinguished in Tara Oceans microbiome data.

NANOMATERIALS

Brushing out uniform nanorods

An ongoing challenge for the synthesis of nanomaterials is to find ways to control their chemistry, architecture, and dimensions while still producing uniform products in reasonable quantities. Pang *et al*. devise a clever strategy that uses bottlebrush block copolymers to guide material growth from the inside out (see the Perspective by Houlton). Because the polymers can be grown with a narrow size distribution, they act as uniform templates for a variety of precursors. A wide range of materials can be grown, including those that have metallic, ferroelectric, photonic, semiconducting, luminescent, thermoelectric, magnetic, or a combination of properties. —MSL

Science, this issue p. 1268; see also p. 1204

IMMUNOLOGY

Uncovering how a drug works

The drug dimethyl fumarate (DMF) is used to treat autoimmune diseases, including psoriasis and multiple sclerosis, and may act by modifying cysteine residues in proteins. A better understanding of how it works is required because its use has led to life-threatening infections in some patients. Blewett *et al*. identified cysteine residues in human T cell proteins that reacted with DMF. They found that modification of the kinase PKCθ by DMF prevented PKCθ from mediating full T cell activation. —JFF

Sci. Signal. **9**, rs10 (2016).

IN OTHER JOURNALS

Edited by **Sacha Vignieri** *and* **Jesse Smith**

The smell of fire rouses Eastern pygmy possums from torpor.

ECOPHYSIOLOGY

Wake up!

Torpor occurs when an endothermic animal's core body temperature is reduced below a critical threshold. Similar to hibernation, torpid animals' physiological and neural systems have reduced activity in this energy-saving state. Generally, it is thought that animals in this condition have little ability to respond to threats. Nowack *et al.* show, however, that Eastern pygmy possums were roused from torpor by the smell of fire, in some cases completely emerging and initiating directed climbing. Though this movement occurred well below suboptimal body temperature, animals were not able to move at the coldest temperatures. These results show that torpid animals may be able to respond to threats, especially gradual ones, such as fire, but that ambient temperature is a key driver of their ability to do so. —SNV

Sci. Nat. 10.1007/s00114-016-1396-6 (2016).

BRAIN CONNECTIONS

How the mind controls the body

Can our brain influence our internal organs? Dum *et al.* used retrograde transneuronal transport of rabies virus to identify the brain regions that project to a part of the adrenal gland called the medulla. Two networks are the major sources of neuronal input to this organ, which plays an important role in dealing with physical and emotional stress. One network includes

PHOTOS (FROM LEFT): CHRISTIAN SARDET; DAVE WATTS/ VISUALS UNLIMITED

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the primary motor cortex and specific regions in the frontal lobe of the brain. The other network involves distinct parts of the anterior cingulate cortex, an area that regulates cognition and emotion. The central commands that control the adrenal medulla originate from brain regions that are involved in multiple functions, including body movement, cognitive control, and affective processing. —PRS

Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1605044113 (2016).

MITOCHONDRIAL GENETICS

Effects of mtDNA and nuclear genetic variation

Because mitochondrial DNA (mtDNA) is predominately inherited through the mother, it is hypothesized that in the case of mtDNA mutations, males might be more affected by genetic incompatibilities. Examining mtDNA and nuclear variation and interactions in fruit flies, Mossman *et al*. show both genotype- and sex-specific effects on gene expression. Effects on gene expression profiles were greater in females for all examined genetic differences, suggesting that females are more sensitive to genetic variation. In contrast, a greater number of genes exhibited some effects in males, but the degree of difference was lower and not significant relative to the impacts of genetic variation in females. —LMZ

Genetics 10.1534/genetics.116.192328 (2016).

DIVERSITY

Mentoring as value added

The benefits of faculty-mentored undergraduate research experiences are well known. Interinstitutional research training programs ensure that these opportunities are available to all science, technology, engineering, and math students, but how do you convince faculty to mentor undergraduates from other institutions? Morales *et al.* surveyed faculty members at 13 U.S. institutions to identify factors leading to their willingness to mentor undergraduate students from other universities and the incentives that they value most in return. Increasing diversity was the main motivation for faculty participation, although incentives varied based on faculty career stage and funding status. Overall, results suggest that promoting faculty awareness of the positive impacts of mentoring students from underrepresented backgrounds and/or underresourced institutions, especially in terms of increasing diversity, would increase faculty engagement. —MM

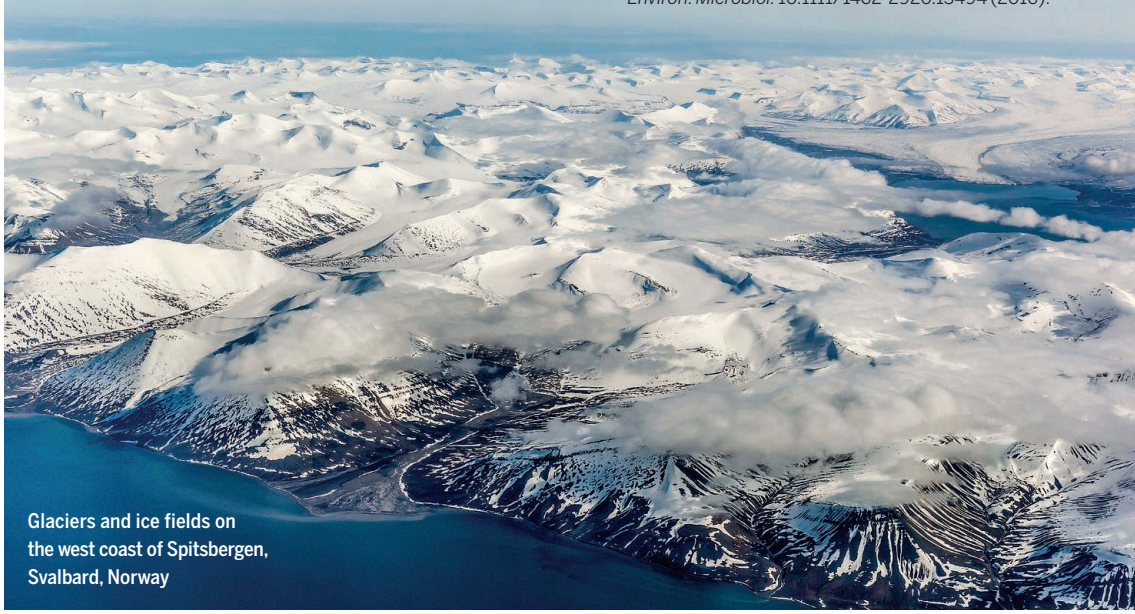
CBE Life Sci. Educ. 10.1187/cbe.16-01-0039 (2016).

MICROBIOLOGY

Grown in the land of the ice and snow

Although seemingly inhospitable, glaciers host a range of microbial activities. Algal blooms are common on the surfaces of snow and ice, for example, which can lower albedo and increase melting. Lutz *et al.* identified the algal communities, along with archaea and bacteria, on 12 glaciers and permanent snowfields in Norway and Sweden. Correlations between nutrient ratios and metabolite production point to commonalities in community function between sites. The synthesis of some darkening carotenoid compounds, for example, is critically dependent on the availability of nitrogen. If atmospheric nitrogen deposition increases as expected, more frequent algal blooms may lead to more glacial melting. —NW

Environ. Microbiol. 10.1111/1462-2920.13494 (2016).



Glaciers and ice fields on the west coast of Spitsbergen, Svalbard, Norway

PHOTO: OBERTHARDING/ALAMY STOCK PHOTO

CERAMICS

Probing the boundaries of ceramics

Ceramics are important but underappreciated materials for numerous technologies. Diercks *et al.* investigate the connection between electrical properties and local chemistry in neodymium-doped ceria. They show that macroscopic changes in properties such as electrical conductivity are tied to the chemical composition of the grain boundaries, which make up a tiny fraction of the overall volume. High-precision maps provide a method for understanding the relationship between microscopic and macroscopic properties, which can be exploited for optimizing materials design. —BG

J. Mater. Chem. A 10.1039/c5ta10064j (2016).

AGING

Regeneration of NAD keeps mouse muscles young

Evidence for a critical role of nitric acid dihydrate (NAD) metabolism in aging is accumulating. Frederick *et al*. studied mice with muscle-specific depletion of nicotinamide phosphoribosyltransferase (Nampt), an enzyme needed to restore concentrations of NAD in working muscle. Muscle in young animals tolerated a large reduction in the amount of NAD without obvious loss of function. However, older control animals had decreased muscle concentrations of NAD, which correlated with decreased performance, and these effects were more pronounced in animals lacking Nampt. Restoration of NAD concentrations by feeding older animals nicotinamide riboside partially restored muscle function. Accordingly, overexpression of Nampt in muscle helped prevent the age-dependent decline in muscular function. Thus, maintenance of proper NAD metabolism in muscle appears to be needed for sustained function. —LBR

Cell Metab. 10.1016/j.cmet.2016.07.005 (2016).

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ALSO IN *SCIENCE* JOURNALS

Edited by **Caroline Ash**

GENOME EDITING

A useful AID for genome editing

The prokaryotic CRISPR-Cas adaptive immune mechanism has revolutionized genome editing, but some glitches remain. The Cas nuclease is directed to its target site by a guide RNA, which then nicks the DNA. Repair of this cut can generate small unwanted insertions and deletions. Nishida *et al*. circumvent the problem of inaccurate repair by using a mutated form of the Cas nuclease from a different species, fused to an ortholog of activation-induced cytidine deaminase (AID), which is part of the vertebrate immune system (see the Perspective by Conticello and Rada). The AID ortholog catalyzes nucleotide substitutions at the targeted locus without cutting the DNA, thus reducing off-target effects and improving the efficiency of the CRISPR-Cas toolbox. —GR

Science, this issue p. 1248; see also p. 1206

FOOD SYSTEM

Good to eat, good for the planet

Food production, processing, and use are responsible for about a quarter of global greenhouse gas emissions. In a Perspective, Garnett considers how the food system can be changed to become more sustainable while also providing for healthy diets. Existing studies highlight the high environmental impacts and damaging health effects of typical Western diets, which are increasingly consumed around the world. But solutions are less clear-cut because existing research only provides a partial picture of what constitutes a sustainable diet. Furthermore, to be successful, policies must take account of the values that not only shape diets but also underlie research and advocacy. —JFU

Science, this issue p. 1202

ANTIBIOTICS

Treatment by the earful

Otitis media, commonly called ear infection, is a ubiquitous childhood malady that accounts for many pediatrician visits and antibiotic prescriptions. However, it is not always easy to get oral antibiotics into small children. Moreover, systemic antibiotics can cause the development of resistance and other side effects. To address these problems, Yang *et al.* developed a hydrogel-based system that permits delivery of antibiotics directly into the ear and demonstrated its effectiveness in a chinchilla model of nontypeable *Haemophilus influenzae* ear infection, a common cause of otitis media. No drug was detected in the animals' blood, confirming the specificity of this local delivery method. —YN

Sci. Transl. Med. **8**, 356ra120 (2016).

INFECTION

Pathogen stimulates gut oxygen

Several bacterial pathogens have mobile elements with genes for a syringe-like mechanism called T3SS. The assembled T3SS injects virulence effectors into host cells. Lopez *et al*. found that a T3SS-injected virulence factor produced by a mouse pathogen provokes inflammation, repair responses, and crypt hyperplasia in the host's gut epithelium. Mature colon cells use oxygen for their physiological role in water absorption, but the immature hyperplastic colonocytes do not need oxygen for growth. Hence, during hyperplasia, there is increased availability of oxygen for aerobic pathogens to grow, and the resident anaerobic microbiota is excluded. The pathogens also benefit from using carbon sources that are unavailable to organisms that rely on fermentation for growth. —CA

Science, this issue p. 1249

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RESEARCH ARTICLE SUMMARY

GENOME EDITING

Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems

Keiji Nishida, Takayuki Arazoe, Nozomu Yachie, Satomi Banno, Mika Kakimoto, Mayura Tabata, Masao Mochizuki, Aya Miyabe, Michihiro Araki, Kiyotaka Y. Hara, Zenpei Shimatani, Akihiko Kondo*

INTRODUCTION: To combat invading pathogens, cells develop an adaptive immune response by changing their own genetic information. In vertebrates, the generation of genetic variation (somatic hypermutation) is an essential process for diversification and affinity maturation of antibodies that function to detect and sequester various foreign biomolecules. The activation-induced cytidine deaminase (AID) carries out hypermutation by modifying deoxycytidine bases in the variable region of the immunoglobulin locus that produces antibody. AID-generated deoxyuridine in DNA is mutagenic as it can be miss-recognized as deoxythymine, resulting in C to T mutations. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) is a prokaryotic adaptive immune system that records and degrades invasive foreign DNA or RNA. The CRISPR/Cas system cleaves and incorporates foreign DNA/RNA segments into the genomic region called the CRISPR array. The CRISPR array is transcribed to produce crispr-RNA that serves as guide RNA (gRNA) for recognition of the complementary foreign DNA/RNA in a ribonucleoprotein complex with Cas proteins, which degrade the target. The CRISPR/Cas system has been repurposed as a powerful genome editing tool, because it can be programmed to cleave specific DNA sequence by providing custom gRNAs.

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RATIONALE: Although the precise mechanism by which AID specifically mutates the immunoglobulin locus remains elusive, targeting of AID activity is facilitated by the formation of a single-stranded DNA region, such as a transcriptional RNA/DNA hybrid (R-loop). The CRISPR/Cas system can be engineered to be nuclease-inactive. The nuclease-inactive form is capable of unfolding the DNA double strand in a protospacer adjacent motif (PAM) sequence-dependent manner so that the gRNA binds to complementary target DNA strand and forms an R-loop. The nuclease-deficient CRISPR/Cas system may serve as a suitable DNA-targeting module for AID to catalyze site-specific mutagenesis.

RESULTS: To determine whether AID activity can be specifically targeted by the CRISPR/Cas system, we combined dCas9 (a nuclease-deficient mutant of Cas9) from *Streptococcus pyogenes* and an AID ortholog, PmCDA1 from sea lamprey, to form a synthetic complex (Target-AID) by either engineering a fusion between the two proteins or attaching a SH3 (Src 3 homology) domain to the C terminus of dCas9 and a SHL (SH3 interaction ligand) to the C terminus of PmCDA1. Both of these complexes performed highly efficient site-directed mutagenesis. The mutational spectrum was analyzed in yeast and demonstrated that point mutations were dominantly induced at cytosines within the range of three to five bases surrounding the –18 position upstream of the PAM sequence on the noncomplementary strand to gRNA. The toxicity associated with the nuclease-based CRISPR/Cas9 system was greatly reduced in the Target-AID complexes. Combination of PmCDA1 with the nickase Cas9(D10A) mutant, which retains cleavage activity for noncomplementary single-stranded DNA, was more efficient in yeast but also induced deletions as well as point mutations in mammalian cells. Addition of the uracil DNA glycosylase inhibitor protein, which blocks the initial step of the uracil base excision repair pathway, suppressed collateral deletions and further improved targeting efficiency. Potential off-target effects were assessed by whole-genome sequencing of yeast as well as deep sequencing of mammalian cells for regions that contain mismatched target sequences. These results showed that off-target effects were comparable to those of conventional CRISPR/Cas systems, with a reduced risk of indel formation.

CONCLUSION: By expanding the genome editing potential of the CRISPR/Cas9 system by deaminase-mediated hypermutation, Target-AID demonstrated a very narrow range of targeted nucleotide substitution without the use of template DNA. Nickase Cas9 and uracil DNA glycosylase inhibitor protein can be used to boost the targeting efficiency. The reduced cytotoxicity will be beneficial for use in cells that are sensitive to artificial nucleases. Use of other types of nucleotide-modifying enzymes and/or other CRISPR-related systems with different PAM requirements will expand our genome-editing repertoire and capacity. ■

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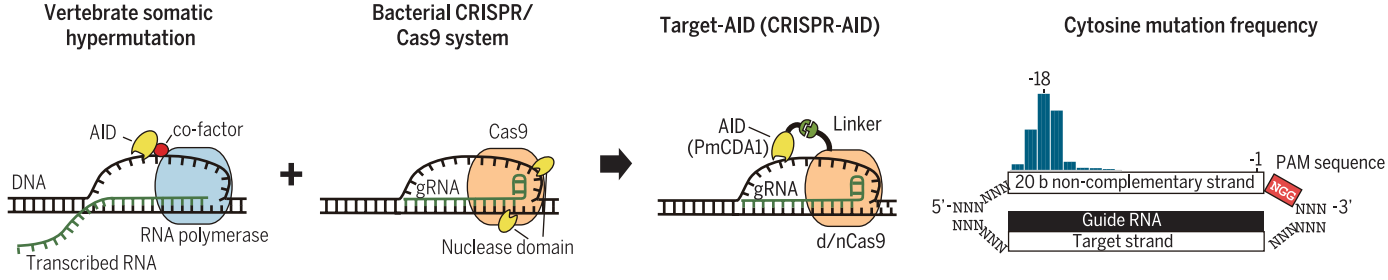

A crippled CRISPR/Cas targets AID. In vertebrate adaptive immunity, cytosine deaminase (AID or PmCDA1) induces somatic hypermutation at single-stranded DNA regions formed during transcription. The bacterial CRISPR/Cas9 immunity system recognizes and cleaves invasive DNA in a gRNA-dependent manner. AID and nuclease-deficient CRISPR/Cas9 are engineered to form a hybrid complex (Target-AID) that performs programmable cytosine mutations in a range of a few bases surrounding the –18 position upstream of PAM sequence of the noncomplementary DNA strand.

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RESEARCH ARTICLE

GENOME EDITING

Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems

Keiji Nishida,[1] Takayuki Arazoe,[1] Nozomu Yachie,[2,3,4] Satomi Banno,[1] Mika Kakimoto,[1] Mayura Tabata,[1] Masao Mochizuki,[1] Aya Miyabe,[1] Michihiro Araki,[1] Kiyotaka Y. Hara,[5] Zenpei Shimatani,[1] Akihiko Kondo[1,6]*

The generation of genetic variation (somatic hypermutation) is an essential process for the adaptive immune system in vertebrates. We demonstrate the targeted single-nucleotide substitution of DNA using hybrid vertebrate and bacterial immune systems components. Nuclease-deficient type II CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated) and the activation-induced cytidine deaminase (AID) ortholog PmCDA1 were engineered to form a synthetic complex (Target-AID) that performs highly efficient target-specific mutagenesis. Specific point mutation was induced primarily at cytidines within the target range of five bases. The toxicity associated with the nuclease-based CRISPR/Cas9 system was greatly reduced. Although combination of nickase Cas9(D10A) and the deaminase was highly effective in yeasts, it also induced insertion and deletion (indel) in mammalian cells. Use of uracil DNA glycosylase inhibitor suppressed the indel formation and improved the efficiency.

The activation-induced cytidine deaminase (AID) is responsible for targeted hypermutation (*1*) by modifying the deoxycytidine of the variable region of the immunoglobulin locus (*2*, *3*). Although the precise mechanism by which AID specifically targets the immunoglobulin locus remains elusive, targeting of AID is facilitated by the formation of a single-stranded DNA region, such as a transcriptional RNA/DNA hybrid (R-loop) (*4*, *5*). AID generates deoxyuridine in DNA, which is further processed by uracil DNA glycosylase for class-switch recombination (*6*) as well as repair (*7*). CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) is a prokaryotic adaptive immune system (*8*). The CRISPR/Cas system records and cleaves invasive foreign DNA. A ribonucleoprotein complex of Cas nuclease and guide-RNA (gRNA) binds to complementary target DNA sequences, forms an R-loop, and then cleaves the DNA. The CRISPR/Cas system has been developed as a genome editing tool (*9*, *10*). Here, by using hybrid vertebrate and bacterial immune systems, we provide genome engineering tools that enable targeted nucleotide substitution without integration or the use of template DNA.

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Cytidine deamination is targeted by CRISPR/Cas system

To determine whether the single-stranded DNA region formed by the CRISPR/Cas system also serves as a preferable substrate for AID, we created a synthetic complex (Target-AID) of dCas9 (a nuclease-deficient mutant of Cas9) (*11*) from *Streptococcus pyogenes* and an AID ortholog, PmCDA1 from sea lamprey by either a fusion protein or attaching a SH3 (Src 3 homology) domain (*12*) to the C terminus of dCas9 and a SHL (SH3 interaction ligand) to the C terminus of PmCDA1 (Fig. 1A). The protein complex was expressed in *Saccharomyces cerevisiae*, which lacks an endogenous AID-related system. Chimeric gRNAs (*11*) were designed (*13*) to target a genomic negative selectable marker gene, CAN1, whose loss of function results in resistance to the drug canavanine. The rate of occurrence of the canavanine-resistant mutant was counted as an on-target mutation. To assess the potential genome-wide, nonspecific mutator phenotype (off-target mutation), another negatively selectable marker, LYP1, was monitored by resistance to S-aminoethyl-L-cysteine. Although the expression of PmCDA1 increased the background mutation frequency, the specific mutation frequency of CAN1 increased by more than 1000-fold over PmCDA1 alone when dCas9-SH3 and SHL-PmCDA1 were expressed simultaneously (Fig. 1B, 4). Specific mutation was observed even without synthetic attachment of dCas9 and PmCDA1 (Fig. 1B, 3), suggesting that the single-stranded DNA formed by dCas9-gRNA is a preferred substrate for PmCDA1. The fusion protein of dCas9 and PmCDA1 was also effective, especially when a large peptide linker (100 amino acids) was used (Fig. 1B, 6). Most mutations were found at g782, which is located at the –16 position upstream of the protospacer adjacent motif (PAM) sequence (*11*), except for PmCDA1 or dCas9 alone (Fig. 1C and table S1), which indicates that the steric effect of the protein complex affects the efficiency but not the location of the mutation. This also suggests that a limited region of DNA is made accessible for PmCDA1 by dCas9-gRNA. Most mutations were found as point mutations at a G and C, which suggests that deamination occurred at the noncomplementary strand of the C (table S1). The fusion protein with the extended linker version (Fig. 1B, 6) was used in the following studies because of its simplicity.

DNA nicking promotes deamination-mediated mutagenesis

Because nucleotide deamination also occurs spontaneously, cells are capable of correcting most deaminated bases, and only a small portion of deamination normally results in mutagenesis (*7*). To exploit the full potential of Target-AID, we sought ways to bypass the nucleotide repair pathway without compromising overall genome integrity. Because the repair pathway requires base excision and template-dependent polymerization of nucleotides, we hypothesized that the introduction of a single-strand break (nick) in the DNA close to the deaminated site may perturb the repair system and result in a greater rate of mutagenesis. The Cas9 protein contains two nuclease domains, and each cleaves the opposite strand of DNA independently (*11*). Substituting aspartic acid with alanine in position 10 and histidine with alanine at position 840 prevents cleavage of the noncomplementary and complementary strands of DNA, respectively, to produce the Cas9 nickases nCas9(D10A) and nCas9(H840A) (*11*). Both nickases alone demonstrated a modest increase in the specific mutation rate, whereas the fusion protein of nCas9(D10A) and PmCDA1 consistently demonstrated an almost saturating mutation frequency (Fig. 2A), indicating that introducing nick on the opposite strand of deamination site facilitates mutagenesis, either by promoting the repair of the nicked, nonedited strand using the edited strand as a template or by providing better accessibility for PmCDA1. The mutational spectrum of nCas9(D10A)-PmCDA1 was similar to that of dCas9-PmCDA1 and distinct from those of Cas9 nickases or the complete nuclease alone (fig. S1). nCas9(D10A) has been shown to induce C-T substitution on the noncomplementary strand of target sites in cancer cell lines that overexpress endogenous cytidine deaminases, suggesting that nCas9-induced base substitution is comediated by the deaminase activity (*14*). This is consistent with our observation that the single-stranded

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DNA formation by dCas9-gRNA is sufficient to recruit PmCDA1 (Fig. 1B, 3) and that the mutation is induced at C on the noncomplementary strand, which is facilitated by nickase activity of nCas9(D10A). The combination of nCas9(H840A) and PmCDA1 reduced mutation frequency, suggesting that nicking on the same strand of the deamination site may prevent the mutagenic effect, presumably by facilitating the excision of deaminated base. It also appeared that Target-AID, using nCas9(D10A) and PmCDA1, was much less toxic than the full CRISPR/Cas9 nuclease, which causes growth defects or cell death in yeast (Fig. 2B). The low efficiency of full Cas9 nuclease in yeast may be attributed to its toxicity. This facilitates emergence of "escaper cells" that have lost the system (*15*, *16*).

Mutation spectrum induced by Target-AID

To detect the presence of associated mutations, deep sequencing of the target-flanking region

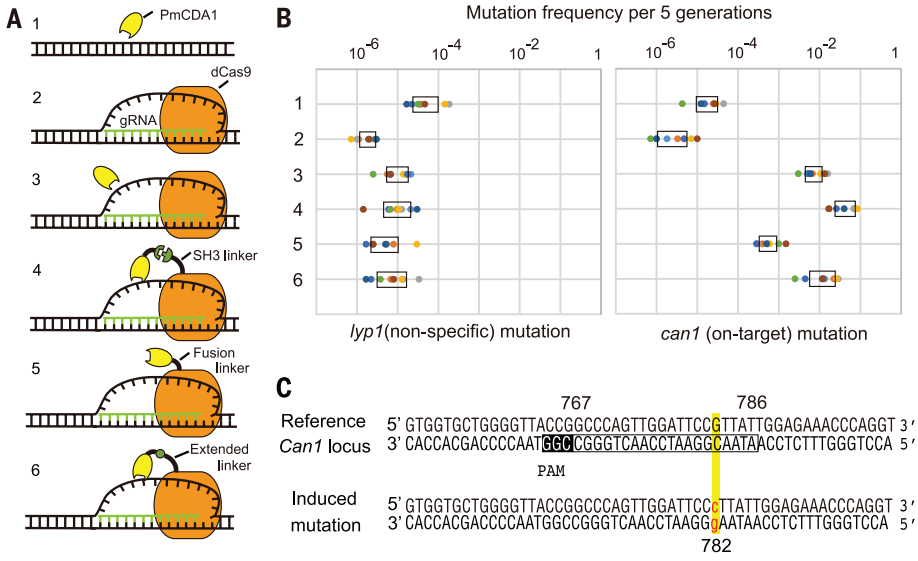

Fig. 1. Complex formation of dCas9-gRNA and PmCDA1 induces targeted mutation. (**A**) Schematics of the complex forms of dCas9, gRNA, the target DNA, and PmCDA1 examined in this study. (1) PmCDA1 alone. (2) dCas9-gRNA alone. (3) PmCDA1 and dCas9-gRNA without direct attachment. (4) PmCDA1-SHL attached to dCas9-SH3-gRNA via SH3 and SHL interaction (SH3 linker). (5) PmCDA1 fused to dCas9 with short linker (17 amino acids; NLS plus 2x glycine-serine peptide linker). (6) PmCDA1 fused to dCas9 with long linker (100 amino acids). (**B**) Yeast cells expressing each protein complex numbered as in (A), with gRNA targeting a single site (786 to 767) in CAN1, were analyzed by a colony formation assay. S-aminoethyl-L-cysteine and canavanine select for the cells with the *lyp1* mutation (nonspecific mutation) and the *can1* mutation (on-target mutation), respectively. Mutation frequency per five generations is plotted. Eight biological replicates are shown in different colors. Box bars indicate 95% confidence interval for a sample mean by *t* test statistical analysis. (**C**) Partial nucleotide sequence of CAN1 with the target site (box) and the PAM sequences (inverted) are indicated with open reading frame nucleotide number. Representative sequence of the canavanine-resistant mutants induced by Target-AID is aligned. Mutation site and mutated nucleotide are highlighted by yellow and red, respectively. A mutation list can be found in table S1.

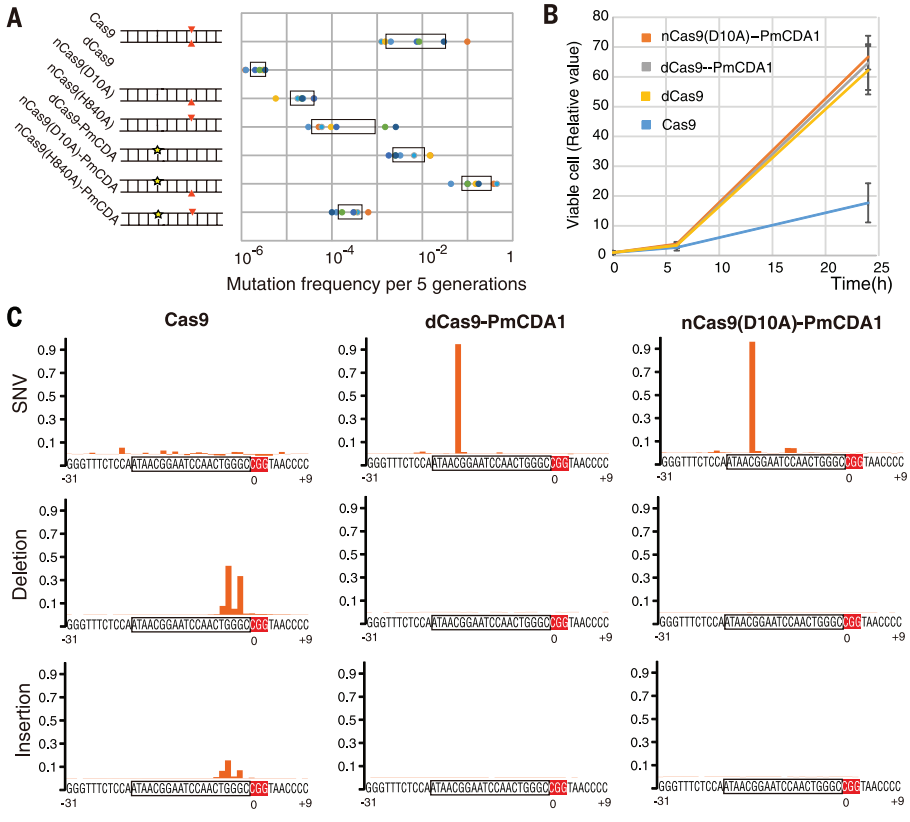

Fig. 2. Effect of nickase activity on Target-AID. (**A**) Forms of fusion protein of Cas9 nuclease mutants [Cas9, dCas9, nCas9(D10A), or nCas9(H840A)] and PmCDA1 are indicated on the left. The schematic illustration in the middle depicts the process of DNA modification by the fusion proteins. Ladder indicates target DNA. Red arrowhead indicates DNA-strand break introduction. Yellow star indicates cytosine deamination introduction. Yeast cells expressing each protein, with gRNA targeting a single site (791 to 772) in CAN1, were analyzed for canavanine resistance, and the mutation frequencies are plotted. Seven biological replicates are shown in different colors. Box bars indicate the 95% confidence interval for a sample mean by *t* test statistical analysis. (**B**) Yeast cells expressing Cas9, dCas9 dCas9-PmCDA1 or nCas9(D10A)-PmCDA1, and gRNA targeting CAN1 were measured for cell viability. Viable cells were counted and plotted based on colony formation at each time point. Relative values (1 at 0 hour) of the mean of the biological triplicate are plotted. Error bars indicate SD. (**C**) Mutation spectra of canavanine-resistant cells produced by Cas9, dCas9-PmCDA1, or nCas9-PmCDA1, analyzed by deep sequencing at the targeted genomic site (32681 to 32700 at chromosome V or 786 to 767 in Can1). Frequencies of SNVs and deletions or insertions at each nucleotide position were plotted from the −31 to the 9-bp position relative to target PAM sequence. The nucleotide sequence is shown with the target sequence (box) and the PAM sequence (inverted red).

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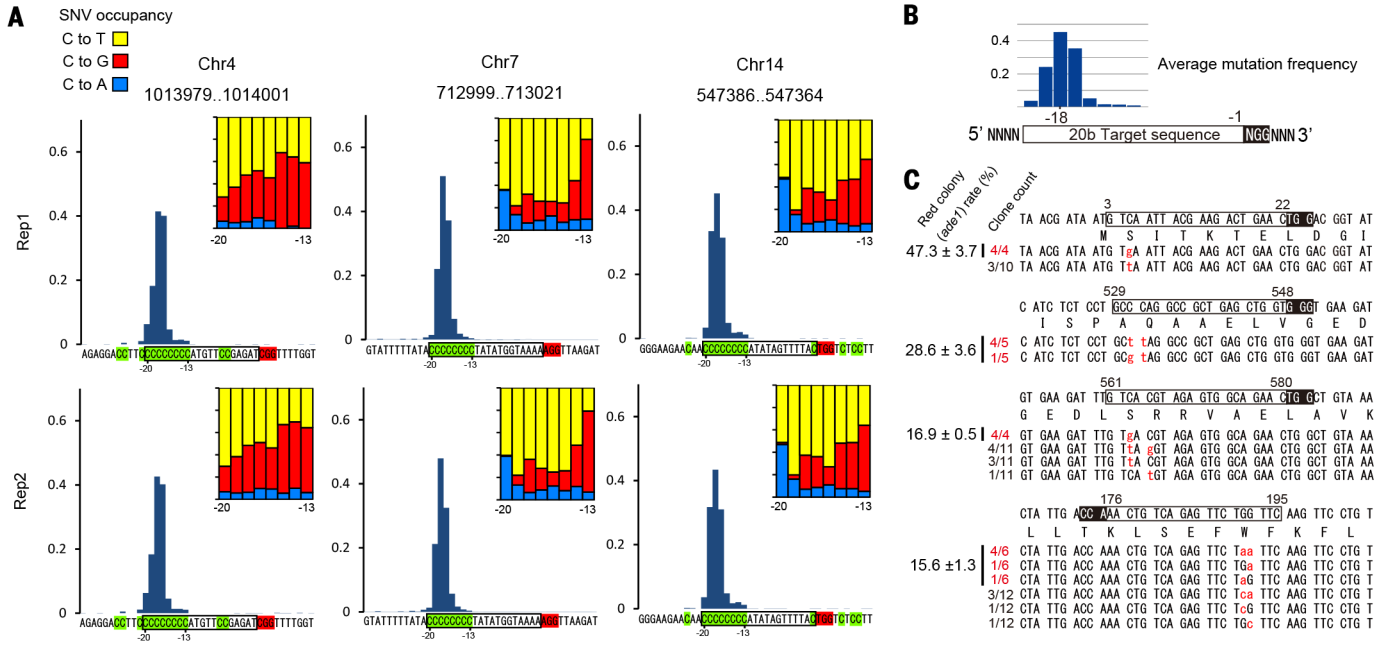

Fig. 3. Mutable position and target design for nCas9(D10A)-PmCDA1. (**A**) Mutation spectra of the targeted poly-C–containing sequences. Three intergenic target sequences that contain poly-C at 5′ half were edited by nCas9(D10A)-PmCDA1 and analyzed by deep sequencing. Frequencies of SNV at each position from −31- to 9-bp position relative to target PAM sequence were plotted. The upper right insets indicate the ratio of nucleotide changes at positions from −20 to −13 as C to T (yellow), C to G (red), and C to A (blue). Two biological replicates (Rep1 and Rep2) are shown. (**B**) Mutational position and frequency by Target-AID. Blue bars indicate average mutation frequency of A at each base position in the 20-base target sequence. (**C**) Four target sites in *Ade1* gene were selected for editing by Target-AID based on the criteria that the cytosine mutations in the −19 to −16 position may introduce a stop codon, resulting in a red colony phenotype. Red colony frequency (mean ± SD of biological triplicate) for each target site is indicated on the left. Sequences of identified mutations are aligned with the number of clones over the number of total sequenced clones for each colony color (red or none). Reference wild-type sequences with translated amino acid sequences are shown with the target site (box) and the PAM sequence (inverted). The bottom target sequence is complementary to the sequence shown.

was performed for canavanine-selected cells mutated at the *Can1* locus by Cas9, dCas9-PmCDA1, or nCas9-PmCDA1 (Fig. 2C). Cas9 induced deletions and insertions at the −1 to −5 positions. dCas9-PmCDA and nCas9-PmCDA induced a point mutation predominantly at the −16 position with a frequency of 94.7 and 96.1%, respectively. The second most frequent single-nucleotide variants (SNVs) were at the −22 position for dCas9-PmCDA and the −10 position for nCas9-PmCDA, with a frequency of 1.7 and 4.3%, respectively. Outside of the −22 to −10 position, no SNVs over 1% were detected for dCas9-PmCDA1, and one SNV at the +24 position for nCas9-PmCDA1 was found (1.2%). Insertions and deletions (indels) were rarely observed for both dCas9-PmCDA and nCas9-PmCDA with the highest frequency of 0.18 and 0.24%, respectively, at the −16 position.

The high efficiency of Target-AID using nCas9(D10A) and PmCDA1 allowed us to obtain mutants without canavanine-selection and to perform nonbiased sequencing analyses, which shows that the mutations were focused at cytosine bases located 15 to 19 bases upstream of the PAM sequence on the noncomplementary strand to gRNA, while the length of the gRNA target sequence (i.e., the 5′ end position of gRNA) did not affect mutation location (fig. S2). C to G and C to T mutations of the noncomplementary strand were observed with nearly equal frequency (9 of 18 and 8 of 18, respectively), whereas C to A was rare (1 of 18).

To obtain a comprehensive mutational spectrum for nCas9-PmCDA1, three intergenic target sites that were C-rich were selected (Fig. 3A). At the three targets, a peak of SNV frequency was consistently observed at the −18 position (41 to 51%), with lower frequencies at the −17 and −19 positions (18 to 40%). The SNV frequencies decreased at the −16 position (4.5 to 6.5%) and −20 position (1.3 to 6.3%) and further decreased in the surrounding area. Frequencies of more than 1% were rarely observed outside the −13 to −21 positions. This suggests that the mutational efficiency is highly dependent on the relative position within the target sequence. It also implies that processive deamination of poly-C by PmCDA1 is limited, if it occurs at all. On the other hand, changed nucleotides showed positional bias within the poly-C region (Fig. 3A, insets). C to T mutations were more common at the 5′ side of the poly-C region, whereas the C to G mutation ratio increased on the 3′ side. In *Saccharomyces cerevisiae*, the translesion DNA polymerase eta prefers to insert C opposite an abasic lesion, resulting in a G insertion at an abasic site (*17*), whereas a T insertion at an abasic site is common in most other organisms (*18*). The 3′ side of the poly-C region is more likely to be subjected to error-prone repair by the translesion polymerase eta, whereas mutations on the 5′ side are left as the deaminated base (i.e., uracil), which is then recognized as T in DNA synthesis.

To assess the general effectiveness of Target-AID, four sites were systematically selected from the *Ade1* gene [918 base pairs (bp)] to introduce a stop codon, following the criteria that C to G or C to T mutations should be introduced at −16 to −19 positions upstream of the PAM sequence (Fig. 3, B and C). The four targets resulted in an approximately 47, 29, 17, and 16% rate of *ade1* gene disruption (red colony phenotype), respectively (Fig. 3C). Sequencing analysis of both red and white colonies showed that the genetic mutation frequency was even greater because not all mutations resulted in gene disruption. All sequenced mutations were localized at the −16 to −18 position (Fig. 3C). There seems to be no strong context preference as reported previously for PmCDA1 (*19*).

Multiplex and biallelic editing by Target-AID

Multiplex editing was performed for the ADE1 and CAN1 genes by simultaneously expressing ADE1 and CAN1 targeting gRNAs. The independent mutation frequencies for ADE1 and CAN1 were ~54 and 51%, respectively, and double-phenotypic mutants occurred ~31% of the time (fig. S3A). Sequencing analysis revealed that all

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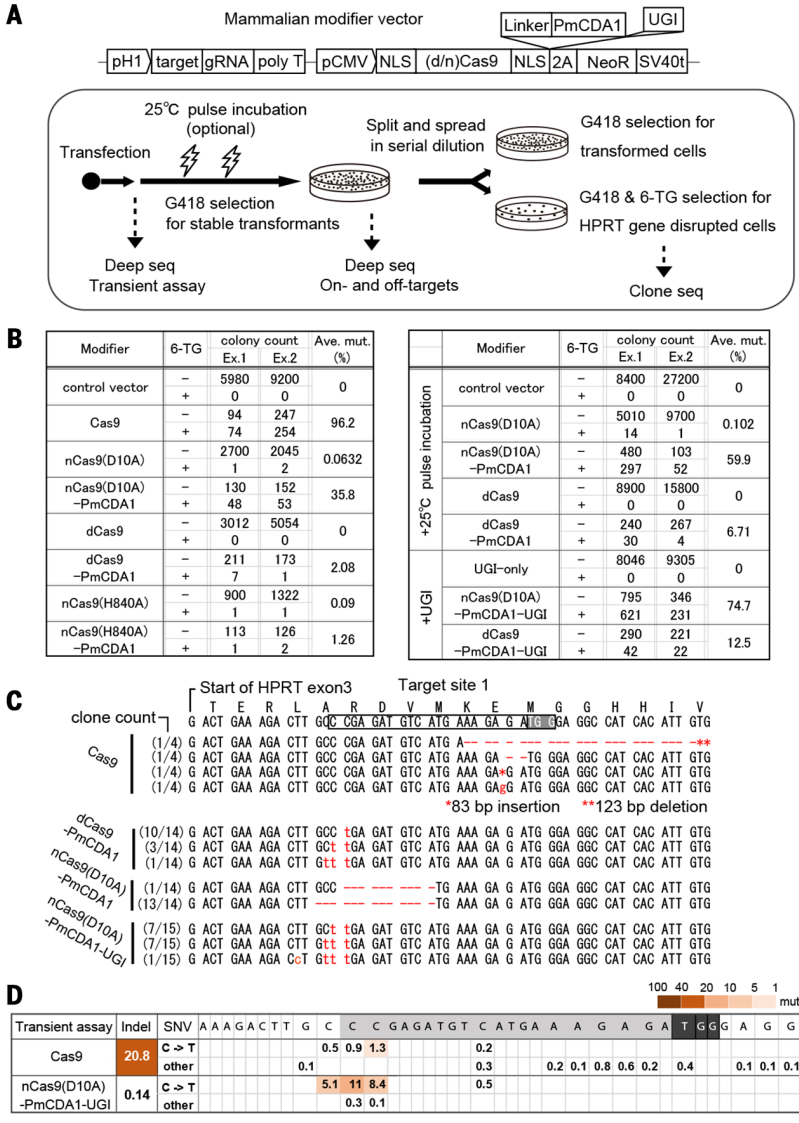

B

Modifier	6-TG	colony count Ex.1	colony count Ex.2	Ave. mut. (%)
control vector	−	5980	9200	0
	+	0	0	
Cas9	−	94	247	96.2
	+	74	254	
nCas9(D10A)	−	2700	2045	0.0632
	+	1	2	
nCas9(D10A) −PmCDA1	−	130	152	35.8
	+	48	53	
dCas9	−	3012	5054	0
	+	0	0	
dCas9 −PmCDA1	−	211	173	2.08
	+	7	1	
nCas9(H840A)	−	900	1322	0.09
	+	1	1	
nCas9(H840A) −PmCDA1	−	113	126	1.26
	+	1	2	

	Modifier	6-TG	colony count Ex.1	colony count Ex.2	Ave. mut. (%)
+25℃ pulse incubation	control vector	−	8400	27200	0
		+	0	0	
	nCas9(D10A)	−	5010	9700	0.102
		+	14	1	
	nCas9(D10A) −PmCDA1	−	480	103	59.9
		+	297	52	
	dCas9	−	8900	15800	0
		+	0	0	
	dCas9 −PmCDA1	−	240	267	6.71
		+	30	4	
+UGI	UGI-only	−	8046	9305	0
		+	0	0	
	nCas9(D10A) −PmCDA1-UGI	−	795	346	74.7
		+	621	231	
	dCas9 −PmCDA1-UGI	−	290	221	12.5
		+	42	22	

Fig. 4. Target-AID in mammalian cells. (**A**) Schematics of the mammalian experiment. The mammalian modifier vector expressing gRNA and the modifier protein is depicted on the top. Linker plus PmCDA1 is inserted before 2A peptide. UGI is also inserted between PmCDA1 and 2A peptide. Experimental flow is depicted at the bottom. CHO cells were transfected using each modifier vector and harvested after 3 days for transient assay or transferred to G418-containing media for selection of the transformed cells. Pulse incubations at 25°C are optionally introduced. After G418 selection, cells were analyzed by deep sequencing. For the HPRT gene target, cells were further tested for 6-TG resistance. The resistant cells were counted and then analyzed by Sanger sequencing. (**B**) 6-TG–resistant (HPRT null) frequencies induced by each modifier plasmid are shown. Cells were spread onto media with (+) or without (−) 6-TG in serial dilution, and resistant colonies were counted at optimal colony density for each sample. Experiment was repeated (Ex.1 and Ex.2) and combined to give average mutation frequency [Ave. mut (%)]. (**C**) Mutation alignment of the targeted HPRT gene locus. About 0.4-kb fragment around the target site of 6-TG resistant cells were PCR-amplified and subcloned for sequencing. Frequencies of the same mutant sequence were indicated as clone count over total reads of the same vector construct. Mutations are shown in red. Large deletion or insertion is indicated by an asterisk. (**D**) Mutation spectra obtained by transient transfection. Cells were transfected with a vector expressing Cas9 or nCas9(D10A)-PmCDA1-UGI and gRNA targeting HPRT. Whole-cell culture was subjected to deep sequencing without any selection or enrichment. Total indel frequency (Indel) within the indicated region (33 nucleotides surrounding the target site) and SNV frequencies above 0.1% at each nucleotide position are shown and highlighted as indicated at the top right. Target sequence is shadowed and PAM sequence is inverted.

clones (10 of 10) contained mutations at both sites, whereas phenotypic alterations were linked to specific types of mutations (fig. S3B).

Biallelic editing was performed by using yeast diploid strain YPH501 (fig. S4). Occurrence of biallelic disruption of Ade1 and Can1 were analyzed by their recessive phenotype of red colony and canavanine resistance, respectively. Biallelic mutants were obtained more frequently than expected if independent allelic mutagenesis was assumed (fig. S4A), whereas sequencing analysis verified heteroallelic mutant from phenotypically wild-type cells (fig. S4B). These imply that microhomology-mediated repair or gene conversion is induced during deamination-mediated mutagenesis, which results in a higher rate of biallelic mutagenesis (*20*).

gRNA mismatch tolerance of Target-AID

Off-targeting effect was assessed by introducing single mismatches at each base position of the gRNA sequence (fig. S5). Mismatches proximal to PAM sequence (3, 6, or 9 bases away) were not tolerated, whereas mismatches at 12 or 15 base position showed a reduced but detectable mutation rate (27- or 35-fold reduction, respectively). Mismatch at the 18 base position, where deamination is expected to occur on the opposite strand, was not tolerated. Mismatches at more than 20 base positions showed no additional effect. These results are consistent with Cas9 specificity and thus will be improved by Cas9 protein engineering (*21*).

Whole-genome sequencing of Target-AID–treated yeast

To further assess nonspecific mutator effects of the deaminase in this system, whole genome sequencing was performed for each of three clones expressing dCas9, nCas9, Cas9, PmCDA1, dCas9-PmCDA1, and nCas9-PmCDA1 (tables S2 and S3). Deaminase-expressing strains (PmCDA1, dCas9-PmCDA1, and nCas9-PmCDA1) were found to contain seven unique SNVs and two structural variants in a total of nine strains, whereas one SNV and three structural variants were found in a total of nine no-deaminase strains (dCas9, nCas9, and Cas9). A slight increase in SNVs in the deaminase-expressing strains may be attributed to the mutator effect of the deaminase. Overexpression of deaminases are mutagenic, and PmCDA1 expression in a yeast strain lacking uracil DNA glycosylase (ung1-) has been shown to induce genome-wide mutation (*20*). On the other hand, vertebrate cells that possess AID family proteins do not suffer from AID-related mutagenicity at wild-type expression levels. Compared with DNA double-strand breaks, DNA deamination is a more frequent spontaneous DNA lesion and much easier to repair precisely. Only intensive deamination or a compromised repair system may cause mutation. Cas9 expression did not show obvious off-target or nonspecific mutagenic effects in yeast, possibly because random DNA double-strand breaks may lead to cell death in yeast and thus be underrepresented.

DNA nicking plus deamination induces deletion in mammalian cells

To investigate the feasibility of Target-AID in mammalian cell, Chinese hamster ovary (CHO) cell was transfected using a vector expressing Cas9, nCas9 (D10A), nCas9(D10A)-PmCDA, dCas9-PmCDA,

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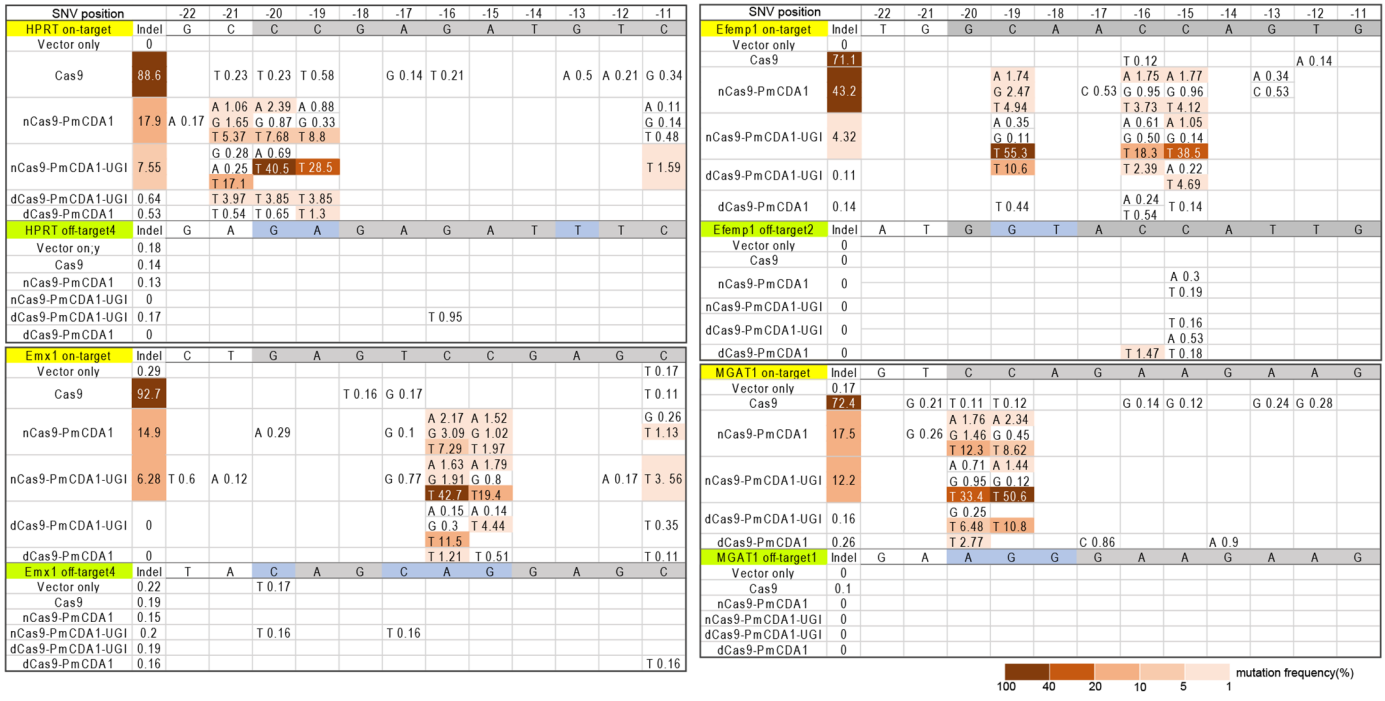

Fig. 5. Deep-sequencing analysis of target and off-target mutations induced by Cas9 and a series of Target-AID in CHO cells. Using the indicated modifier vector (left column) expressing gRNA targeting HPRT1, EMX1, Efemp1, or MGAT1, transformed cell culture was analyzed 7 days after transfection. The on-target site (yellow) and the top six off-target sites determined by CCtop (http://crispr.cos.uni-heidelberg.de/index.html) as listed in tables S5 to S8 were analyzed by deep sequencing. Full sets of off-target analysis are shown in figs. S5 to S8, whereas the most highly mutated off-target sites (green) for each target site are shown here. Target sequence and mismatched bases are highlighted in gray and light blue, respectively, with nucleotide position (−22 to −11) relative to PAM sequence on the top. There is no mismatch in the position from −12 to −1 of the all off-target sites examined. Total indel frequency within the 43 bases (20 bases of target region plus upstream 10 bases and downstream 13 bases, including PAM) is shown in the second column from the left. SNV frequencies above 0.1% at each nucleotide position (−22 to −11) are separately shown with each mutant nucleotides. Mutation frequencies are highlighted as indicated at the bottom right.

or nCas9(H840A)-PmCDA, with a gRNA targeting the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene. As HPRT converts a purine analog 6-thioguanine (6TG) into a toxic derivative, homozygous disruption of the HPRT gene confers 6TG resistance to the cell. After transfection, equal numbers of cells were spread and grown to form colonies in the presence or absence of 6TG to calculate mutation frequency (Fig. 4A). nCas9(D10A)-PmCDA1 induced a higher number of resistant colonies than dCas9-PmCDA1 (Fig. 4B). Sequencing analysis showed base substitutions for clones from dCas9-PmCDA1, as expected (Fig. 4C). However, nCas9(D10A)-PmCDA1 induced short deletions with a central focus on the 5′ distal side of the target sequence, where deamination was expected to occur. This is likely due to a combination of nick and deamination that cannot be repaired properly in mammalian cell.

Suppression of deletion by uracil DNA glycosylase inhibitor

To assess and bypass the repair pathway, we introduced uracil DNA glycosylase inhibitor protein (UGI) (*22*), which blocks removal of uracil in DNA and the subsequent repair pathway. UGI was expressed as a fusion protein with d/nCas9-PmCDA1 to demonstrate improved mutation frequency (Fig. 4B), as well as a substantial change in the mutation spectra, showing a majority of C to T substitutions instead of deletions (Fig. 4C, nCas9(D10A)-PmCDA1-UGI). Pulse incubations at 25°C after transfection improved the mutation frequency to 59.9 and 6.71% for nCas9-PmCDA1 and dCas9-PmCDA1, respectively (PmCDA1 is originally from sea lamprey, which is presumably adapted to low temperatures). Transient transfection was tested by harvesting the cell culture 3 days after transfection without any marker selection. Deep sequencing showed that nCas9-PmCDA1-UGI induced 8.4, 11, and 5.1% of C to T base substitutions at −19, −20 and −21 positions, respectively, whereas Cas9 induced 20.8% indels in the same condition (Fig. 4D).

Off-target assessment in mammalian cells

General effectiveness and potential off-target effect was assessed by using three target loci—MGAT1 (*23*), Efemp1 (*24*), and EMX1 (*25*)—that were previously tested as CRISPR/Cas9 targets in CHO cells or in human cells, in addition to HPRT locus. After G418 selection for transformants, cells were harvested and subjected to deep sequencing. Potential off-target genomic regions were selected using CCtop (http://crispr.cos.uni-heidelberg.de/index.html) (*26*) (tables S6 to S9), and the top six off-target sites were analyzed (Fig. 5 and figs. S6 to S9). Compared with nCas9-PmCDA1, which induced indels as well as SNVs, nCas9-PmCDA1-UGI effectively induced C to T mutations within the −15 to −21 region of the four target sites with a lower frequency of indels. dCas9-PmCDA1-UGI showed fewer indels and a moderate frequency of C to T mutations. There were possible off-target mutations detected at lower than 1.5% for Target-AID throughout the analyzed off-target sites (Fig. 5 and figs. S5 to S8).

Conclusion

We demonstrated targeted nucleotide substitution without the use of template DNA by expanding the potential of the CRISPR/Cas9 system. The Target-AID performed a very narrow range of targeted nucleotide substitution within a few bases. Its reduced cytotoxicity will be beneficial, especially for use in bacteria and other cell types that are sensitive to artificial nucleases (*16*). A combination of nickase Cas9(D10A) and PmCDA1 was highly efficient in yeast, whereas it induced deletions as well as point mutations in mammalian cells, suggesting that the mutation spectrum is affected by the difference of the repair pathway. The rat apolipoprotein B mRNA editing enzyme, catalytic polypeptide (rAPOBEC1), another cytidine deaminase, has also been reported to serve as a programmable base editor

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(BE) by fusing it to the N terminus of dCas9 (27). Consistent with our study, the use of UGI fusion and D10A nickase increased efficiency in human cells. There are also different features as well. Using Target-AID, three to five bases surrounding the −18 position upstream of the PAM sequence are substantially subjected to mutation, whereas five bases surrounding the −15 position upstream of PAM (or position 4 to 8 from the end distal to the PAM of a 20-base protospacer) are the major target for BE. This may be attributed to the difference of the enzymatic characteristics (PmCDA1 versus rAPOBEC1) or the different attachments to Cas9 (C terminus versus N terminus). Possible RNA-editing activity is also to be assessed, because APOBEC proteins have RNA-editing activity physiologically (2). Additionally, PmCDA1 exhibited temperature sensitivity that can be exploited to control its activity. Although direct comparison will be needed, these two systems may complement each other to extend the repertoire of possible editing sites. Using other CRISPR-related systems or other modifier enzymes, such as adenosine deaminase, will also broaden the editing capacity and further enrich the genome editing toolbox.

Materials and methods

DNA manipulation

Plasmids available from Addgene are listed in table S4. The human-optimized *Streptococcus pyogenes* Cas9 gene-containing plasmid p415-GalL-Cas9-CYC1t and the chimeric gRNA–containing plasmid p426-SNR52p-gRNA.CAN1.Y-SUP4t (13) were kindly provided by Church laboratory and obtained from Addgene (Cambridge, MA, USA). Nuclease-deficient Cas9 D10A and H840A mutations were introduced using a PCR-based method. The target gRNA sequences were replaced using a PCR method. A glycine-serine peptide linker (GGGGS), a SH3 domain, and a 3xFlag tag (DYKDHDGDYKDHDIDYKDDDDK) were added at the C terminus after the SV40 nuclear localization signal (NLS) (PKKKRKV) in Cas9. The DNA coding sequences for PmCDA1, the SH3 domain, and the 3xFlag tag were synthesized (Eurofin Genomics, Tokyo, JPN). The DNA fragments were assembled and modified using standard methods. The nucleotide sequence for the entire coding region is in fig. S10.

For CHO transfection plasmid, human codon-optimized PmCDA1 was synthesized on pcDNA3.1 backbone (Genscript, NJ, USA). Human-optimized Cas9 coding sequence was inserted downstream of CMV promoter, followed by SV40 NLS, dead SH3 domain, 3xFlag, PmCDA1, and UGI by PCR sewing and Gibson assembly method. Neomycin-resistant gene was linked via 2A peptide (EGRGSLLTCGDVEENPGP) at the end of the coding region. The nucleotide sequence for the entire coding sequence is in fig. S11.

Targeted mutagenesis

Saccharomyces cerevisiae BY4741 (*MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0*) (Open Biosystems, Huntsville, AL, USA) was used in this study. Yeast culture and transformation were performed according to standard methods. For the mutational analysis, the yeast cells were grown at 30°C in synthetic complete media with an indicated carbon source and an appropriate auxotrophic compound complemented by the plasmids. The cells were first inoculated in a 2% glucose medium and grown to saturation, diluted 16-fold into a 2% raffinose medium, and grown to saturation again. The cells were then diluted 16-fold or 32-fold into 2% raffinose medium plus 0.2 or 0.02% galactose medium for induction. To calculate the mutation rate per generation, cell culture was diluted 32-fold every 24 hours. After the indicated time, the cell culture was sampled and serially diluted 10-fold in water and spotted or spread onto a synthetic medium plate supplemented with the appropriate dropout mix and the appropriate drug. For CAN1 mutational analysis, arginine was omitted and 60 mg/l canavanine was added. For LYP1 mutational analysis, lysine was omitted and 100 mg/l of S-aminoethyl-L-cysteine was added. The mutation frequency of ADE1 was counted by colony color because the loss of function of ade1 results in the accumulation of red pigment when subjected to an adenine-limited condition. The plates were incubated at 28°C for 2 to 3 days and the colonies were counted. Sample size was determined based on countable colony number in a single spot (up to 40) or dish (up to 400). The *t* test statistical analysis was done using Excel software (Microsoft, WA, USA). The plate images were acquired using an Image Quant LAS 4000 (GE Healthcare Japan, Tokyo, Japan) or a Cybershot DSC-WX100 digital camera (Sony, Tokyo, Japan) and were processed using Adobe CS2 software. For the sequencing analysis, the average-sized colonies were randomly picked and directly amplified by PCR and were analyzed by Sanger sequencing using a 3130xL Genetic Analyzer (Applied Biosystems, CA, USA) according to the manufacturer's instructions.

Viability test

Yeast cells were precultured in synthetic complete media with 2% raffinose minus leucine and uracil for overnight, then diluted by 32-fold and supplemented with 0.02% galactose to induce protein expression. Cells were harvested at each time point. Ten-fold serial dilution of harvested culture was spotted onto synthetic complete media minus leucine and uracil and observed for colony formation for 2 days.

Mutation spectra analysis

Yeast strains harboring galactose-inducible modifier gene plasmid (Cas9, nCas9, dCas9, dCas9-CDA1, nCas9-CDA1, or CDA1) containing LEU2 marker and gRNA-expressing plasmid containing Ura3 marker were precultured in 5 ml SC–Leu–Ura+Ade media containing 2% galactose overnight at 30°C with rotation. Each cell sample was washed once with SC–Leu–Ura+Ade media containing 2% raffinose, resuspended in the same raffinose media at 0.1 OD600 nm, and incubated overnight at 30°C with rotation. For the modifier induction, cells were resuspended into SC–Leu–Ura+Ade media containing 2% galactose at 0.1 OD600 nm and incubated overnight at 30°C with rotation. Each sample was then spread on SC–Leu–Ura–Arg+Ade and SC–Leu–Ura–Arg+Ade containing 60 μg/ml canavanine plates. From each plate, colonies were scraped and pooled with sterile water and genomic DNA was extracted using YeaStar Genomic DNA Kit (Zymo Research). Target genomic locus was amplified by PCR with primers containing common sequence ends and reamplified by common Illumina adapter primers containing index tags for sample multiplexing. PCR bands were size-selected using E-Gel SizeSelect 2% Agarose Gel (Thermo Fisher Scientific) and quantified by KAPA Library Quantification Kits for Illumina (KAPA Biosystems). Sequencing libraries were multiplexed at equal molar ratio and analyzed by Illumina MiSeq Reagent Kit V2 (250×250 bp paired-end sequencing). Sequence reads were aligned to the primer sequences and the target genomic regions using BLAST+ program (short-read option; E-value cutoff of 1e−3) to demultiplex the read samples and to identify mutations and indels. Fractions of mutations, base insertions and deletions were separately calculated for every base position of target genomic region and subtracted by those of the PmCDA1 dataset derived from corresponding screening condition to normalize sequencing errors.

Whole-genome sequencing

Yeast strains harboring each expression construct (dCas9, nCas9, Cas9, PmCDA1, dCas9-PmCDA1, and nCas9-PmCDA1) with gRNA targeting Can1 were preincubated in 2% raffinose medium overnight, diluted by 32-fold into 0.02% galactose medium for induction and grown overnight. Cells were spread onto canavanine-containing plate medium to isolate single colonies. Each three independent colonies were inoculated into YPDA medium containing 300 ug/ml canavanine. Overnight culture was collected and genomic DNA was extracted using Wizard Genomic DNA purification Kit (Promega, WI, USA) following manufactures' instructions. Genomic DNA was then fragmented by sonication using Bioruptor UCD-200 TS Sonication System (Diagenote, NJ, USA) to obtain fragments with size distribution centering at 500 to 900 bp. Genomic DNA library was prepared by using NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, MA, USA) and labeled by Dual Index Primers Set 1. Size selection of the library was done using Agencourt AMPure XP (Beckman Coulter, CA, USA) to obtain tagged fragments with length ranging from 600 to 800 bp. Size distribution was evaluated by the Agilent 2100 Bioanalyzer system (Agilent Technologies, CA, USA). DNA was quantified using Qubit HS dsDNA HS Assay Kit and fluorometer (Thermo Fisher Scientific, MA, USA). Sequencing was performed using MiSeq sequencing system (Illumina, CA, USA) and MiSeq Reagent Kit v3 to obtain 2 × 300 bp read length, expecting approximately 30-fold coverage for the genome size. The sequence reads were paired and overlapping reads within a read pair were merged then trimmed based on a quality limit of 0.001

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with a maximum of 2 ambiguities. Reads were mapped to *Saccharomyces cerevisiae* S288C reference genome by the following setting (Masking mode = no masking, Mismatch cost = 2, Insertion cost = 3, Deletion cost = 3, Length fraction = 0.5, Similarity fraction = 0.9, Global alignment = No, Auto-detect paired distances = Yes, Nonspecific match handling = ignore). Local realignment was done with default settings (Realign unaligned ends = Yes, Muti-pass realignment = 2). InDels and Structural Variants were detected with the following settings (*P*-value threshold = 0.001, Maximum number of mismatches = 3, Filter variants = Yes, Maximum number of reads = 10). Local realignment was redone using the Variant track of InDels and Structural Variants and Force realignment to guidance-variant track. The variant calling was performed with the following settings (Ignore positions with coverage = 1000, Ploidy = 1, Required variant probability = 90, Ignore broken pairs = No, Ignore Nonspecific matches = Region length 50, Minimum coverage = 10, Minimum count = 2, Minimum frequency = 67%, Base quality filter = No, Read detection filter = Yes, Relative read direction filter = 1%, Significance = 1%, Read position filter = 1%, Remove pyro-error variants = No). Compare variant within group was done with threshold of 1% using the Variant Detection files of all three clones of 6 different modifiers (dCas9, nCas9, Cas9, PmCDA1, dCas9-PmCDA1, and nCas9-PmCDA1). Mutations appeared in both of two groups (d/n/wtCas9-only and PmCDA1-only) were assigned as common mutations.

CHO cell experiments

CHO-K1 adherent cells (ECACC 85051005) were used in this study. Cells were cultured in Ham's F12 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Biosera, Nuaille, France) and 100 μg/mL Penicilline-Streptomycin (Life Technologies, Carlsbad, CA, USA) at 37°C with a humidified 5% CO2 atmosphere. For the transfection, cells were plated at 0.5×105 cells per well of 24-well plates and cultured for 1 day. The cells were transfected with 1.5 μg plasmid and 2 μL Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) per well according to manufacturer's instructions. Five hours after transfection, the medium was changed to Ham's F12 medium containing 0.125 mg/mL G418 (InvivoGen, San Diego, CA, USA), and the cells were incubated for 7 days. For pulse incubation at 25°C, the cells were first transfected and incubated at 37°C for 1 day. Cells were then transferred to 25°C and incubated for 1 day, followed by 37°C incubation for 2 days. This process was repeated twice. To calculate the mutation frequency of the HPRT gene, the cells were released from the well using trypsin-EDTA (Life Technologies, Carlsbad, CA, USA) and serially diluted and spread to obtain countable colony density onto Ham's F12 medium containing G418 or G418 plus 5 g/mL 6-TG (Tokyo Chemical Industry, Tokyo, Japan). After 7 days, the number of resistant colonies was counted. The mutation frequency was calculated as the rate of 6TG resistant colonies over G418 resistant colonies. For sequencing analysis, cells were trypsinized and pelleted by centrifugation. Genomic DNA was extracted from the pellets using the NucleoSpin Tissue XS kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. PCR fragments including HPRT target site was amplified from the genomic DNA using a forward primer GGCTACATAGAGGGATCCTGTGTCA and a reverse primer ACAGTAGCTCTTCAGTCTGATAAAA. PCR products were cloned into *E.coli* vector and analyzed by Sanger sequencing.

Deep sequencing of target and off-target region of CHO cells

Target region-containing fragment (~1.5 kb) was first PCR-amplified using 1st primer pairs from the extracted genomic DNA. The second nested PCR was performed to obtain adapter-added amplicon (~0.3 kb) by using the first PCR products as template and 2nd primer pairs containing adapter sequences. The primes used in this study are listed in table S5. The amplicon was labeled using NEBNext Multiplex Oligos for Illumina (Index Primers Set 1 and Dual Index Primers Set 1) (New England Biolabs, MA, USA). Deep sequencing was performed using MiSeq sequencing system (Illumina, CA, USA) to obtain paired 300 bp read length and more than 100,000 reads per sample on average.

Data analysis was done using CLC Genomics Workbench 7.0 (CLC bio, Aarhus, Denmark). The sequence reads were paired and trimmed based on a quality limit of 0.05 with a maximum of two ambiguities and then overlapping reads within a read pair were merged. Reads were mapped to each reference sequence obtained from CHOgenome.org (www.chogenome.org/) by the following setting (Masking mode = no masking, Mismatch cost = 2, Insertion cost = 3, Deletion cost = 3, Length fraction = 0.2, Similarity fraction = 0.5, Global alignment = No, Auto-detect paired distances = Yes, Nonspecific match handling = ignore). The samples containing low-quality reads (Efem1 on-target, Efemp1 off-target 5, MGAT1 off-target 1 and EMX1 off-target 4) were reanalyzed by the following setting instead. The sequence reads were paired and overlapping reads within a read pair were merged then trimmed based on a quality limit of 0.01 with a maximum of 2 ambiguities. Reads were mapped with the following settings (Masking mode = no masking, Mismatch cost = 2, Insertion cost = 3, Deletion cost = 3, Length fraction = 0.6, Similarity fraction = 0.9, Global alignment = No, Auto-detect paired distances = Yes, Nonspecific match handling = ignore). The variant calling was performed with the following settings (Ignore positions with coverage = 1,000,000, Ignore broken pairs = No, Ignore Nonspecific matches = Region length 50, Minimum coverage =10, Minimum count = 2, Minimum frequency = 0.1%, Base quality filter = No, Read detection filter = Yes, Relative read direction filter = 1%, Significance = 1%, Read position filter = 1%, Remove pyro-error variants = No). Output file was rearranged using Excel (Microsoft, WA, USA).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S11
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RESEARCH ARTICLE

INFECTION

Virulence factors enhance *Citrobacter rodentium* expansion through aerobic respiration

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Citrobacter rodentium uses a type III secretion system (T3SS) to induce colonic crypt hyperplasia in mice, thereby gaining an edge during its competition with the gut microbiota through an unknown mechanism. Here, we show that by triggering colonic crypt hyperplasia, the *C. rodentium* T3SS induced an excessive expansion of undifferentiated Ki67-positive epithelial cells, which increased oxygenation of the mucosal surface and drove an aerobic *C. rodentium* expansion in the colon. Treatment of mice with the γ-secretase inhibitor dibenzazepine to diminish Notch-driven colonic crypt hyperplasia curtailed the fitness advantage conferred by aerobic respiration during *C. rodentium* infection. We conclude that *C. rodentium* uses its T3SS to induce histopathological lesions that generate an intestinal microenvironment in which growth of the pathogen is fueled by aerobic respiration.

Attaching and effacing (AE) pathogens are defined by virulence characteristics encoded by a pathogenicity island called the locus of enterocyte effacement (LEE) (*1*, *2*), which contains genes encoding a type III secretion system (T3SS) (*3*) and an adhesin termed intimin (*4*). The T3SS injects the intimin receptor Tir into the host cell cytosol (*5*), resulting in intimate attachment of bacteria to the effaced epithelial surface (*6*). The LEE-encoded T3SS of the AE pathogen *C. rodentium* injects additional effector proteins that are required for causing transmissible colonic crypt hyperplasia in mice (*7*, *8*) (fig. S1). After the development of colonic crypt hyperplasia, *C. rodentium* blooms in the lumen of the murine large bowel (*9*). The LEE-encoded T3SS is required for this rapid luminal expansion possibly by allowing *C. rodentium* to compete with the microbiota for carbon sources, because the T3SS provides no benefit in germ-free mice (*10*). These data suggest that the T3SS places *C. rodentium* in a microenvironment that somehow provides a growth advantage during its competition with the resident microbiota, but it remains obscure which resources might become available in this niche to fuel pathogen expansion.

Nitrate respiration is dispensable for pathogen growth during inflammation

Electron acceptors, such as nitrate, are produced as a by-product of the inflammatory host response and boost luminal growth of pathogenic *Salmonella enterica* or commensal *Escherichia coli* by anaerobic respiration in mouse models of colitis (*11*–*13*). Because *C. rodentium* infection triggers colonic crypt hyperplasia, we wanted to determine whether the inflammatory host response would enable the pathogen to grow by anaerobic respiration. The respiratory reductases for nitrate, dimethyl sulfoxide (DMSO), and trimethylamine *N*-oxide (TMAO), as well as the formate dehydrogenases FdnGHI and FdoGHI, contain a molybdopterin cofactor. Thus, to explore a possible role of anaerobic respiration during *C. rodentium* growth in the mouse gut, we constructed a *C. rodentium* mutant lacking a gene required for molybdopterin cofactor biosynthesis (*moaA* mutant) (fig. S2A) (*14*). Mice (C57BL/6) were inoculated with an equal mixture of wild-type *C. rodentium* and an isogenic *moaA* mutant to compare the fitness of both strains. Mice developed intestinal inflammation as indicated by increased transcript levels of proinflammatory markers in the colonic mucosa (fig. S3, A and B). The *C. rodentium* wild type was recovered in significantly ($P < 0.05$) higher numbers than the *moaA* mutant (Fig. 1A). Similar results were observed with genetically susceptible C3H/HeJ mice that experience more severe intestinal inflammation during *C. rodentium* infection (fig. S3, C and D). In contrast, when germ-free mice were inoculated with an equal mixture of the *C. rodentium* wild type and a *moaA* mutant, both strains were recovered in similar numbers (Fig. 1B and fig. S3E), suggesting that either anaerobic respiration or the utilization of formate provided an edge during competition of the pathogen with the resident microbiota.

C. rodentium infection resulted in a markedly increased colonic expression of *Nos2* (fig. S3, B and D), the gene encoding inducible nitric oxide synthase (iNOS), an enzyme necessary for generating nitric oxide, which is converted to nitrate in the intestinal lumen (*11*). To determine whether nitrate respiration enhances growth, we constructed a mutant deficient for the three nitrate reductases encoded by *C. rodentium* (*narG napA narZ* mutant) (fig. S2, B and F). Notably, in mice inoculated with an equal mixture of the *C. rodentium* wild type and a *narG napA narZ* mutant, both strains were recovered in equal numbers from colon contents and feces (Fig. 1A and fig. S3, C and E), suggesting that nitrate respiration did not provide a fitness advantage.

We next examined the possibility that the phenotype of the *moaA* mutant was due to an inability to utilize formate as an electron donor rather than nitrate as an electron acceptor. Because formate dehydrogenases FdnGHI and FdoGHI can couple electron transfer from formate through the quinone pool to nitrate (*15*) or oxygen (*16*), respectively, we envisioned that analysis of this pathway would provide indirect information about the electron acceptor used by *C. rodentium* in the gut. To test whether the nitrate-dependent and/or the oxygen-dependent formate dehydrogenase contributed to growth of *C. rodentium* during colitis, we inoculated mice with an equal mixture of the *C. rodentium* wild type and either an *fdnG* mutant or *fdoG* mutant (fig. S2, C and D). Consistent with our observation that nitrate respiration did not contribute to fitness (Fig. 1A), inactivation of the nitrate-dependent *fdnG* did not reduce growth of *C. rodentium* (Fig. 1B). However, inactivation of the oxygen-dependent *fdoG* reduced fitness of *C. rodentium* in conventional (C57BL/6) mice ($P < 0.05$), whereas little benefit was observed in germ-free (Swiss Webster) mice (Fig. 1B and fig. S3E). The finding that *fdoG* was required for pathogen expansion was intriguing, because it suggested that *C. rodentium* might utilize oxygen as a terminal electron acceptor during luminal growth.

Aerobic respiration drives a luminal pathogen expansion

To test the hypothesis that aerobic respiration fuels *C. rodentium* growth during infection, we deleted the *C. rodentium cydAB* genes (fig. S2E), which encode a high-affinity cytochrome bd oxidase required for aerobic respiration in microaerobic environments. In vitro, the *C. rodentium* wild type was recovered in higher numbers than the *cydAB* mutant when both strains were cocultured under conditions mimicking hypoxia (1% O_2) or normal tissue oxygenation (4 or 8% O_2), but not under anaerobic conditions (0% O_2) or at atmospheric oxygen levels (21% O_2) (Fig. 1C). Next, we compared the fitness of the *C. rodentium* wild type and a *cydAB* mutant by infecting C57BL/6 mice with a 1:1 mixture of both strains. Notably, the *C. rodentium* wild type was recovered in 20,000-fold higher numbers ($P < 0.01$) than the *cydAB* mutant from colon contents 7 days after infection (Fig. 1D), suggesting that aerobic respiration contributed to growth of the pathogen. Expression of *ler*, encoding the master regulator of T3SS synthesis, was reduced in the *cydAB*

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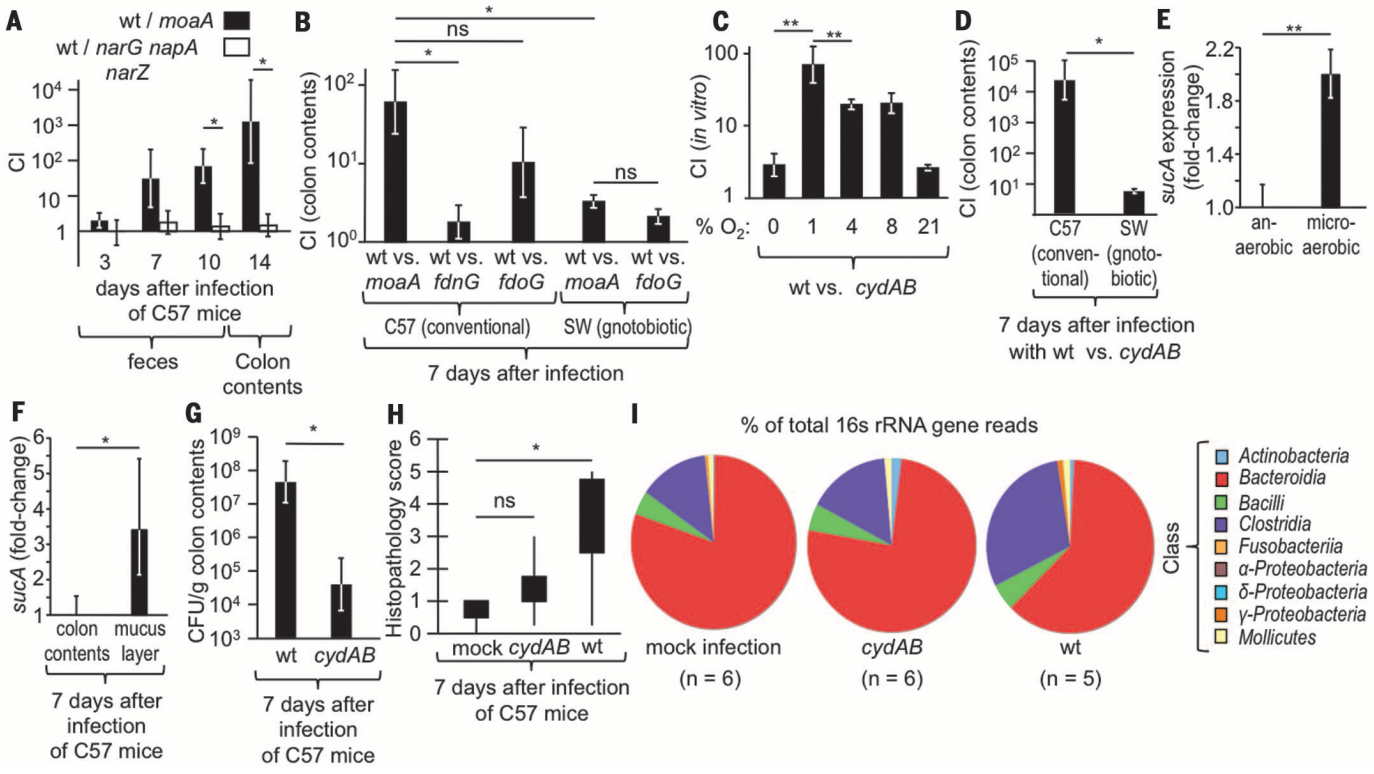

Fig. 1. Oxygen respiration supports *C. rodentium* expansion in the mouse colon. (**A**) C57BL/6 (C57) mice were infected with *C. rodentium* wild type (wt, DBS100) and either a *moaA* mutant (CAL142) or a *napA narG narZ* mutant (CAL93). (**B**) Conventional C57 or germ-free Swiss Webster (SW) mice were infected with wt *C. rodentium* and either a *moaA* mutant, a *fdnG* mutant [CAL210 (pWSK129)], or a *fdoG* mutant (CAL261). *N* is indicated in fig. S3E. (**C**) Competitive in vitro growth (*N* = 8 mice) of *C. rodentium* wild type (wt) and a *cydAB* mutant (CAL247) for 16 hours in minimal medium in the presence of the indicated oxygen levels (% O_2). (**D**) Conventional or germ-free mice were infected with an equal mixture of the *C. rodentium* wild type (wt) and a *cydAB* mutant. (A and D) *N* = 4 mice. (**E**) *C. rodentium* was grown in minimal medium supplemented with mannose as a carbon source under either microaerobic or anaerobic conditions. (**F**) Bacterial RNA was isolated from either mucus scrapings or colon contents of *C. rodentium*–infected mice. (E and F) The transcript levels of *sucA* were quantified by real-time polymerase chain reaction, normalized to 16*S* ribosomal RNA (rRNA) levels, and shown as fold-changes. *N* is shown in fig. S4, B and C. (**G** and **I**) Mice [*N* indicated in (I)] were either mock-treated or infected with the *C. rodentium* wild type (wt) or a *cydAB* mutant (CAL247). (G) Enumeration of *C. rodentium* by plating on selective media. (A to G) Bars represent geometric mean ± SE. (**H**) Boxes in whisker plots represent the second and third quartiles of combined histopathology scores, while lines indicate the first and fourth quartiles. (I) Microbial representation at the class level based on 16*S* rRNA gene sequencing of colon contents 7 days after infection. Color coding for classes is shown on the right. **P* < 0.05; ***P* < 0.01; ns, not statistically significantly different.

mutant, but this difference did not reach statistical significance (fig. S4A). When infection with a 1:1 mixture of the *C. rodentium* wild type and a *cydAB* mutant was repeated in germ-free (Swiss Webster) mice, the fitness advantage provided by *cydAB* was greatly reduced (Fig. 1D). Collectively, these data suggested that aerobic respiration drove an uncontrolled luminal expansion of *C. rodentium* in an environment occupied by the gut microbiota.

To test whether tissue- and mucus-associated bacteria have a respiratory metabolism compared to luminal bacteria, we investigated transcription of the *sucA* gene, which encodes a subunit of 2-ketoglutarate dehydrogenase, an enzyme required for the aerobic tricarboxylic acid (TCA) cycle. Under anaerobic conditions, SucA is no longer required as the normally cyclic TCA pathway switches to a noncyclic series of reactions. Higher expression of *sucA* under aerobic than anaerobic conditions can serve as an indicator for a respiratory central metabolism (*17*). *C. rodentium* *sucA* was transcribed at significantly ($P < 0.05$) higher levels when bacteria were cultured in vitro under microaerobic conditions compared to anaerobic conditions (Fig. 1E and fig. S4B). To investigate *sucA* expression in vivo, we isolated RNA from mucus scrapings or colon contents of mice infected with *C. rodentium*. *C. rodentium* residing in close proximity to tissue (i.e., mucus scrapings) transcribed *sucA* at significantly ($P < 0.05$) higher levels than bacteria located in colon contents (Fig. 1F and fig. S4C). These results provided further support for the idea that bacteria in close proximity to the mucosal surface had an oxidative metabolism in vivo.

Intestinal inflammation caused by *Salmonella enterica* drives a depletion of *Clostridia*, which in turn increases epithelial oxygenation (*18*). We thus wanted to determine whether *C. rodentium*–induced colonic crypt hyperplasia would increase oxygen availability in the gut by driving a depletion of *Clostridia*. The numbers of *C. rodentium* recovered from colon contents of mice infected with the *C. rodentium* wild type were 1000-fold higher ($P < 0.05$) than those recovered from colon contents of mice infected with a *cydAB* mutant (Fig. 1G), which correlated with reduced colonic inflammation ($P < 0.05$) (Fig. 1H and fig. S5). Consistent with a previous report (*9*), infection of mice with the *C. rodentium* wild type resulted in a shift in the microbial community structure (Fig. 1I and fig. S6). The absolute abundances of members of the *Enterobacteriaceae* (fig. S7A) and *Erythrobacteraceae* (fig. S7B) were significantly ($P < 0.05$) increased in mice infected with the *C. rodentium* wild type, a shift in the community structure previously associated with increased oxygen availability in the gut (*19*). However, infection with the *C. rodentium* wild type resulted in an increased ($P < 0.05$) abundance of *Clostridia* (Fig. 1I), which was most pronounced for members of the *Lachnospiraceae* belonging to the genus *Dorea* (fig. S7C) and the genus *Coprococcus* (fig. S7D). These changes in the microbial population profile were reduced or absent in mice

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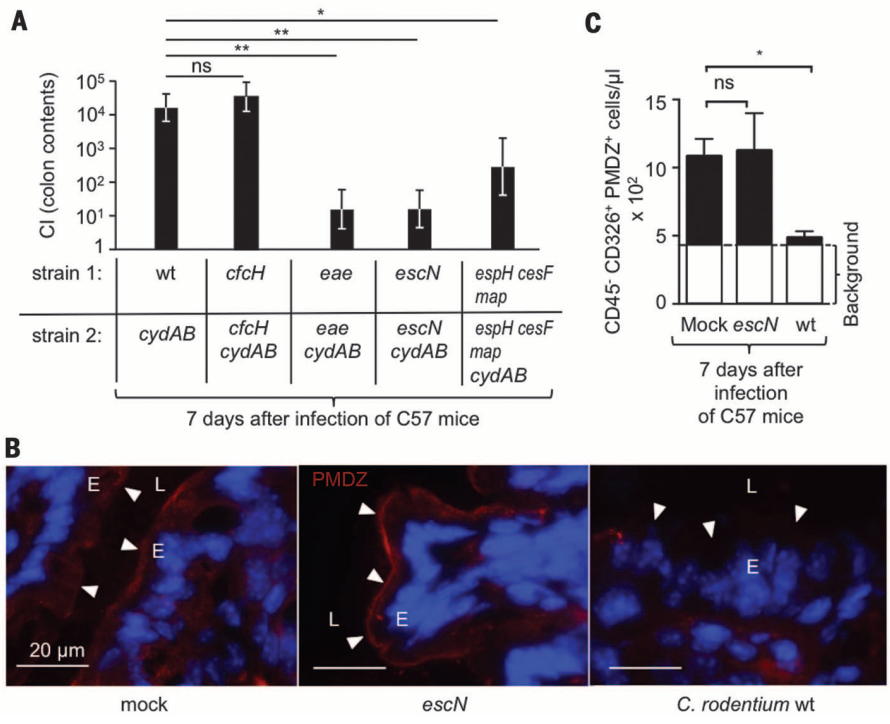

Fig. 2. A functional T3SS increases epithelial oxygenation and drives a CydAB-dependent *C. rodentium* expansion. (**A**) Mice (*N* is indicated in fig. S8A) were infected with the indicated *C. rodentium* strain mixtures. Bars represent geometric means of the competitive index (CI) ± SE. (**B**) Representative images of colonic sections stained to detect pimonidazole hypoxia stain (red fluorescence) and counterstained with DAPI (4′,6-diamidino-2-phenylindole) nuclear stain (blue fluorescence). Arrowheads point to the mucosal surface. E, epithelial cell; L, lumen. (**C**) Pimonidazole staining ($PMDZ^+$) was quantified by flow cytometry in colonic epithelial ($CD45^-$ $CD326^+$) cells. White bars indicate the level of background staining observed in mice that were not injected with PMDZ. Bars represent geometric mean ± SE. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not statistically significantly different; wt, DBS100; *cydAB*, CAL247; *cfcH*, BMM12; *cfcH cydAB*, BMM11; *eae*, CAL290; *eae cydAB*, CAL291; *escN*, CAL286; *escN cydAB*, CAL287; *espH cesF map*, BMM30; *espH cesF map cydAB*, BMM40.

infected with a *cydAB* mutant (Fig. 1I and figs. S6 and S7). In conclusion, our data were not consistent with the hypothesis that colonic crypt hyperplasia increased oxygen availability by depleting *Clostridia*.

T3SS-mediated niche construction

C. rodentium attaches to murine colonic epithelial cells (colonocytes) using a type IV pilus encoded by the *cfc* operon (*20*). To test whether this adhesin contributed to establishing a niche in which the pathogen could grow by CydAB-mediated respiration, we constructed a *cfcH* mutant (fig. S1F). Genetic ablation of type IV pilus biosynthesis did not change the CydAB-dependent growth advantage 7 days after infection of mice (Fig. 2A and fig. S8A), possibly because a *cfcH* mutant still occupied a niche near the epithelium (fig. S8B).

C. rodentium intimately attaches to colonocytes in a T3SS-dependent fashion using intimin. To test whether the T3SS and intimin contributed to CydAB-dependent growth, we constructed mutations in *escN*, encoding a component of the T3SS, and *eae*, the gene encoding intimin (fig. S1G and S1H). The fitness advantage conferred by CydAB-dependent respiration 7 days after infection was significantly reduced in *C. rodentium* strains lacking *eae* or *escN* (Fig. 2A and fig. S8A), suggesting that in the absence of intimate attachment or a functional T3SS, oxygen respiration no longer conferred a marked fitness advantage. However, at 3 days after infection, when intestinal inflammation was just beginning to develop (fig. S8, C and D), inactivation of *escN* did not significantly reduce the fitness advantage conferred by the *cydAB* genes (fig. S8E). Because the *escN* mutation did not reduce the fitness advantage conferred by the *cydAB* genes in the absence of overt crypt hyperplasia (i.e., at 3 days after infection), we considered the possibility that enhanced access to oxygen might not depend on intimate attachment, but might require T3SS-mediated host responses.

Colonocytes mature as they migrate from the bottom of the crypt to the luminal surface. The mature surface colonocyte population functions in water absorption, which is driven by electrolyte transport (*21*). To energize absorption of electrolytes, mature colonocytes respire butyrate in their mitochondria (*22*), a process consuming oxygen and rendering the mucosal surface hypoxic (< 7.6 mmHg pO_2 or < 1% O_2) (*23*, *24*) (fig. S1). To investigate whether T3SS-induced colonic crypt hyperplasia, which developed by seven days after infection (fig. S9A and S9B), would alter epithelial oxygenation in vivo, we visualized the "physiologic hypoxia" of surface colonocytes using the exogenous hypoxic marker pimonidazole (*25*) (Fig. 2B). Notably, a lack of hypoxia staining at the mucosal surface suggested that infection with the *C. rodentium* wild type significantly ($P < 0.05$) reduced epithelial hypoxia by 7 days after infection (Fig. 2, B and C, and fig. S10), indicative of a marked increase in epithelial oxygenation. This increased epithelial oxygenation was T3SS-dependent, because it was not observed in mice infected with the *escN* mutant (Fig. 2B and 2C), which did not develop inflammation in the colon (fig. S8).

T3SS-induced colonic crypt hyperplasia is characterized by an excessive intestinal epithelial repair response [reviewed in (*26*)]. To further investigate the mechanism by which the T3SS increases epithelial oxygenation, we deleted the *map*, *cesF*, and *espH* genes to reduce damage to colonocytes (fig. S2H). Map and EspH are T3SS effector proteins that damage mitochondria (which might impair respiration of butyrate) or activate caspase-3 to induce cytotoxicity, respectively (*27*, *28*), whereas the *cesF* gene encodes a chaperone for the mitochondria-associated T3SS effector EspF (*29*). Deletion of the *map*, *cesF*, and *espH* genes significantly ($P < 0.05$) reduced the fitness advantage conferred by CydAB-dependent respiration 7 days after infection of mice (Fig. 2A), but it was not clear whether this was simply due to an overall lower level of colonization (fig. S8A). When mice were infected with either the *C. rodentium* wild type or an *espH cesF map* mutant, both strains were detected in association with the mucosal surface, although the *espH cesF map* mutant appeared impaired for colonization (fig. S11A) and the *C. rodentium* wild type was recovered in higher numbers than a *espH cesF map* mutant from colon contents 7 days after infection (Fig. 3A). Only infection with the *C. rodentium* wild type significantly reduced epithelial hypoxia, while the colonic surface remained hypoxic in mice infected with a *espH cesF map* mutant (fig. S11, B and C).

Whereas mature colonocytes at the luminal surface respire butyrate, undifferentiated colonocytes in the crypts exhibit the Warburg metabolism of dividing cells, which is characterized by fermenting glucose to lactate (*30*). *C. rodentium* infection induces epithelial regeneration and repair mechanisms, which drives a marked expansion of undifferentiated colonocytes, resulting in crypt elongation and the presence of undifferentiated colonocytes at the luminal surface (*31*, *32*) (fig. S1). Because the Warburg metabolism does not consume oxygen, we wanted to determine whether reduced hypoxia of surface colonocytes observed during *C. rodentium* infection (Fig. 2, B and C, and fig. S11, B and C) was due to an accumulation of undifferentiated colonocytes at the luminal surface. Infection with a *espH cesF map* mutant triggered significantly ($P < 0.05$) less mitotic divisions in the colonic epithelium than infection with wild-type *C. rodentium* (fig. S8C),

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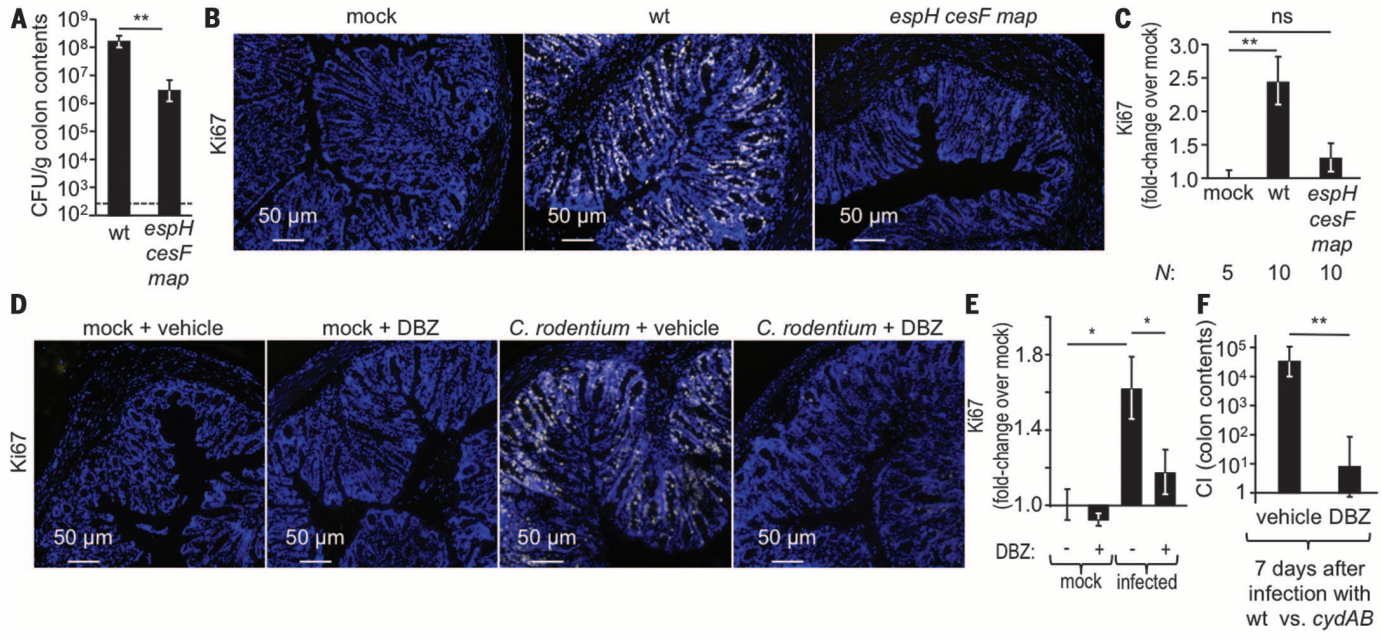

Fig. 3. Colonic crypt hyperplasia drives a CydAB-dependent *C. rodentium* expansion. (**A** to **C**) C57BL/6 mice were mock infected or infected with the *C. rodentium* wild type (wt, DBS100) or an *espH cesF map* mutant (BMM30). (**D** to **F**) C57BL/6 mice were mock infected or infected with a 1:1 mixture of the *C. rodentium* wild type (wt) and a *cydAB* mutant (CAL247). Mice were treated with either dibenzazepine (DBZ) or vehicle control. (A to F) Organs were collected 7 days after infection. (A) Colony-forming units (CFU) recovered from colon contents. (B and D) Representative images of colonic sections were stained to detect Ki67 (yellow fluorescence) and counterstained with DAPI nuclear stain (blue fluorescence). (C and E) Ki67 staining was quantified by image analysis. (F) Enumeration of wt and *cydAB* mutant recovered from colon contents was used to calculate the CI. (A, C, E, and F) Bars represent geometric mean ± SE. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not statistically significantly different.

suggesting that deletion of the *espH*, *cesF*, and *map* genes might reduce crypt hyperplasia. To test this idea, we stained colonic sections from mock-infected mice or mice infected with the *C. rodentium* wild type or an *espH cesF map* mutant with Ki67, a cellular marker for proliferation. Infection with the *C. rodentium* wild type induced a marked expansion of Ki67-positive colonocytes, resulting in crypt elongation, a thickening of the colonic mucosa, and an accumulation of Ki67-positive colonocytes along the length of the crypt and at the luminal surface (Fig. 3, B and C). This host response was blunted in mice infected with a *C. rodentium espH cesF map* mutant (Fig. 3, B and C), which correlated with increased epithelial hypoxia (fig. S11, B and C).

Colonic crypt hyperplasia drives aerobic pathogen expansion

We next wanted to determine whether colonic crypt hyperplasia was a driver of CydAB-dependent pathogen expansion. To this end, mice were mock infected or infected with a 1:1 mixture of the *C. rodentium* wild type and *cydAB* mutant and then treated with dibenzazepine (DBZ) or with vehicle control. DBZ is a Notch and Wnt (wingless-related integration site) signaling pathway inhibitor that prevents colonic crypt hyperplasia during *C. rodentium* infection (*31*). Ki67 staining suggested that mice infected with the *C. rodentium* strain mixture developed colonic crypt hyperplasia, which was significantly ($P < 0.05$) blunted by DBZ treatment (Fig. 3, D and E), although DBZ treatment did not reduce the severity of other histopathological changes (fig. S12, A and B). Notably, inhibition of colonic crypt hyperplasia by DBZ treatment abrogated the fitness advantage conferred by the *cydAB* genes (Fig. 3F), suggesting that crypt hyperplasia was a main driver of aerobic pathogen expansion.

Our finding that the LEE-encoded T3SS provides *C. rodentium* access to oxygen in vivo helps explain why the mechanism by which this trademark virulence factor confers a benefit to AE pathogens has not been apparent from in vitro studies (*33*, *34*), because such experiments are commonly performed in a 95% air (79% N_2/21% O_2) atmosphere, supplemented by 5% CO_2, thus providing 19.95% O_2 (150 mmHg). This oxygenation is considerably higher than normal tissue oxygenation (23 to 70 mmHg or 3 to 10% O_2) (*35*) or oxygenation of colonocytes in vivo (< 7.6 mmHg or < 1% O_2) (*23*, *24*). Although oxygen emanating from the mucosal surface is a very limited resource in the lumen of the large intestine (*36*), epithelial oxygenation was markedly elevated during T3SS-induced colonic crypt hyperplasia. As a result, T3SS-induced colonic crypt hyperplasia drove growth of *C. rodentium* through CydAB-mediated aerobic respiration, presumably because a respiratory metabolism enables the pathogen to utilize carbon sources more effectively than competing microbes that rely on fermentation for growth. In conclusion, our results revealed how histopathological changes triggered by a trademark virulence factor of AE pathogens create a unique nutrient niche to fuel an uncontrolled luminal expansion of *C. rodentium*.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S12
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REPORTS

QUANTUM SIMULATION

Site-resolved measurement of the spin-correlation function in the Fermi-Hubbard model

Maxwell F. Parsons, Anton Mazurenko, Christie S. Chiu, Geoffrey Ji, Daniel Greif, Markus Greiner*

Exotic phases of matter can emerge from strong correlations in quantum many-body systems. Quantum gas microscopy affords the opportunity to study these correlations with unprecedented detail. Here, we report site-resolved observations of antiferromagnetic correlations in a two-dimensional, Hubbard-regime optical lattice and demonstrate the ability to measure the spin-correlation function over any distance. We measure the in situ distributions of the particle density and magnetic correlations, extract thermodynamic quantities from comparisons to theory, and observe statistically significant correlations over three lattice sites. The temperatures that we reach approach the limits of available numerical simulations. The direct access to many-body physics at the single-particle level demonstrated by our results will further our understanding of how the interplay of motion and magnetism gives rise to new states of matter.

Quantum many-body systems exhibiting magnetic correlations underlie a wide variety of phenomena. High-temperature superconductivity, for example, can arise from the correlated motion of holes in an antiferromagnetic (AFM) Mott insulator (*1*, *2*). It is possible to probe physical analogs of such systems using ultracold atoms in lattices, which introduce a degree of control that is not available in conventional solid-state systems (*3*, *4*). Recent experiments exploring the Hubbard model with cold atoms are accessing temperatures where AFM correlations form but have only observed these correlations via measurements that were averages over inhomogeneous systems (*5*, *6*). The recent development of site-resolved imaging for fermionic quantum gases (*7*–*13*) provides the ability to directly detect the correlations and fluctuations present in a fermionic quantum many-body state. As demonstrated in experiments with both bosons (*14*, *15*) and fermions (*12*, *13*, *16*), microscopy gives access to the spatial variation in observables that occurs in an inhomogeneous system, yielding precise comparisons with theory. The low energy scales in cold-atom systems also allow for time-resolved observations of many-body dynamics, which typically occur on millisecond time scales. In bosonic systems, temporal and spatial resolution have been combined to observe strongly correlated quantum walks (*17*), to measure entanglement entropy (*18*), and to study the dynamics of magnetic correlations (*19*, *20*).

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Here, we report trap-resolved observations of magnetic correlations in a Fermi-lattice system. Fermionic atoms in a two-dimensional (2D) optical lattice can be well described by the Hubbard Hamiltonian, a simple model in which there is a competition between the nearest-neighbor tunneling energy t and the on-site interaction energy U. Despite the apparent simplicity of the Hubbard model, it has a rich phase diagram, containing, for example, the transition from a metal to a Mott insulator. AFM spin correlations begin to appear near half-filling when the temperature scale becomes comparable to the exchange energy, which in the strongly interacting regime is $J = 4t^2/U$. In the thermodynamic limit, what happens as the temperature is lowered further depends on the dimensionality of the system: In three dimensions, there is a finite-temperature phase transition to a state with long-range AFM order, whereas in two dimensions, such an order is prohibited by the Mermin-Wagner-Hohenberg theorem (*21*). Nonetheless, AFM correlations do arise, decaying exponentially with a correlation length ξ that diverges as the temperature goes to zero as $\xi \simeq \exp(\alpha J/T)$, where α is of order unity (*22*). We use quantum gas microscopy to reveal precisely these correlations, which for our finite-size 2D system are expected to lead to long-range order at a finite temperature, where ξ becomes comparable to the system size.

Our experiments begin with a low-temperature, 2D gas of fermionic ^{6}Li atoms in a mixture of the two lowest hyperfine states ($|\uparrow\rangle$ and $|\downarrow\rangle$), as described in (*12*). By adjusting a magnetic bias field in the vicinity of the Feshbach resonance located at 832G, we set the s-wave scattering length to $210a_0$, where a_0 is the Bohr radius (*23*). Using a 30-ms linear ramp, the atoms are adiabatically loaded into an isotropic, square optical lattice with a depth of $s_0 = 7.7(5)E_r$, where the recoil energy is $E_r/h = 25.6$kHz with Planck constant h. We detect magnetic correlations by removing atoms in either spin state and measuring the resulting charge correlations with site-resolved imaging (*24*), as shown in Fig. 1. Because our imaging technique removes doubly occupied sites, both doubly occupied and unoccupied sites show up as empty sites after imaging. However, proper linear combinations of the different particle and

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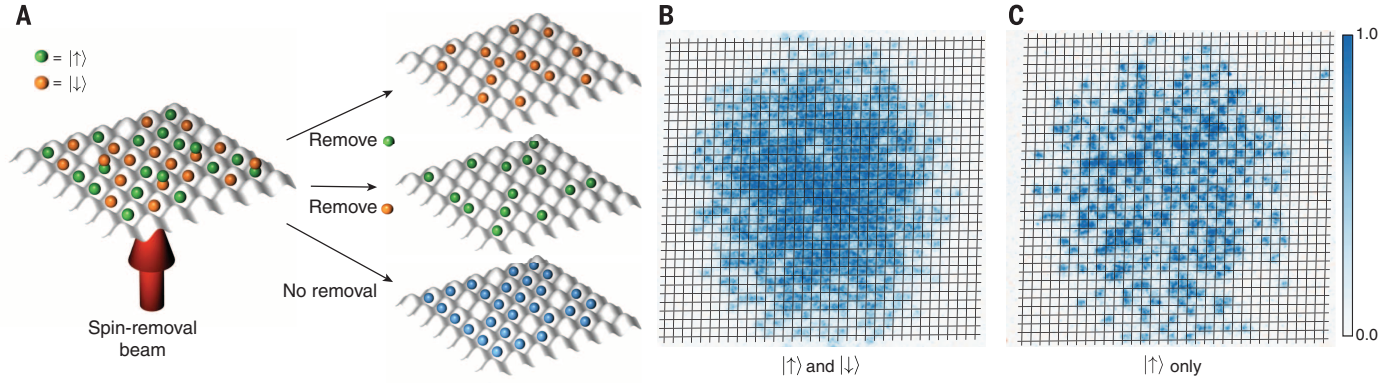

Fig. 1. Experimental technique for measuring spin correlations. (**A**) After loading the atoms into an optical lattice, we use a spin-removal technique to map the spin correlations onto charge correlations, which can then be detected using site-resolved imaging. The two spin states are denoted by green and orange balls. By driving cycling optical transitions for either spin state with the spin-removal beam, we can eject one spin state from the trap. We can then combine charge correlations measured in images where we remove each spin state and where no removal is performed to compute the local spin correlation (*24*). (**B**) A typical image where no atoms are removed. (**C**) A typical image with one of the spins removed. Atoms in doubly occupied sites are removed in both the spin-removal and imaging procedures as a result of light-assisted collisions.

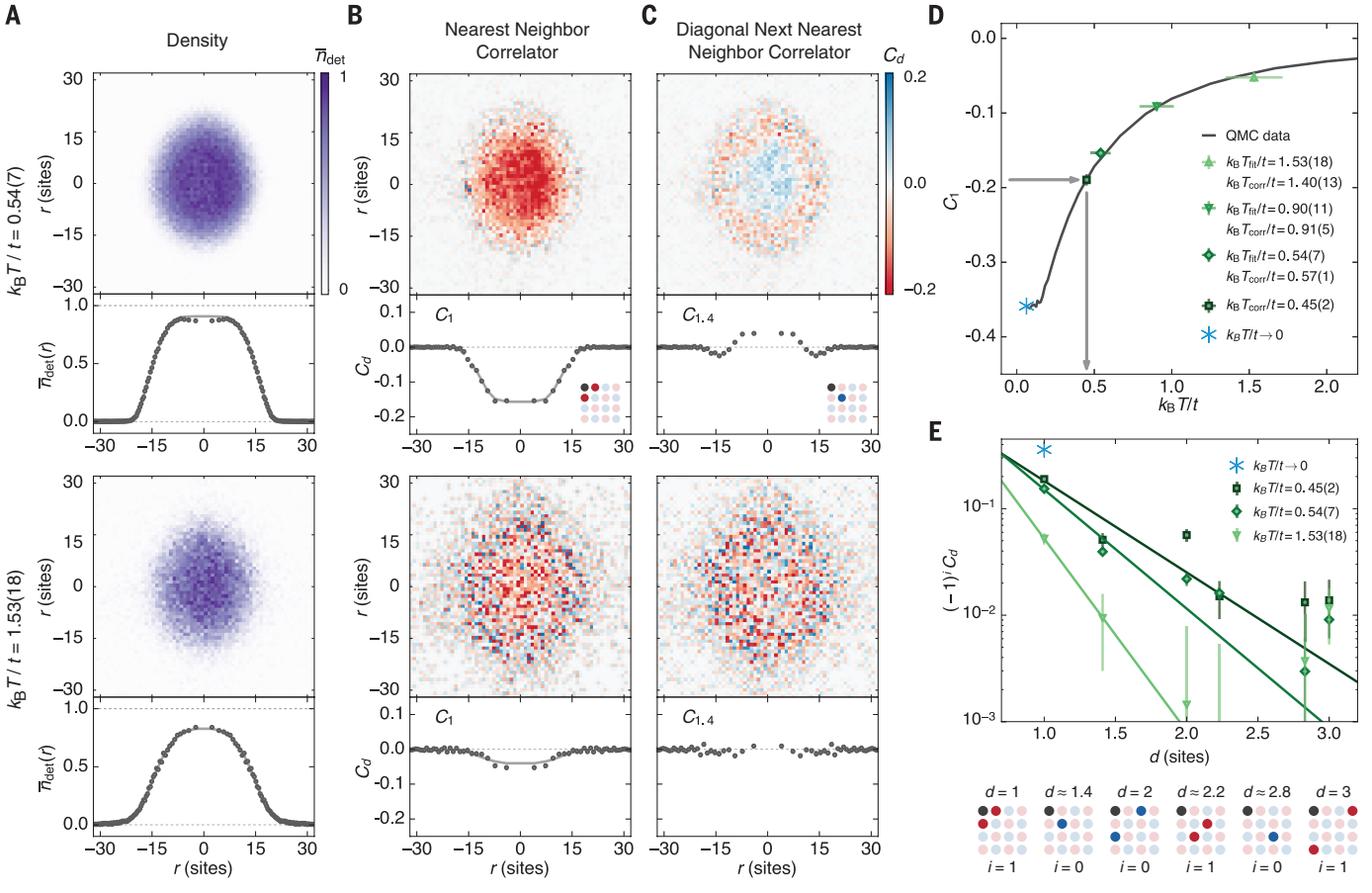

Fig. 2. Local observation of density and spin correlations. (**A** to **C**) Spatial maps and azimuthally averaged profiles (mirrored about $r = 0$ and corrected for ellipticity) of the detected density, nearest-neighbor correlator and diagonal next-nearest neighbor correlator for a cold (top) and hot (bottom) cloud. A combined fit determines the temperature T and chemical potential μ (solid lines). (**D**) Green symbols show the nearest-neighbor correlator in the center of the cloud for samples prepared at different temperatures. Listed are the values of k_BT_{fit}/t from fits of a numerical linked-cluster expansion to the radial profile and k_BT_{corr}/t obtained by comparing the central correlator value to a quantum Monte Carlo calculation at half-filling (solid line) (*22*). For the coldest data in (D) and (E) (squares), the NLCE theory error is too large for a fit, and we report only the QMC result. (**E**) An exponential fit to the correlator in the center of the cloud versus d allows us to extract the correlation length for data sets at three different temperatures, giving 0.24(9), 0.39(2), and 0.51(4) sites for decreasing temperature. The asterisk denotes the nearest-neighbor correlator value from the QMC calculation in (D) as $T \to 0$. Error bars on $\bar{n}_{det}(r)$ and $C_d(r)$ are standard errors after averaging at least 20 sets of combined correlation maps and averaging azimuthally (*24*). All data shown are at $U/t = 8.0(1)$. Horizontal errors in (D) are fit errors.

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hole correlators (measured both with and without spin-dependent removal) will account for the contribution to the signal from imperfect unity filling (*24*). From this, we determine the spin correlator (*24*)

$$C_{\mathbf{a}}(\mathbf{r}) = 4(\langle S^z_{\mathbf{r}} S^z_{\mathbf{r}+\mathbf{a}}\rangle - \langle S^z_{\mathbf{r}}\rangle\langle S^z_{\mathbf{r}+\mathbf{a}}\rangle) \quad (1)$$

Here, $S^z_{\mathbf{r}} = \frac{1}{2}(n^{\uparrow}_{\mathbf{r}} - n^{\downarrow}_{\mathbf{r}})$, with $n^{\sigma}_{\mathbf{r}}$ denoting the number of particles of spin σ on the site at **r**. We take an average of $C_{\mathbf{a}}(\mathbf{r})$ over all **a** where $|\mathbf{a}| = d$ to obtain $C_d(\mathbf{r})$. The nearest-neighbor, diagonal next-nearest-neighbor, straight next-nearest-neighbor, etc., correlators are given by $C_1(\mathbf{r})$, $C_{1.4}(\mathbf{r})$, and $C_2(\mathbf{r})$, etc. From images where neither spin was removed, we directly obtain a spatial map of the single-occupation probability $\overline{n}_{\mathrm{det}}(\mathbf{r})$, which also corresponds to the local moment $C_0(\mathbf{r})$.

After loading atoms into the lattice, we observe AFM correlations for nearest neighbors and diagonal next-nearest neighbors. These correlations are strongest in the cloud center, where the local chemical potential is set to approximately half-filling. The spatial maps $\overline{n}_{\mathrm{det}}(\mathbf{r})$, $C_1(\mathbf{r})$, and $C_{1.4}(\mathbf{r})$ for colder (top) and hotter (bottom) temperatures are shown in panels A, B, and C, respectively, of Fig. 2. For these data, the interaction is tuned to $U/h = 6.82(10)$kHz, with $t/h = 850(100)$Hz ($U/t = 8.0(1)$). The chemical potential is tuned to approximately $\mu = U/2$ in the center of the cloud for the colder data by adjusting the atom number to maximize $\overline{n}_{\mathrm{det}}$ in the center. In Fig. 2A, the suppression of $\overline{n}_{\mathrm{det}}$ in the center of the cloud is caused by the formation of doubly occupied sites and indicates that the chemical potential in the center of the cloud slightly exceeds $U/2$. To heat the cloud, we hold the atoms in the optical dipole trap for 4 s before loading the lattice. After heating, the maximum detected occupation decreases from 0.89(1) to 0.84(1), with a slight broadening of the density profile, whereas the largest magnitude of the nearest-neighbor correlator decreases from 0.154(3) to 0.052(6). In this regime, where the exchange energy is much smaller than both U and the bandwidth, an increase in temperature quickly saturates the entropy available in the spin degree of freedom while creating little entropy in the charge degree of freedom, making the nearest-neighbor correlator much more sensitive than the density to temperature changes. For the colder data, we observe significant negative correlations in $C_{1.4}(\mathbf{r})$ away from half-filling, which requires further theoretical investigation.

We take azimuthal averages along the equipotentials of the underlying harmonic trap to obtain $\overline{n}_{\mathrm{det}}(r)$ and $C_d(r)$. The resulting profiles are simultaneously fit to the results of a numerical linked-cluster expansion (NLCE) of the 2D Hubbard model under a local density approximation (LDA) (*24*–*26*). From these fits, we obtain a temperature $k_BT/t = 0.54(7)$ and chemical potential $\mu/U = 0.52(1)$ for the cooler data and $k_BT/t = 1.53(18)$ and $\mu/U = 0.33(1)$ for the hotter data. The excellent agreement with theory provides a strong indication that the local density approximation and the assumption of thermal equilibrium are valid.

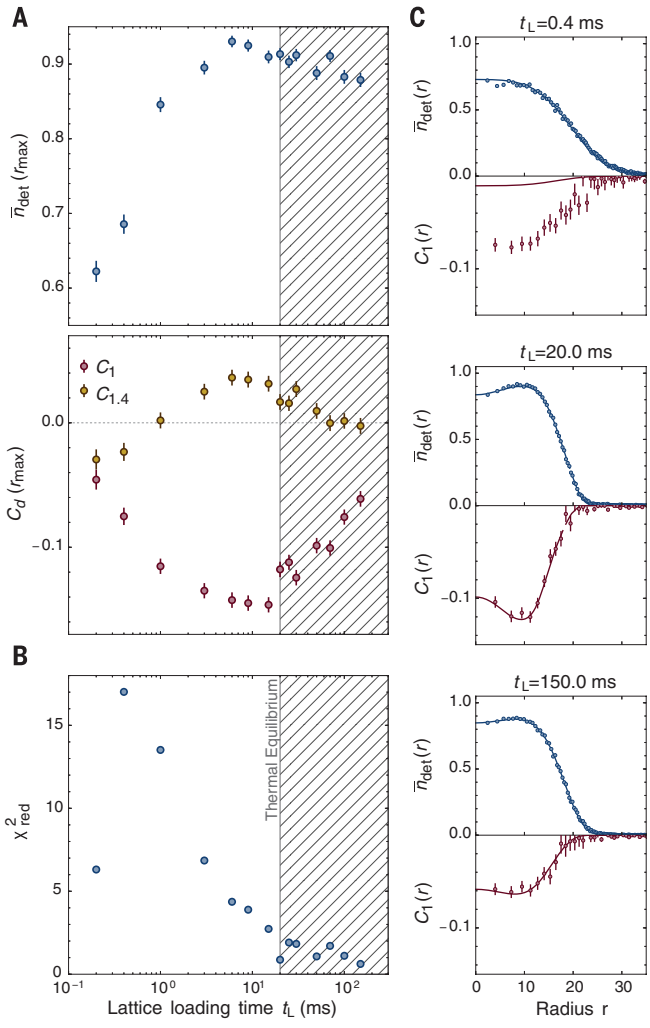

Fig. 3. Thermalization dynamics during lattice loading. (**A**) (Upper) Detected density. (Lower) The nearest- and diagonal next-nearest-neighbor spin correlator. Both are measured at r_{max} as a function of lattice loading time t_L, where r_{max} is the radius where $\overline{n}_{\mathrm{det}}$ is maximized. (**B**) Computed χ^2_{red} of simultaneous fits of the density and nearest-neighbor correlator profiles to NLCE data. A value $\chi^2_{\mathrm{red}} \approx 1$ indicates a good fit, consistent with our model, which assumes thermal equilibrium. χ^2_{red} settles to approximately 1 at a lattice loading time of 20 ms, indicated by the shaded region. (**C**) Sample profile fits for three different loading times.

By evaporatively cooling further before lattice loading, we are able to prepare samples with even larger nearest-neighbor correlations. However, for this data, the NLCE theory error is too large away from half-filling for the fit to converge, owing to the low temperature. Because the averaged correlator in the center may not be at exactly half-filling, by comparing this value for the coldest data set to a quantum Monte Carlo (QMC) calculation at half-filling (*22*), we can determine an upper bound on the temperature. The correlator value of −0.190(8) gives $k_BT/t < 0.45(2)$, the lowest temperature reported for a Hubbard-regime cold-atom system. The QMC calculation predicts that the nearest-neighbor correlator settles as $T \to 0$ to a value of −0.36; our largest measured nearest-neighbor correlation is therefore 53% of the largest predicted value. In Fig. 2D, we plot our largest measured value of the correlator for samples prepared at different temperatures, the temperature derived from the NLCE fits where they converge (*x* axis), and the QMC upper bound. We find very good agreement between our data and theoretical prediction, which is consistent with half-filling at the cloud center.

We see statistically significant antiferromagnetic correlations to distances of three sites, and the sign of every measured correlator value is consistent with antiferromagnetic ordering. Our ability to measure correlations at all length scales allows us to directly extract the correlation length (Fig. 2E). Samples are prepared at three different temperatures, with the atom number optimized to achieve half-filling in the center of the cloud. Values for the correlator are taken by averaging the spatial maps over a region in the center of the cloud with a six-site radius. To determine the correlation length, we perform an exponential fit of $(-1)^i C_d$ in the center of the cloud versus d, where i = 0 (1) if d is such that the two sites are on the same (different) sublattice. The correlation lengths are 0.24(9), 0.39(2), and 0.51(4) sites for temperatures of $k_BT/t = 1.53(18)$, 0.54(7), and 0.45(2), respectively. The asterisk in Fig. 2, D and 2 shows the QMC prediction of −0.36 for the nearest-neighbor correlator at half-filling as $T \to 0$.

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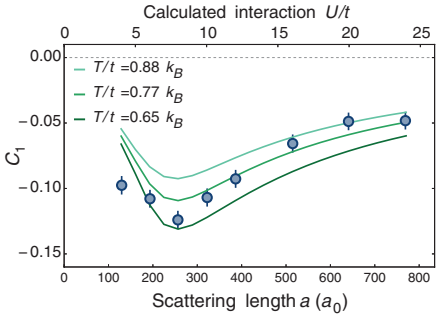

Fig. 4. Varying the interaction strength. Nearest-neighbor correlator at half-filling for varying scattering length. The top *y* axis gives computed values of U/t for each scattering length (*24*). The solid lines are isothermal theory curves from the NLCE theory.

Quantum gas microscopy also allows for a detailed study of the thermalization of the atomic cloud when loading into the lattice. We investigate the formation of spin correlations and the thermalization of the density distribution for different lattice loading times in Fig. 3. For these data the lattice is ramped on linearly with a varying duration t_L. We determine the radius $r_{\max}$, where $\overline{n}_{\det}(r)$ is maximized. For a cloud in thermal equilibrium with $r_{\max}$ not in the center of the cloud, $r_{\max}$ corresponds to half-filling ($\mu = U/2$). Figure 3A shows $\overline{n}_{\det}(r_{\max})$ and $C_d(r_{\max})$ as a function of loading time. The detected density grows from 0.6 at very short loading times and settles at about 0.9 for $t_L = 10$ ms. The loading time required for the density to settle also corresponds to the maximum absolute values for both the nearest-neighbor and diagonal next-nearest-neighbor spin correlators. The matching time scales suggest that the suppression of magnetic correlations at $t_L < 10$ ms is caused by the low initial density and not by exchange dynamics. The density at short loading times is determined by the confinement of the optical dipole trap preceding the lattice loading (*12*). For loading times larger than 10 ms, both $(-1)^i C_d(r_{\max})$ and $\overline{n}_{\det}(r_{\max})$ decay, consistent with heating. The faster decay of $(-1)^i C_d(r_{\max})$ is further indication that the spin correlators are much more sensitive than the density to temperature in this regime of parameters.

We also study thermalization by fitting the data for different loading times to the NLCE theory and performing a reduced chi-squared (χ^2_{red}) analysis. Figure 3B shows χ^2_{red} versus loading time, and Fig. 3C shows individual NLCE fits at t_L of 0.4 ms, 20 ms, and 150 ms from top to bottom. The value of χ^2_{red} settles to approximately 1 on a 20-ms time scale, which is slightly longer than the settling times for the density and spin correlator. The value of χ^2_{red} remains near unity up to our largest loading times, showing that the density and spin-correlator distributions remain consistent with thermal equilibrium.

Whereas in Bose-Hubbard systems AFM correlations appear only in the Heisenberg limit $U \gg t$, Fermi-Hubbard systems exhibit AFM correlations at all U/t, with a maximum in the correlations occurring near $U/t = 8$. For large U/t, AFM correlations are suppressed because the exchange energy becomes small compared with the temperature. For $U/t < 8$, where the interaction energy is smaller than the bandwidth, charge fluctuations destroy the magnetic correlations. We study these effects by varying the scattering length for fixed $t/h = 970(110)$ Hz. In Fig. 4, we plot $C_1(r_{\max})$ versus the scattering length, along with the predictions of the NLCE theory for three different temperatures. We show the calculated U/t from Wannier functions in the lowest band; for our parameters, corrections to this single-band approximation could play a role (*27*). The data show the expected dependence on U/t from the simple picture mentioned above. We also compare our data with theoretical isothermal curves at half-filling. In this comparison, additional factors should be considered. First, the atom number is fixed, so the chemical potential in the center of the cloud varies with U/t. Second, we anticipate the loading entropy to be approximately fixed, as opposed to the temperature, so the data are not expected to strictly follow a single isotherm. The comparison of data with the theory reflects differences between the thermodynamic preparation of atomic and conventional solid-state systems.

Our ability to observe the in situ, site-resolved distribution of spin correlations at all distances has enabled high-precision comparison with numerical studies and detailed verification that the atomic sample behaves in a manner consistent with thermal equilibrium. These experimental benchmarks on thermal equilibrium affirm our understanding of the entropy distribution, paving the way for the implementation of entropy redistribution techniques to achieve finite-system-size long-range order (*28*, *29*). Implementation of such techniques would require precise trap-shaping protocols, which have been fruitfully demonstrated in bosonic quantum gas microscopes (*30*). Numerical simulations provide evidence that a pseudogap phase in the hole-doped 2D Hubbard model arises in conjunction with long-range AFM correlations (*31*) and should therefore be accessible in our experiment in the near future. At lower temperatures of $k_B T/t \sim 0.03$ a d-wave superconductor is expected (*32*). Further thought is required to understand how the real-space observables that we can measure might shed light on these low-temperature phases. Beyond equilibrium physics, we could also exploit our ability to take temporally resolved snapshots of the correlations in a many-body wave function, allowing for in-depth studies of nonequilibrium physics beyond the capability of existing theoretical tools (*33*).

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SUPPLEMENTARY MATERIALS

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Supplementary Text
Figs. S1 to S6
Table S1
Reference (*36*)

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QUANTUM SIMULATION

Spin- and density-resolved microscopy of antiferromagnetic correlations in Fermi-Hubbard chains

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The repulsive Hubbard Hamiltonian is one of the foundational models describing strongly correlated electrons and is believed to capture essential aspects of high-temperature superconductivity. Ultracold fermions in optical lattices allow for the simulation of the Hubbard Hamiltonian with control over kinetic energy, interactions, and doping. A great challenge is to reach the required low entropy and to observe antiferromagnetic spin correlations beyond nearest neighbors, for which quantum gas microscopes are ideal. Here, we report on the direct, single-site resolved detection of antiferromagnetic correlations extending up to three sites in spin-1/2 Hubbard chains, which requires entropies per particle well below $s^* = \ln(2)$. The simultaneous detection of spin and density opens the route toward the study of the interplay between magnetic ordering and doping in various dimensions.

The Fermi-Hubbard model, which describes interacting fermions on a lattice, supports a rich phase diagram at low temperatures. Despite the conceptual simplicity of the Hubbard model, parts of its phase diagram, especially away from half-filling, and its connection to high-temperature superconductivity are still under debate (*1*). Controlled experiments with ultracold fermions in optical lattices might provide new insight (*2*). For one particle per lattice site, the so-called half-filling regime of a balanced two-component fermion mixture, and repulsive interactions, the Hubbard model features a crossover from a metallic to a Mott insulating state when the temperature is lowered. For even lower temperatures, antiferromagnetic correlations are expected to develop in the Mott insulating phase owing to the superexchange mechanism (*2*–*5*). The paramagnetic Mott insulating state has been observed in seminal ultracold atom experiments involving trap-averaged quantities and, recently, at the single-atom level (*6*–*12*). Detailed experimental studies of the thermodynamics of the Hubbard model also revealed its equation of state in the density sector down to temperatures at which short-range spin-ordering might occur (*13*, *14*). Unfortunately, the experimental preparation of low-entropy lattice fermions has proven to be extremely challenging, making the observation of longer ranged antiferromagnetism difficult. Important progress in revealing magnetic ordering in the Hubbard model has been reported with the observation of nearest-neighbor correlations via singlet-triplet spin oscillations (*15*–*17*) and short-range correlations deduced from optical Bragg spectroscopy (*18*). However, the detection of the onset of magnetic order is complicated by the inhomogeneity of the trapped samples, in which different phases coexist. Microscopic control or detection helps to overcome this limitation, and the analog of antiferromagnetic correlations has been measured in small systems of up to three fermions (*19*). Recently, local non–spin-resolved detection of ultracold fermions in single-lattice sites has been demonstrated (*20*–*23*), and the nonuniform entropy distribution in band and Mott insulating states has been observed in the density sector (*11*, *12*, *24*).

Here, we report on a site- and spin-resolved study of antiferromagnetic correlations extending over up to three sites in one-dimensional (1D) spin-1/2 Hubbard chains realized with ultracold lithium-6 in an optical superlattice. Using our spin and onsite atom number–sensitive quantum gas microscope, we directly measured spin correlations together with density fluctuations in the system.

The fermionic atoms in each of the 1D lattice tubes are well described by the single-band Hubbard Hamiltonian

$$\hat{H} = -t\sum_{i,\sigma}(\hat{c}^{\dagger}_{i,\sigma}\hat{c}_{i+1,\sigma} + \text{h.c.}) + U\sum_{i}\hat{n}_{i,\uparrow}\hat{n}_{i,\downarrow} + \sum_{i,\sigma}\varepsilon_i\hat{n}_{i,\sigma} \quad (1)$$

Here, the fermion creation operator is denoted by $\hat{c}^{\dagger}_{i,\sigma}$ and the annihilation operator is denoted by $\hat{c}_{i,\sigma}$ at site i for each of the two spin states $\sigma = \uparrow,\downarrow$. The operator $\hat{n}_{i,\sigma} = \hat{c}^{\dagger}_{i,\sigma}\hat{c}_{i,\sigma}$ counts the number of atoms with spin σ on the respective site. Three competing energy scales govern the physics of this system: intersite nearest-neighbor hopping with strength t, onsite interactions of strength U, and local trap-induced energy offsets ε_i. Here, t is controlled via the lattice depth, whereas U can be tuned independently in the experiment by using the broad Feshbach resonance of lithium-6 between the lowest hyperfine states $|\downarrow\rangle = |F = 1/2, m_F = -1/2\rangle$ and $|\uparrow\rangle = |1/2, 1/2\rangle$ (*25*), where F and m_F define the hyperfine state of the atoms. In the experiments reported here, we exclusively worked with repulsive interactions $U > 0$, for which the Hubbard model supports finite-range antiferromagnetism with correlations suddenly appearing at distances beyond nearest neighbors for entropies per particle below the "critical" value of $s^* = S/Nk_B = \ln(2)$ (*26*–*28*), where S is the entropy, N is the atom number, and k_B is the Boltzmann constant. True long-range order is absent in the 1D Hubbard model even at zero temperature (*4*, *29*), and the algebraic decay of the correlations is strong even on a distance of a few sites (*27*, *28*). In the limit of very strong repulsive interactions and half-filling, the emerging spin order is intuitively understood from the mapping of the Hubbard model to a Heisenberg antiferromagnet with superexchange coupling $J = 4t^2/U$ (*3*). For weaker interactions, particle-hole fluctuations become important, and the ground state is characterized by a spin density wave. In one dimension, the model is Bethe ansatz integrable (*4*, *29*), and precise predictions for the finite entropy spin correlations and density fluctuations have been reported in the parameter regime relevant to cold-atom experiments (*27*, *28*).

The experiments started with the preparation of a low-temperature balanced spin mixture of the $|\uparrow\rangle$ and $|\downarrow\rangle$ states in a single 2D lattice plane (*24*). The final temperature and atom number was controlled by magnetic field–driven spill-out evaporation at repulsive interactions (*30*). We set the final interaction strength using a homogeneous magnetic offset field in order to control the scattering length in the vicinity of the Feshbach resonance centered at 832 G (*25*). Afterward, we ramped up the large spacing component (site separation $a_{sl} = 2.3$ μm) of a superlattice (*31*, *32*) in the y direction to prepare independent 1D tubes. Next, we slowly turned on a lattice with spacing $a_l = 1.15$ μm along the tubes in the x direction using a 100-ms linear ramp to 11 E_R, where $E_R = \hbar^2/8ma_l^2$ denotes the recoil energy of the lattice for atoms of mass m and $\hbar$ is Planck's constant. The hopping strength is $t = \hbar \times 125(9)$ Hz at this final lattice depth. The lattice filling was controlled by varying the evaporation parameters. To simultaneously detect the spin and density degrees of freedom of the 1D Hubbard chains locally, we froze the dynamics by rapidly increasing the lattice depth along the tubes to 42 E_R within 1 ms, followed by a turn-off of the magnetic offset field in 20 ms. We obtained spin resolution using the superlattice potential and a magnetic field gradient in the y direction in a Stern-Gerlach–like setting. The magnetic field gradient shifted the potential minima experienced by the two spin states of opposite magnetic moment, and the subsequent adiabatic ramp-up of the short scale component of the y superlattice with well separation a_l caused a separation of the spins into the two different sites of the local double well (Fig. 1A). Applying this technique to a spin-polarized gas,

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we inferred a splitting fidelity of 98% limited by superlattice phase fluctuations of 25 mrad (*30*). Last, we ramped up a 3D pinning lattice for detection and reconstructed the lattice site occupations from fluorescence images (Fig. 1, B and C) after deconvolution with the measured point-spread-function (*24*, *30*). The above detection procedure enables us to detect the position of all spins, doublons, and holes in the system with single-lattice-site resolution, obtaining complete information about the system in Fock space.

First, we analyzed the spin correlations $C(d) = 4(\langle \hat{S}_i^z \hat{S}_{i+d}^z \rangle - \langle \hat{S}_i^z \rangle \langle \hat{S}_{i+d}^z \rangle)$ between the spin operators $\hat{S}_i^z = \frac{\hat{n}_{i,\uparrow} - \hat{n}_{i,\downarrow}}{2}$ versus distance d. To this end, we fixed the s wave scattering length to 671(10)a_B, where a_B is the Bohr radius, corresponding to $U/t = 12.6$ and took a high statistics data set of 1200 individual pictures. We focused on the central region of the inhomogeneous sample, defining two spatial regions of interest for the analysis. The first one, referred to as the loose filter, involves all sites with an average density $\hat{n}_i = \hat{n}_{i,\uparrow} + \hat{n}_{i,\downarrow}$ in the range $\hat{n}_i = 1 \pm 0.3$. Here, we benefit from higher statistics, but there is a considerable effect of lower-density regions in the data. The second, so-called tight filter selects one specific tube and takes into account only sites closer to unity density with $\hat{n}_i = 1 \pm 0.1$ (*30*). The strong nearest-neighbor correlations of $C(1) = -0.220(5)$ and $C(1) = -0.34(9)$ observed for the loose and tight filtering correspond to 37 and 58%, respectively, of the expected zero-temperature signal in the Heisenberg limit (Fig. 2) (*27*, *28*). These data are based on the average over all measurements taken for $U/t > 8$ (Fig. 3). We observed significant correlations over a distance of up to three sites of the staggered correlator $C_s(d) = (-1)^d C(d)$. A comparison between the experimentally measured correlator $C(d)$ and finite-temperature quantum Monte Carlo (QMC) calculations for homogeneous Hubbard chains at half-filling allows the determination of the entropy and temperature of the lattice gas (*30*). We inferred an effective local entropy per particle of $s = 0.61(1)$ in the loosely filtered case, reducing to $s = 0.51(5)$ for the tight filter, both significantly below $s^* = \ln(2) \approx 0.69$. In a uniform system at half-filling, this lowest entropy corresponds to a temperature of $k_B T/t = 0.22(4)$ at $U/t = 12.6$ (*30*).

In order to explore the properties of the Hubbard chains at different interaction strengths U/t, we measured spin correlations and particle-hole fluctuations for varying onsite interactions U, while keeping the lattice ramp and final lattice depth constant at 11 E_R (Fig. 3). We compared the measurements to QMC results for a homogeneous system at half-filling for different temperatures and entropies. The dependence of the correlations on the interactions is rather different when comparing isothermal and isentropic state preparation. In the former case, a maximum of the correlations is expected at intermediate interactions U/t, where part of the entropy is carried by density modes (*33*), whereas at large interactions, the correlations decrease owing to the smaller energy scale of spin excitations given by the superexchange coupling J. In the isentropic case of constant entropy, spin correlations saturate toward strong interactions, where the energetic gap between spin and density modes is large. At intermediate interaction strengths, the correlation behavior depends on the entropy, and a weakly pronounced maximum exists for intermediate entropies around $s^* = \ln(2)$ (Fig. 3A), whereas below $s = 0.6$, a monotone increase of the correlations with interaction strength is expected. Experimentally, we observed a saturation behavior of the spin correlations for $U/t > 8$. The inferred temperature dropped from $k_B T = 0.6t$ to $0.3t$ while increasing U/t from 8 to 16, as expected for adiabatic cooling. At intermediate interactions, $U/t \approx 5$, we observed reduced spin

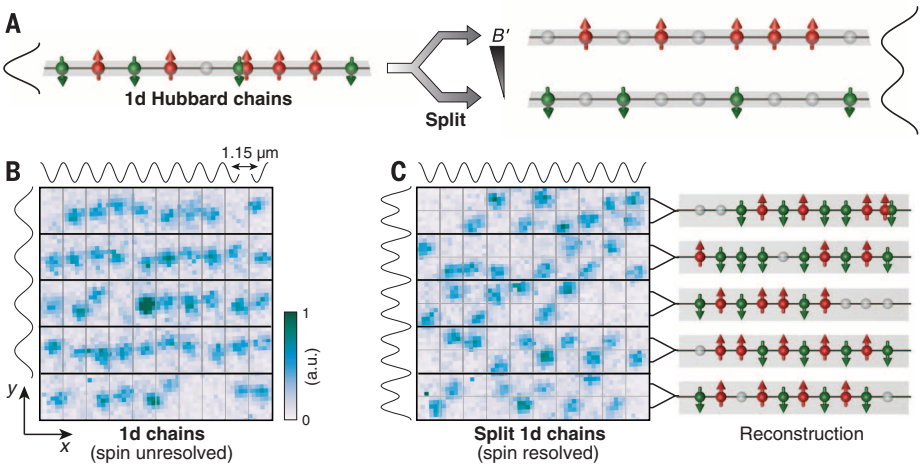

Fig. 1. Schematic of the spin- and density-resolved detection. (**A**) Schematic of the spin-resolved imaging. Each site of the Hubbard chain was split spin-dependently into a local double-well potential by applying a magnetic field gradient B'. This allows for the simultaneous detection of up spins ($|\uparrow\rangle$, red), down spins ($|\downarrow\rangle$, green), doublons (up and down spins overlapping), and holes (gray spheres) and thus for a full characterization of the Hubbard chains. (**B**) Typical fluorescence image of atoms in five mutually independent 1D tubes imaged before splitting. The lattice potentials are indicated by the black lines next to the images, with a spacing along the tubes oriented in the x direction of 1.15 μm and a transverse intertube separation of 2.3 μm. The increasing fluorescence level is shown by darker colors in relative units (color bar). The imaging slightly displaces the atoms from their original positions and also allows for the detection of doubly occupied sites (saturated signal in the center) (*24*). (**C**) Typical image with spin-resolved detection. A superlattice in the y direction (indicated on the left of the image) was used to split each chain in a spin-dependent manner. The $|\downarrow\rangle$ spins were pulled down, whereas the $|\uparrow\rangle$ spins were pulled upward. (Right) Illustration of the reconstructed Hubbard chains.

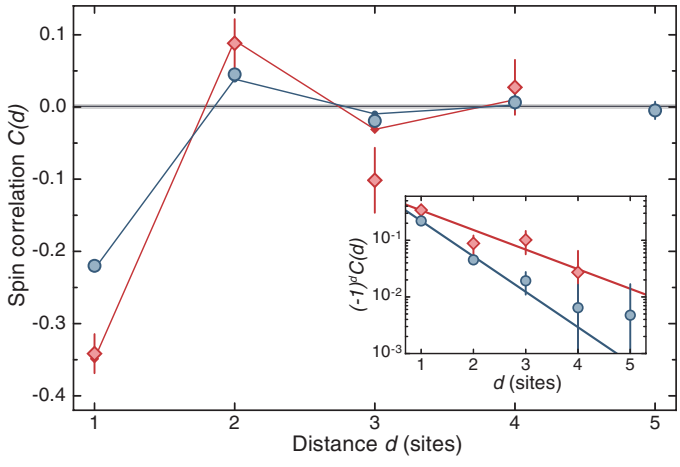

Fig. 2. Antiferromagnetic spin correlations versus distance. Measured spin correlations at $U/t = 12.6$ for the loosely (blue circles) and more tightly filtered data (red diamonds). The staggered behavior directly visualizes the antiferromagnetic nature of the correlations $C(d)$. Correlations up to three sites are statistically significant. The transverse correlations (gray line) vanish within their 1 SEM uncertainty (light gray shading). The red and blue lines connecting filled symbols are QMC results for a homogeneous system at half-filling corresponding to entropies per particle of $s = 0.51(5)$ and $s = 0.61(1)$, respectively. (Inset) Decay of the staggered spin correlator $C_s(d) = (-1)^d C(d)$ in a logarithmic plot together with an exponential fit $C_s(d) \propto \exp(-d/\xi)$, revealing decay lengths of $\xi = 0.69(6)$ sites and $\xi = 1.3(4)$ sites for the two data sets. For low entropies, an exponential decay is expected to be strictly valid only at large distances. However, within the statistical uncertainty of the experimental data, the fit captures the observed behavior. All error bars represent 1 SEM.

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correlations compared with the isentropic prediction at half-filling. We attribute this to a changing entropy distribution in the trap (*8*, *30*) and a measured weak increase of the mean density in the analyzed region by 5%. In the regime of saturated spin correlations, the doublon and hole fractions reached their lowest value of P_d = 5% and P_h = 12%, respectively. The higher hole fraction is mainly caused by the loose filtering, which results in an effective hole doping in the analyzed region of the system; this doping is lower (P_d = 3%, P_h = 7%) for the tightly filtered data.

More insight into the behavior of the spin correlations can be obtained by making use of the full microscopic characterization of the system. To study the antiferromagnetic spin correlations away from half-filling, we show the nearest neighbor correlator $C(1)$ per pair of sites versus their mean density in Fig. 4. These data combine different data sets taken at U/t = 10.3 and also contain measurements at different temperatures, obtained by holding the cloud for up to 2.5 s in the 2D plane. We observed a clear dependence of the spin correlator on the local density, with strongest correlations close to $\langle \hat{n} \rangle = 1$. Away from half-filling, both to higher and lower densities, a strong decrease of the correlations is observed, reflecting that doping reduces spin order (*2*). Generally, the data scatter is much higher than expected just by statistics—that is, at a given density we observe events with a range of significantly different nearest-neighbor spin correlations. This reflects the distribution of entropy within each cloud, as well as between the measurement settings. To further analyze the data, we selected a density interval $\langle \hat{n}_i \rangle = 1 \pm 0.1$ and calculated the normalized variance of the density $\mathrm{Var}(\hat{n})/\langle \hat{n} \rangle$ for all pairs of sites in this window. These fluctuations reflect the entropy in the density sector, whereas the nearest-neighbor spin correlations are a measure of the spin entropy. We show their mutual dependence in the inset of Fig. 4, identifying two distinct regimes of total entropy. In the regime below $s^* = \ln(2)[C(1) \lesssim -0.15]$, the density fluctuations depend only weakly on the total entropy (*27*, *28*), which in turn is stored in the spin fluctuations. Only when these are saturated at s^* do the density fluctuations grow, visible in their steep rise when the spin correlations are just below zero. The freezing of density fluctuations renders them useless as a thermometer in the low-entropy regime, whereas the highly temperature-sensitive (and entropy-sensitive) spin correlations are ideal for this purpose down to T = 0 (*27*).

Such a "spin thermometer" (*30*, *34*) is a crucial step toward optimized cooling (*35*, *36*) to lower entropies required to study, for example, d wave superfluidity away from half-filling (*37*). The demonstrated measurement of all relevant degrees of freedom gives access to spin-density correlators, which are essential to reveal the interplay of magnetism and doping. Furthermore, the combination of superlattices and local detection will allow for the search of an adiabatic path between low-entropy valence bond solids (*15*) or plaquette-resonating valence bond states (*38*, *39*) and the Heisenberg antiferromagnet (*40*),

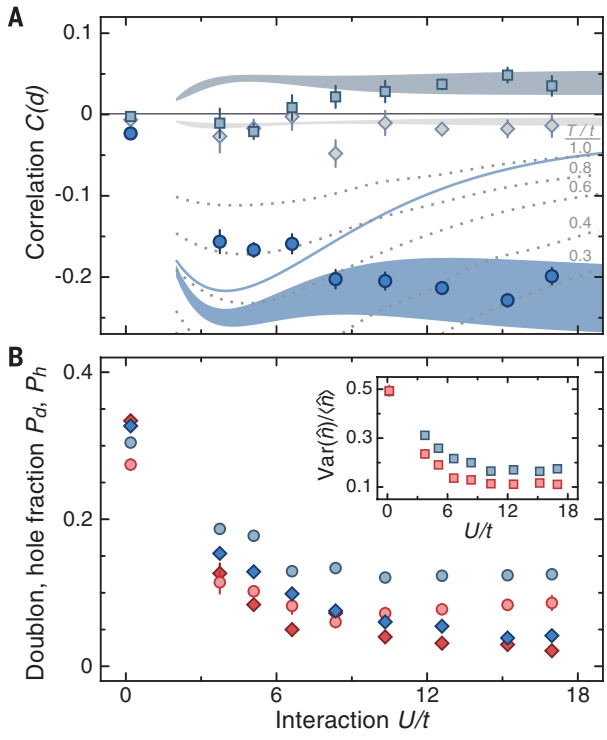

Fig. 3. Spin and density degrees of freedom at different interaction strengths. (**A**) Spin correlations $C(d)$ for distances d = 1 (dark blue), 2 (light blue), and 3 (gray) versus interaction strength U/t. Starting close to zero at vanishing interactions, finite range spin correlations develop and saturate for interaction strengths $U/t > 8$. The shaded areas indicate the QMC predictions in a homogeneous system at half-filling for an entropy per particle between s = 0.60 (lower bound) and 0.65 (upper bound); the solid line is the prediction for $C(1)$ at s^* = ln(2). Dotted lines are isothermals for $C(1)$ at the indicated temperatures. For large U/t, we observed adiabatic cooling, whereas both temperature and entropy decrease in the analyzed spatial region at intermediate U/t. The transverse nearest-neighbor correlations (dark gray line close to zero) is consistently above the d = 3 spin correlator, which supports its statistical significance. Because of limited statistics, only loosely filtered data is shown. (**B**) Evolution of the density degree of freedom. Shown is the evolution of the fraction of holes (circles) and doublons (diamonds) with interaction strength U/t. The hole (P_h) and doublon (P_d) fractions decrease for stronger interactions and then saturate. Data are shown for the loose (blue) and tight (red) filter cases. (Inset) Evolution of the normalized onsite atom number variance $\mathrm{Var}(\hat{n})/\langle \hat{n} \rangle$ The density fluctuations are suppressed already at vanishing interactions owing to effects of Pauli blocking in the metal. This suppression becomes stronger for increasing interactions until the fluctuations saturate. All error bars represent 1 SEM, and the apparent fluctuation of the data are due to day-to-day systematics.

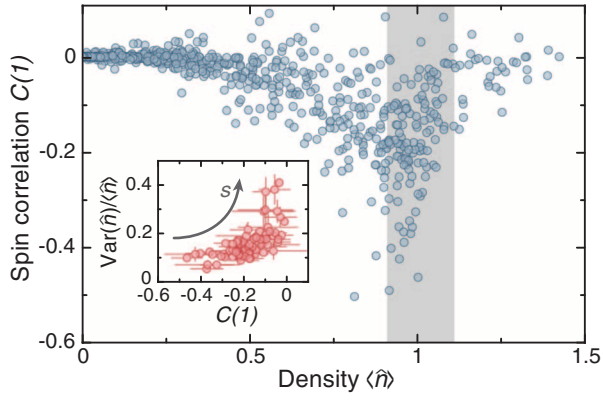

Fig. 4. Interplay of density and spin fluctuations. We show the nearest-neighbor spin correlations $C(1)$ for different densities corresponding to different positions in the trap. The data combine several measurements at an interaction strength of U/t = 10.3, also including higher-temperature data. Every data point corresponds to two neighboring sites, where between 30 and 2000 samples contribute. The strength of spin correlations $C(1)$ peaks just below densities of one is consistent with the half-filling regime taking the detection efficiency of ~95% into account. (Inset) The normalized density fluctuations $\mathrm{Var}(\hat{n})/\langle \hat{n} \rangle$ versus $C(1)$ for a density interval $\langle \hat{n}_i \rangle = 1 \pm 0.1$ as indicated by the gray area in the main plot. Density fluctuations rise steeply for low values of the spin correlator, signaling the saturation of the entropy in the spin sector. This characteristic dependence identifies the strong vertical scatter of the data in the main figure mainly as a result of different local entropies s as indicated by the arrow. For clarity of the presentation, we omit the error bars in the main figure. They are of the same size as the ones in the inset and correspond to 1 SEM.

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also in two dimensions. Realization of the paradigmatic quantum phase transition from such an artificial valence bond solid to a Heisenberg antiferromagnet (*41*) therefore seems within reach of present experiments.

Recently, we became aware of similar experimental results in two dimensions (*42*, *43*).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6305/1257/suppl/DC1
Supplementary Text
Figs. S1 to S5
Table S1
References (*44*–*51*)

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QUANTUM SIMULATION

Observation of spatial charge and spin correlations in the 2D Fermi-Hubbard model

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Strong electron correlations lie at the origin of high-temperature superconductivity. Its essence is believed to be captured by the Fermi-Hubbard model of repulsively interacting fermions on a lattice. Here we report on the site-resolved observation of charge and spin correlations in the two-dimensional (2D) Fermi-Hubbard model realized with ultracold atoms. Antiferromagnetic spin correlations are maximal at half-filling and weaken monotonically upon doping. At large doping, nearest-neighbor correlations between singly charged sites are negative, revealing the formation of a correlation hole, the suppressed probability of finding two fermions near each other. As the doping is reduced, the correlations become positive, signaling strong bunching of doublons and holes, in agreement with numerical calculations. The dynamics of the doublon-hole correlations should play an important role for transport in the Fermi-Hubbard model.

A central question in the study of cuprate high-temperature superconductors is how spin and charge correlations give rise to the wealth of observed phenomena. Antiferromagnetic order present in the absence of doping quickly gives way to superconductivity upon doping with holes or electrons (*1*), suggesting the viewpoint of competing phases. On the other hand, antiferromagnetic correlations can also occur in the form of singlet bonds between neighboring sites. In fact, it has been proposed (*2*) that superconductivity could result, upon doping a Mott insulator, from the condensation of such resonating valence bonds. It has also been argued (*1*) that the pseudogap and "strange metal" regions are supported by a liquid of spin-singlets. This argument has spurred the simultaneous examination of nearest-neighbor spin and charge correlations, which might reveal the underlying mechanisms of pairing and transport.

In recent years, ultracold atomic gases have been established as pristine quantum simulators of strongly correlated many-body systems (*3*–*5*). The Fermi-Hubbard model is of special importance, thanks to its paradigmatic role for the study of high–critical temperature cuprates. At low temperatures and away from half-filling, solving the Fermi-Hubbard model theoretically is very challenging because of the fermion sign problem. Central properties of Fermi-Hubbard physics—from the reduction of double occupancy (*6*, *7*) and of compressibility (*8*, *9*) as the repulsion is increased, to short-range antiferromagnetic correlations (*10*–*12*) and the equation of state (*9*, *13*, *14*)—have been observed in ultracold atom experiments. The recently developed Fermi gas microscopes (*13*, *15*–*19*) have led to the direct observation of two-dimensional (2D) fermionic Mott insulators, band insulators, and metals with single-atom, single-site–resolved detection (*20*, *21*). The strength of this technique, however, is on full display when single-site detection is used to directly measure correlations in the gas, as has been achieved with bosons (*22*–*24*).

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In our study, we used a Fermi gas microscope of ^{40}K atoms to directly observe charge and spin correlations in the 2D Fermi-Hubbard model (*15*, *21*). Spin correlations displaying antiferromagnetic behavior have also been observed very recently with fermionic ^{6}Li in one (*25*) and two (*26*) dimensions. We employ the local resolution to simultaneously obtain correlations in the entire range from zero doping (half-filling) to full doping (zero filling), as the density varies in the underlying trapping potential. The microscope measures the parity-projected density on a given lattice site—that is, doubly occupied sites (doublons) appear empty. For a two-spin mixture of fermions in the lowest band of the optical lattice, the parity-projected density is described by the magnetic moment operator (*21*) $\hat{m}_{z,i}^2 = \left(\hat{n}_{\uparrow,i} - \hat{n}_{\downarrow,i}\right)^2$, where $\hat{n}_{\sigma,i} = \hat{c}_{\sigma,i}^{\dagger}\hat{c}_{\sigma,i}$ is the number operator and $\hat{c}_{\sigma,i}$ ($\hat{c}_{\sigma,i}^{\dagger}$) are fermion annihilation (creation) operators for spin $\sigma = \uparrow, \downarrow$ on site i. Many repeated measurements yield the average local moment on each site (Fig. 1, A and D), which is a thermodynamic quantity that quantifies the interaction energy. This is evident when one rewrites the interaction energy term $U\hat{n}_{\uparrow,i}\hat{n}_{\downarrow,i}$ as $\frac{U}{2}(\hat{n}_{\uparrow,i} + \hat{n}_{\downarrow,i} - \hat{m}_{z,i}^2)$. The Fermi-Hubbard Hamiltonian can be written in terms of local moments as

$$\hat{H} = -t\sum_{\langle i,j\rangle,\sigma}\left(\hat{c}_{\sigma,i}^{\dagger}\,\hat{c}_{\sigma,j} + \text{h.c.}\right) - \frac{U}{2}\sum_{i}\hat{m}_{z,i}^2 - \mu\sum_{i}\left(\hat{n}_{\uparrow,i} + \hat{n}_{\downarrow,i}\right) \quad (1)$$

which is a form that highlights the particle-hole symmetry of the Hamiltonian. Here, $\langle i,j\rangle$ denotes nearest-neighbor sites i and j, t is the nearest-neighbor hopping amplitude, U is the on-site interaction energy, and μ is the chemical potential. At moderate temperatures and depending on the fillings $n_i = \langle\hat{n}_{\uparrow,i} + \hat{n}_{\downarrow,i}\rangle$, this model can yield metallic, band insulating, or Mott insulating states. At half-filling ($n_i = 1$) and at temperatures below the superexchange scale $4t^2/U$, quasi-long-range antiferromagnetic correlations arise. For a fixed temperature, these correlations are expected to be maximal when $U \approx 8t$, where the interaction energy equals the single-particle bandwidth. Upon doping, a pseudogap phase emerges; at even lower temperatures, one expects a d-wave superconducting state (*1*). Although the superexchange scale is about a factor of 2 lower than the temperatures achieved here, site-resolved detection of short-range correlations should already reveal precursory signs of physics at this energy scale.

Figure 1A shows a typical measurement of the site-resolved average local magnetic moment from ~90 individual experimental realizations at U/t = 7.2(1). Atoms are confined in a radially symmetric trapping potential. Under the local density approximation, this results in a varying local chemical potential and, thus, a spatially varying filling n throughout the sample. We prepared samples where the maximum filling, which occurs in the center of the trap, lies above n = 1. From radially averaged profiles (Fig. 1D), the half-filling point is identified as the radial position where the moment reaches its maximum. This follows from the particle-hole symmetry of the moment operator $\hat{m}_{z,i}^2$, a property that holds for all of its averages and cumulants (*21*).

Because the local moment satisfies the operator identity $(\hat{m}_{z,i}^2)^2 = \hat{m}_{z,i}^2$, fluctuations of the local moment do not yield additional information.

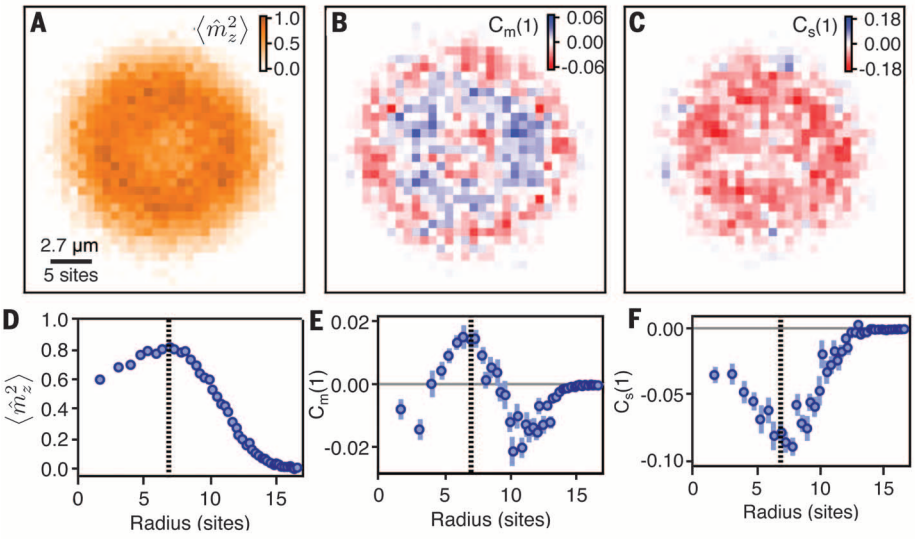

Fig. 1. Local moment and nearest-neighbor charge and spin correlations. An ultracold atom realization of the Fermi-Hubbard model for U/t = 7.2(1) is shown. (**A** to **C**) Local moment, nearest-neighbor moment correlator, and nearest-neighbor spin correlator, respectively, as functions of position, averaged over ~90 shots. The spatial variations reflect the varying local doping due to the underlying trapping potential. (**D** to **F**) Radial averages of (A), (B), and (C), respectively. The half-filling point is marked by vertical dotted lines.

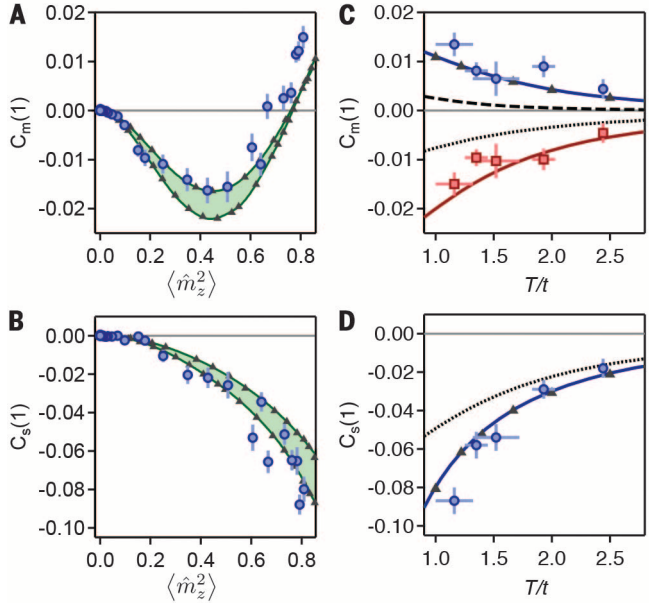

Fig. 2. Spin and moment correlators as functions of doping and temperature for U/t = 7.2(1). (**A** and **B**) Nearest-neighbor moment correlator [$C_m(1)$] (A) and spin correlator [$C_s(1)$] (B) as functions of the local moment, denoted by blue circles. Results from NLCE (and DQMC) for temperatures T/t = 0.89 and 1.22 are shown in green lines (and gray triangles), with the intermediate temperature range indicated by green shading. (**C**) The maximum and minimum of the moment correlator as functions of temperature are denoted by blue circles and red squares, respectively. Corresponding results are obtained from NLCE (solid blue line and solid red line, respectively), for the noninteracting gas (black dashed and dotted lines, respectively), and from DQMC for the correlator at half-filling (gray triangles). (**D**) Nearest-neighbor spin correlator at half-filling as a function of temperature (blue circles). Solid blue line, NLCE results; gray triangles, DQMC results; black dotted line, noninteracting gas. For all graphs, theory curves are not adjusted for the experimental imaging fidelity of 95%.

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However, correlations of the moment between different sites do (27). We experimentally measured the moment correlator at a separation of one site, $C_m(1)$, defined as

$$C_m(1) = \frac{1}{4}\sum_{j\in nn_i}\left(\left\langle \hat{m}^2_{z,i}\hat{m}^2_{z,j}\right\rangle - \left\langle \hat{m}^2_{z,i}\right\rangle\left\langle \hat{m}^2_{z,j}\right\rangle\right) \quad (2)$$

where the sum is over all four nearest neighbors. The locally resolved correlator $C_m(1)$ at each site i and its radial average are shown in Fig. 1, B and E, respectively. $C_m(1)$ displays nonmonotonic behavior, changing sign as the filling is lowered.

The local moment correlator, however, is not sensitive to the sign of the spin $\hat{S}_{z,i} = \frac{1}{2}(\hat{n}_{\uparrow,i} - \hat{n}_{\downarrow,i})$. One important spin-sensitive correlator is $\langle \hat{S}_{z,i}\hat{S}_{z,j}\rangle$, which can reveal antiferromagnetic ordering, expected to occur at half-filling and at low temperatures. This correlator can be expressed as $\frac{1}{2}\sum_\sigma \langle \hat{p}_{\sigma,i}\hat{p}_{\sigma,j}\rangle - \frac{1}{4}\langle \hat{m}^2_{z,i}\hat{m}^2_{z,j}\rangle$ (28), where $\hat{p}_{\sigma,i} = \hat{n}_{\sigma,i} - \hat{n}_{\uparrow,i}\hat{n}_{\downarrow,i}$, which we measured by removing one spin state via resonant light, before imaging. All terms can be obtained experimentally in separate runs and are averaged separately. Analogous to the nearest-neighbor moment correlator $C_m(1)$, we define the nearest-neighbor spin correlator at site i

$$C_s(1) = \sum_{j\in nn_i}\left(\langle \hat{S}_{z,i}\hat{S}_{z,j}\rangle - \langle \hat{S}_{z,i}\rangle\langle \hat{S}_{z,j}\rangle\right) \quad (3)$$

Figure 1, C and F, show the locally resolved nearest-neighbor spin correlation $C_s(1)$ and its corresponding radial average, respectively. The fact that $C_s(1)$ is negative suggests antiferromagnetic correlations, as expected (29–31). However, even without interactions, Pauli-blocking of like spins suppresses $C_s(1)$. One can see this by noting that $C_s(1)$ contains density correlations of either spin species separately $\left[\langle \hat{n}_{\sigma,i}\hat{n}_{\sigma,j}\rangle - \langle \hat{n}_{\sigma,i}\rangle^2\right]$, which are negative even for the noninteracting gas thanks to Pauli suppression. For the lowest temperatures reached, we observed a maximum absolute spin correlation of about a factor of 2 larger than that of a noninteracting Fermi gas.

Figure 2, A and B, show the nearest-neighbor moment and spin correlations versus the measured local moment $\langle \hat{m}^2_{z,i}\rangle$. This representation allows for comparison with theory under minimal assumptions. As a thermodynamic quantity, the moment can replace the role of the chemical potential μ. All thermodynamic variables can then be viewed as functions of the local moment, the spin correlation at half-filling, U, and t. In fact, the local spin correlation at half-filling is itself a thermometer that does not require any fit (32). Also shown in Fig. 2, A and B, are numerical linked-cluster expansion (NLCE) (33) and determinantal quantum Monte Carlo (DQMC) (34) calculations (28), which display similar behavior as the experimental data. Note that there are no free parameters; the temperature T/t = 1.16(16) is obtained from the spin correlation at half-filling.

As expected, the antiferromagnetic spin correlations are maximum at half-filling and decrease in absolute value with increased doping. Moment correlations instead are negative at low to intermediate fillings, crossing zero around a moment of 0.75 (doping ≈0.21) before turning positive toward half-filling. This implies that moments change their character from effectively repulsive (antibunching) to effectively attractive (bunching). The antibunching and bunching behaviors in the moments, as well as the antiferromagnetic spin correlations, become more pronounced as the temperature is lowered. Figure 2C shows the moment correlation at half-filling (maximum positive value), as well as its minimum value versus temperature. The spin correlator at half-filling (minimum value) (Fig. 2D) displays a similar temperature dependence, reaching −0.09 at the lowest temperatures in our experiment. This is about 30% of the maximum spin correlation expected for the spin-½ Heisenberg model at zero temperature in two dimensions (35).

To interpret the moment correlations, one may recast them in terms of the two-point correlator

$$g_2(r) = \frac{\langle \hat{m}^2_z(r)\hat{m}^2_z(0)\rangle}{\langle \hat{m}^2_z(r)\rangle\langle \hat{m}^2_z(0)\rangle} \quad (4)$$

which measures the probability of finding two moments a distance r from each other. In the absence of correlations, g_2 = 1. At low filling, for which the doublon density is negligible and the moment $\langle \hat{m}^2_z\rangle = \langle \hat{n}\rangle - 2\langle \hat{n}_\uparrow\hat{n}_\downarrow\rangle \approx n$ is essentially the density, $g_2(r)$ measures density correlations. These are nontrivial even for the spin-polarized noninteracting Fermi gas, where fermion statistics lead to anticorrelations at short distances, reflecting the fact that two fermions cannot occupy the same site. This leads to Pauli suppression of g_2 that persists to a distance on the order of the average interparticle spacing, a feature known as the Pauli hole. Although implications of this fermion antibunching have been observed in the suppression of density fluctuations (36, 37) and momentum space correlations (38, 39), the real space suppression $g_2(r)$ has not been observed in situ before. In a noninteracting two-spin mixture, the anticorrelations are halved, as only two identical fermions experience the Pauli hole. However, repulsive interactions between opposite spins also suppress $g_2(r)$, leading to a combined Pauli and correlation hole.

In Fig. 3A, we show the directly measured $g_2(1)$ as a function of moment at an intermediate interaction of U/t = 7.2. The strong suppression of $g_2(1)$ at low fillings (large interparticle spacing) is observed and is stronger than Pauli suppression alone, reflecting short-range anticorrelations due to repulsive interactions. The data are well described by NLCE and DQMC calculations (Fig. 3A).

Whereas $g_2(r)$ measures the probability of finding two moments a distance r from each other, near half-filling, where $\langle \hat{m}^2_z\rangle \sim 1$, the correlations arise mainly from sites where the moment is zero (i.e., sites with holes and doublons). The number of holes and doublons, which appear empty after imaging, is given by $\langle 1 - \hat{m}^2_z\rangle$. The corresponding two-point correlation function $\bar{g}_2(r)$ of these antimoments is thus

$$\bar{g}_2(r) = \frac{\langle (1-\hat{m}^2_z(r))(1-\hat{m}^2_z(0))\rangle}{\langle 1-\hat{m}^2_z(r)\rangle\langle 1-\hat{m}^2_z(0)\rangle} \quad (5)$$

In Fig. 3B, we show that $\bar{g}_2(1)$ is strongly enhanced near half-filling beyond the uncorrelated value of 1. $\bar{g}_2(1)$ thus reveals the strong bunching of holes and doublons. There are three contributions

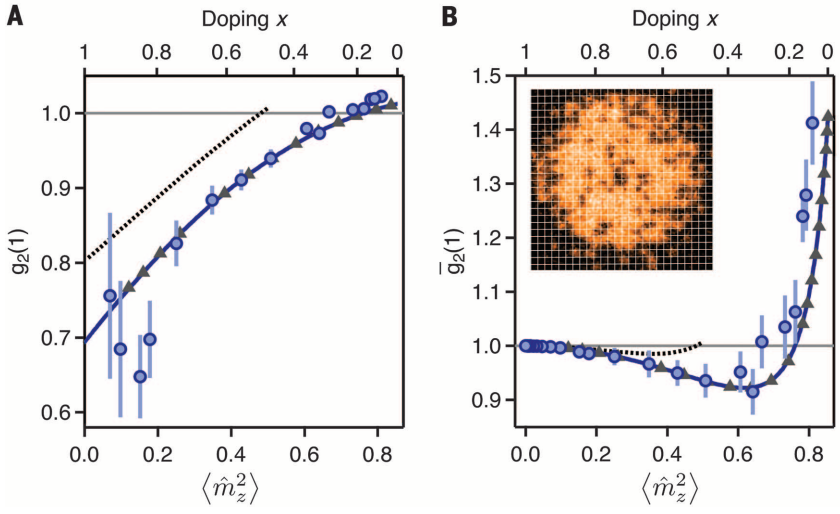

Fig. 3. Two-point correlation functions. g_2 is the correlation function for moments and $\bar{g}_2$ for antimoments at a separation of one lattice site for U/t = 7.2(1). (**A**) $g_2(1)$ for moments. (**B**) $\bar{g}_2(1)$ for antimoments. Blue circles, experimental data; blue solid line, NLCE theory; gray triangles, DQMC theory. Both NLCE and DQMC calculations are performed at T/t = 1.22, and neither are adjusted for the experimental imaging fidelity of 95%. Black dotted lines, noninteracting gas. The doping x as a function of local moment is determined from NLCE theory at T/t = 1.22, without adjustment for imaging fidelity. (Inset) Typical image showing neighboring antimoments (imaged holes) near half-filling.

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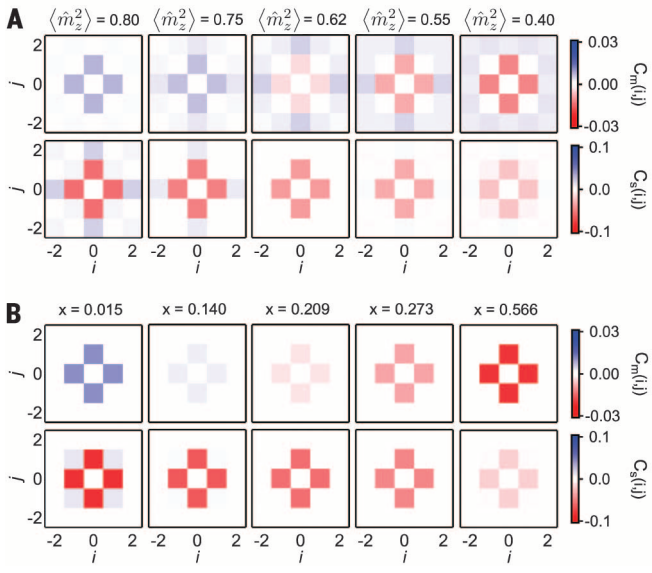

Fig. 4. Spin and moment correlations as functions of distance and doping. (**A**) Moment and spin correlations for U/t = 7.2(1) are shown in the top and bottom rows, respectively, at various values of the local moment. Correlation values are averaged over symmetric points. The moment correlator $C_m(0,1)$ changes sign near a local moment of $\langle \hat{m}_z^2 \rangle \approx 0.75$. The anticorrelation of spins $C_s(0,1)$ is observed to weaken upon increasing doping (decreasing moment). In contrast, the next-nearest-neighbor spin correlator $C_s(1,1)$ changes from positive at zero doping to negative at large doping. (**B**) Moment and spin correlations obtained from DQMC theory for U/t = 7.2(1) and T/t = 1.00 are shown in the top and bottom rows, respectively, at various values of doping x. The nonzero value of the moment and spin correlators at distance $(i,j) = (0,0)$ are omitted for clarity. They are both local quantities determined by the value of the moment. NLCE and DQMC results for the correlators at all distances shown are in good agreement (*28*).

to $\bar{g}_2(1)$: correlations between pairs of holes, between pairs of doublons, and between holes and doublons. One expects neighboring holes and neighboring doublons to show negative correlations due to Pauli suppression and strong repulsion. Hence, the bunching behavior must originate from positive correlations between neighboring doublon-hole pairs. This expectation is confirmed by NLCE and DQMC calculations (*28*).

The strong doublon-hole correlation near half-filling in the presence of antiferromagnetic correlations can be qualitatively captured by a simple two-site Hubbard model, experimentally realized in (*40*). In the strongly interacting limit ($U \gg t$), the doublon density vanishes and the ground state is a spin singlet. However, at intermediate interaction strengths, tunneling admixes a doublon-hole pair into the ground state wave function, with an amplitude ~t/U. Thus, short-range singlet correlations at moderate U/t occur naturally together with nearest-neighbor doublon-hole correlations.

At a separation of one lattice site, we have revealed the competition between the combined Pauli- and interaction-driven repulsion of singly occupied sites and the effective attraction of doublons and holes, which manifests itself in a sign change of the correlator. The ability of the microscope to measure at a site-resolved level also allows investigation of longer-distance correlations. In Fig. 4, A and B, we show the moment and spin correlations $C_m(i,j)$ and $C_s(i,j)$, respectively, as a function of separation distance $i\hat{x} + j\hat{y}$. Near half-filling, even at the temperatures of this graph ($T/t \approx 1.2$), antiferromagnetic spin correlations beyond the next neighbor are visible. With increased doping, they give way to a more isotropic negative spin correlation. For example, $C_s(1,1)$ changes sign from positive at half-filling to negative at large dopings. This resembles the effect of Pauli suppression that is already present for noninteracting fermions. For the moment correlator, we clearly observe the sign change of $C_m(1,0)$ at a doping of $x \approx 0.21$ and that the correlations do not extend substantially beyond one site.

The measurement of nonlocal moment correlations also results in direct access to the associated potential energy fluctuations (ΔE_{pot}). From the Fermi-Hubbard Hamiltonian in Eq. 1, we find that

$$\Delta E_{pot}^2 = \frac{1}{4}U^2\left(\langle \hat{M}^2 \rangle - \langle \hat{M} \rangle^2\right) = \frac{1}{4}U^2 \sum_{i,j}\left(\langle \hat{m}_{z,i}^2 \hat{m}_{z,j}^2 \rangle - \langle \hat{m}_{z,i}^2 \rangle \langle m_{z,j}^2 \rangle\right) \quad (6)$$

where $\hat{M} = \sum_i \hat{m}_{z,i}^2$ is the total moment operator. At half-filling, the contribution to the fluctuations from the nearest-neighbor moment correlations is thus $U^2 C_m(1) \approx 0.8t^2$ for $T/t \approx 1$ and U/t = 7.2(1). This suggests that doublon-hole correlations can arise from coherent tunneling of particles bound in spin singlets.

Away from half-filling, both NLCE and DQMC calculations are currently limited to a temperature range around $T/t \approx 0.5$, not far below what is reached experimentally in this work. Further reduction in experimental temperatures will provide a valuable benchmark for theoretical techniques, especially away from half-filling, where the sign problem arises. The clear importance of doublon-hole correlations will prompt further studies of their dynamics, especially away from half-filling, which could elucidate their role for the transport properties of a possible strange metal phase and potential pseudogap behavior.

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SUPPLEMENTARY MATERIALS

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POLYMER SCIENCE

Quantifying the impact of molecular defects on polymer network elasticity

Mingjiang Zhong,[1,2]* Rui Wang,[2]* Ken Kawamoto,[1]* Bradley D. Olsen,[2]† Jeremiah A. Johnson[1]†

Elasticity, one of the most important properties of a soft material, is difficult to quantify in polymer networks because of the presence of topological molecular defects in these materials. Furthermore, the impact of these defects on bulk elasticity is unknown. We used rheology, disassembly spectrometry, and simulations to measure the shear elastic modulus and count the numbers of topological "loop" defects of various order in a series of polymer hydrogels, and then used these data to evaluate the classical phantom and affine network theories of elasticity. The results led to a real elastic network theory (RENT) that describes how loop defects affect bulk elasticity. Given knowledge of the loop fractions, RENT provides predictions of the shear elastic modulus that are consistent with experimental observations.

Molecular defects fundamentally govern the properties of all real materials (*1*–*3*). The language of crystallography has been successfully used to describe defects and to model their impact in materials with a degree of periodicity, such as silicon, steel, block copolymers, and liquid crystals. However, understanding defects in amorphous materials presents a continued challenge. In polymer networks, the relevant defects are largely of a topological nature: The properties of these amorphous materials depend primarily upon the way the molecules in the material are connected. Understanding the correlation between the network topology and properties is one of the greatest outstanding challenges in soft materials.

Polymer networks can have a wide range of shear elastic moduli (G') from ~10^2 to ~10^7 Pa (*4*, *5*), with different applications requiring moduli across this entire range. Covalent polymer networks are generally formed via kinetically controlled processes; consequently, they possess cyclic topological defects. The classical affine and phantom network theories of network elasticity neglect the presence of such defects (*4*, *5*); they rely on idealized end-linked networks (Fig. 1A) that consider only acyclic tree-like structures, which leads to overestimation of G' (*6*, *7*). In practice, G' is frequently calculated according to the equation $G' = C\nu_{eff}kT$, where kT is the thermal energy, ν_{eff} is the density of elastically effective chains, and C is a constant that has a value of 1 for the affine network model and $1 - 2/f$ for the phantom network model (where f is the functionality of the network junctions). Because polymer networks include elastically defective chains, ν_{eff} is never known precisely, and thus neither theory is able to accurately fit experimental data; a controversy continues over which theory, if either, is correct. Thus, despite decades of advances in polymer network design, our inability to quantitatively calculate the effects of defects on shear elastic modulus and to measure the corresponding defect densities in real polymer networks precludes quantitative prediction of G' and validation of the affine and phantom network models (*4*, *8*–*12*).

To understand how molecular structure affects G' and to use this knowledge to create a predictive theory of elasticity, it is first necessary to quantify the density of topological defects in a polymer network and to determine the impact of these defects on the mechanical properties of the network. Cyclic defects, created from intrajunction reactions during network formation, are chemically and spectroscopically almost identical to noncyclic junctions, making them difficult to distinguish and quantify (*5*, *13*–*16*). We have developed symmetric isotopic labeling disassembly spectrometry (SILDaS) as a strategy to precisely count the number of primary loops (Fig. 1B), the simplest topological defects, in polymer networks formed from $A_2 + B_3$ and $A_2 + B_4$ reactions (*17*–*20*). Furthermore, we have developed Monte Carlo simulations and kinetic rate theories that show that cyclic defects in these polymer networks are kinetically linked, such that experimental measurement of only the primary loops determines the densities of all higher-order defects including secondary (Fig. 1C) and ternary loops (Fig. 1D) (*21*). Here, we measured loop fractions and G' for a series of hydrogels, thus providing quantitative relationships between these parameters. With this information, we examined the classical affine and phantom network theories of elasticity, and we derived a modified phantom network theory—real elastic network theory (RENT)—that accounts for topological molecular defects.

To rigorously determine how molecular topological defects affect elasticity, it is necessary to measure the topological defect density and modulus in the same gel. A class of stable yet chemically degradable gels was developed from bis-azido-terminated polyethylene glycol (PEG) (number-average molecular weight M_n = 4600, dispersity index $Đ$ = 1.02) polymers with nonlabeled or isotopically labeled segments near their chain ends, A_{2H} and A_{2D}, respectively (*22*) (structures are shown in Fig. 1A; for synthesis and characterization details, see figs. S1 to S3 and figs. S17 to S34). Such labeling provides a convenient method for precise measurement of primary loops by SILDaS (*19*). The PEG molecular weight ensures that the polymer solutions used to form gels are well below the entanglement regime (*5*, *12*). The labeled (A_{2D}) and nonlabeled (A_{2H}) polymers (referred to herein as "A_2 monomers") were mixed in a 1:1 molar ratio, and this mixture was allowed to react with a tris-alkyne (B_3) or a tetra-alkyne (B_4) (structures are shown in Fig. 1A) in propylene carbonate solvent to provide end-linked gels via copper-catalyzed azide-alkyne cycloaddition (*23*, *24*). When the reactive group stoichiometry—azide and alkyne in this case—was carefully controlled to be 1:1, spectroscopic analysis demonstrated that dangling functionalities (unreacted azides or alkynes) could be minimized (*19*) such that their impact on elasticity is negligible. Gels with varied fractions of topological defects were synthesized by varying the initial concentrations of A_2 and B_3 or B_4 monomers (*22*).

For measurement of the shear elastic modulus as a function of gel preparation conditions, gel samples 1.59 mm thick were formed in situ in Teflon molds under an inert atmosphere (fig. S4). Gel disks (diameter 12 mm) were punched (Fig. 1A) and loaded onto an oscillatory shear rheometer equipped with parallel-plate geometry. Propylene carbonate was chosen as the solvent

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because of its high boiling point of 242°C, which mitigates solvent evaporation during G' measurement. Representative rheology data for $A_2 + B_3$ ($f = 3$) and $A_2 + B_4$ ($f = 4$) networks are provided in Fig. 2, A and D, respectively (additional results are presented in figs. S5 and S6). Highly reproducible measurements of G' were achieved for several samples of each network. As expected, G' for both the trifunctional gels (Fig. 2B) and the tetrafunctional gels (Fig. 2E) increased with concentration; increased concentration leads to fewer topological defects (*17*–*19*).

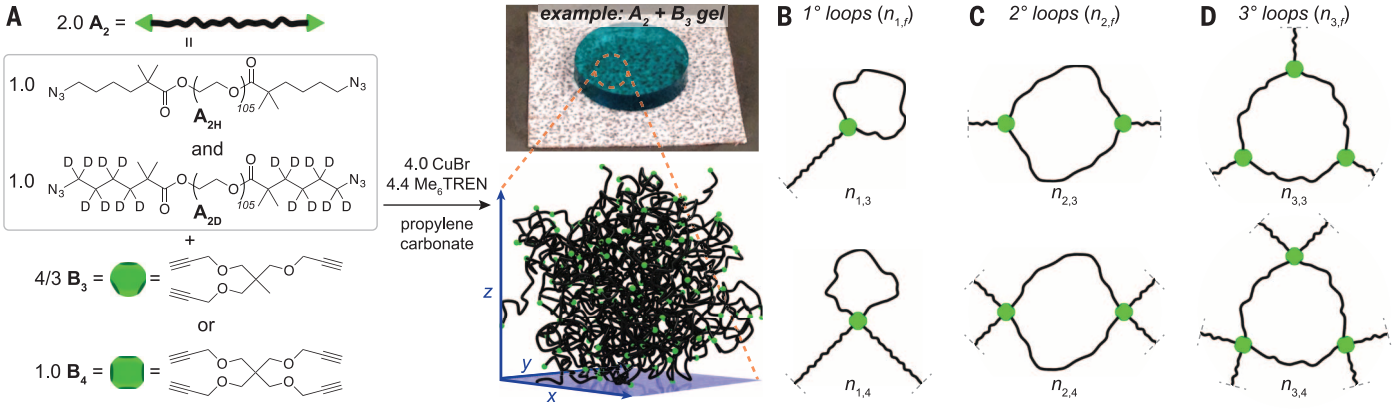

Fig. 1. Cyclic defects in end-linked polymer networks. (**A**) Synthetic schemes for the preparation of $A_2 + B_3$ and $A_2 + B_4$ gels labeled for loop counting analysis. (**B** to **D**) Topological structure of primary loops (the average number of primary loops per f-functional network junction is $n_{1,f}$) (B), secondary loops (the average number of secondary loops per f-functional junction is $n_{2,f}$) (C), and ternary loops (the average number of ternary loops per f-functional junction is $n_{3,f}$) (D) formed in $A_2 + B_3$ ($f = 3$; top) and $A_2 + B_4$ ($f = 4$; bottom) gels, respectively.

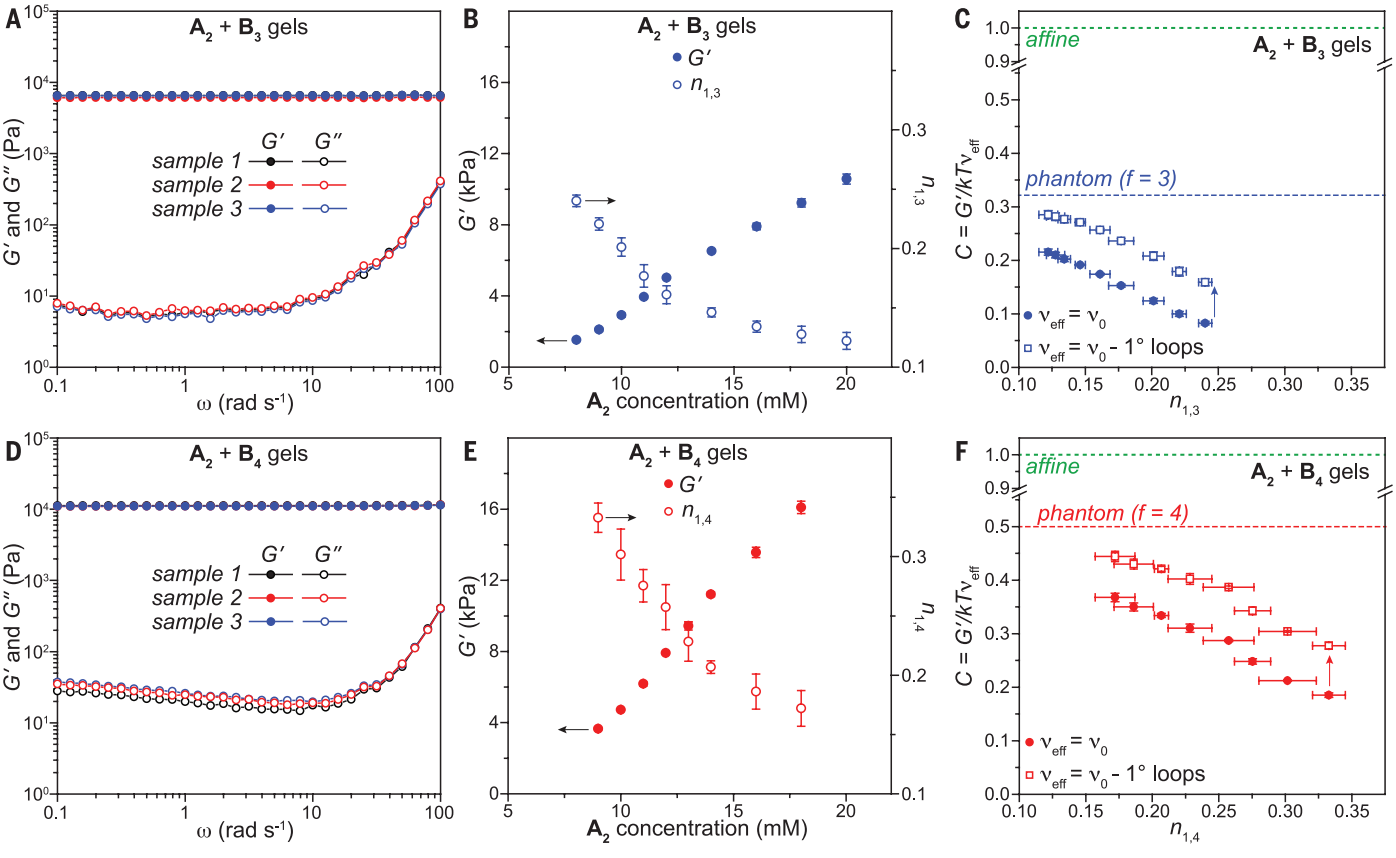

Fig. 2. Shear rheology and loop measurements in PEG gels. (**A** to **C**) Trifunctional ($A_2 + B_3$) gels: (A) Representative rheological curves demonstrating reproducibility of this gelation system (A_2 concentration for all samples, 14 mM). (B) G' and the average number of primary loops per junction in these $f = 3$ networks ($n_{1,3}$) versus A_2 concentration. Error bars of G' represent SD of three measurements; error bars of $n_{1,3}$ represent SD of nine measurements. (C) Experimentally estimated coefficient C with and without first-order affine correction. (**D** to **F**) Tetrafunctional ($A_2 + B_4$) gels: (D) Representative rheological curves demonstrating reproducibility of this gelation system (A_2 concentration for all samples, 14 mM). (E) G' and the average number of primary loops per junction in these $f = 4$ networks ($n_{1,4}$) versus A_2 concentration. Error bars of G' represent SD of three measurements; error bars of $n_{1,4}$ represent SD of nine measurements. (F) Experimentally estimated coefficient C with and without first-order affine correction.

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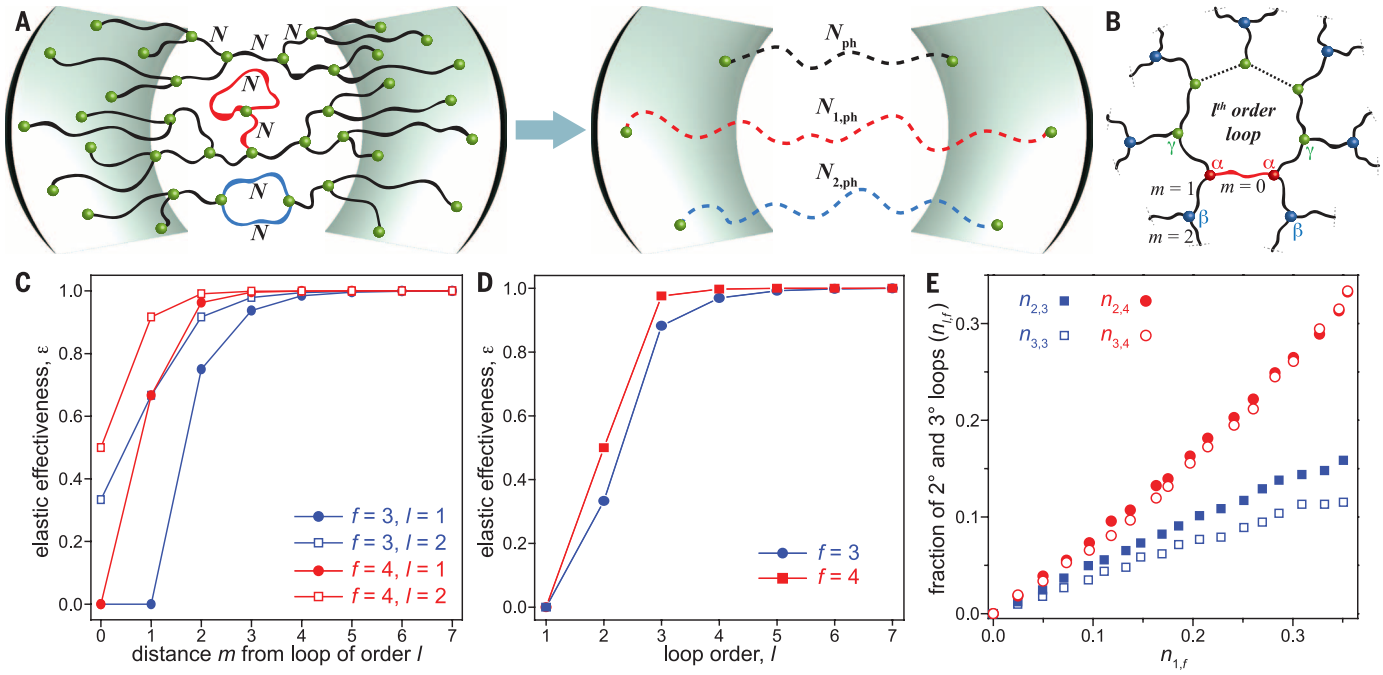

Fig. 3. Real elastic network theory (RENT). (**A**) Schematic depiction of the conversion of independent loops to a mixture of phantom strands with different effective length. (**B**) Schematic that illustrates the calculation of effective phantom length of strands in *l*th-order loops at a distance *m* from the loop. (**C**) Elastic effectiveness of polymer strands as a function of topological distance *m* from a primary ($l = 1$) and secondary ($l = 2$) loops in $f = 3$ or $f = 4$ networks. (**D**) Elastic effectiveness of polymer strands in loops of different order *l* for $f = 3$ and $f = 4$ networks. (**E**) Fraction of secondary and ternary loops ($n_{2,f}$ and $n_{3,f}$) as a function of the number of primary loops per junction ($n_{1,f}$) in $f = 3$ and $f = 4$ networks.

After measurement of G', we used SILDaS to measure the average number of primary loops per junction ($n_{1,f}$) in each gel. Basic hydrolysis cleaved the ester bonds near the chain ends of the polymer and converted the gel to soluble degradation products (*22*) (figs. S7 and S8). These particular products have isotopic labeling patterns with mass/charge ratios (m/z) differing by +8 that depend exactly on the number or primary loops in the parent gel. The relative abundance of each labeled degradation product was quantified by mass spectrometry; the ratios of the abundances were used to obtain $n_{1,f}$ [see (*22*) for details of the loop analysis procedure for both the $A_2 + B_3$ and the $A_2 + B_4$ gels]. As shown in Fig. 2, B and E, $n_{1,f}$ decreases with increased concentration for both $f = 3$ and $f = 4$ networks because of a reduced probability of intrajunction reaction at higher network concentration.

With this simultaneous knowledge of G' and $n_{1,f}$, the elasticity of the gels could be compared to theoretical models. An intuitive approach to obtain ν_{eff} is to simply subtract the primary loop density from the total number density of polymer strands in the gel, ν_0 [see (*22*) for detailed calculation], which is a first-order affine correction. Upon applying this correction, the data approach the phantom network prediction as $n_{1,f}$ decreases (Fig. 2, C and F); however, they are still clearly below the predictions of both phantom and affine network theory.

The results shown in Fig. 2, C and F, suggest that the simple affine correction for primary loops is not sufficient to reach agreement between measured G' and classical elasticity theories. Higher-order loop defects (Fig. 1, C and D) should also be incorporated; because these defects are fractionally elastically effective, simply subtracting their density would not be appropriate. Therefore, to quantitatively understand the effect of each type of topological defect on network elasticity, we developed a real elastic network theory (RENT) [see (*22*) for detailed derivation] that captures the effect of cyclic defects in the phantom network. For a loop-free system (fig. S9), the phantom network strands are ideal chains with N monomers; the ends of the strands are joined at junctions that are allowed to fluctuate around their average position. Junctions are connected to the nonfluctuating boundary of the network through repetitive branch connections, equivalent to a single effective phantom chain (Fig. 3A). However, in the real network, loops disturb the ideal repetitive tree structure: A loop junction is linked to other junctions, which reduces its effective connection to the boundary (Fig. 3A and figs. S10 to S13). Thus, loop junctions are less constrained than ideal nonloop junctions, which results in longer effective phantom lengths of the loop strands (Fig. 3A, right) or, equivalently, fewer elastically effective strands. The effective length m of strands adjacent to loops also increases because of their connection to the boundary paths through loop strands (Fig. 3B). To quantify these effects, RENT assumes that different cyclic defects are independent of each other; the gel can be envisioned as an "ideal loop gas," which enables an isolated treatment of each cyclic defect [the implications of this assumption are discussed below (*21*)]. The network is therefore a mixture of phantom strands with different effective length in parallel (Fig. 3A). The impact of each strand on the elastic modulus is linearly additive, yielding

$$\frac{G'}{kT} = \sum_i \frac{N}{N_{i,\mathrm{ph}}}\nu_i = \frac{f-2}{f}\sum_i \varepsilon_i \nu_i = \frac{f-2}{f}\left(\nu_{\mathrm{id}} + \sum_{l=1}^{\infty}\sum_{m=0}^{\infty}\nu_{l,f}^{m}\varepsilon_{l,f}^{m}\right) \quad (1)$$

where N is the actual degree of polymerization of each strand, and ν_i is the actual density of the type i strand in the network with effective phantom length $N_{i,\mathrm{ph}}$. The elastic effectiveness $\varepsilon_i = N_{i,\mathrm{ph}}/N_{\mathrm{ph}}$ is defined as the ratio of $N_{i,\mathrm{ph}}$ and the ideal phantom strand $N_{\mathrm{ph}} = fN/(f-2)$. Polymer strands are divided into the ideal strands (with density ν_{id} and elastic effectiveness $\varepsilon_{\mathrm{id}} = 1$) and nonideal strands (with density $\nu_{l,f}^{m}$ and elastic effectiveness $\varepsilon_{l,f}^{m}$, including both the loop strands and the strands adjacent to loops). The indices l and m denote the loop order and the topological distance of the strand from the loop (see Fig. 3B; $m = 0$ denotes the loop strand), respectively.

To predict G', the elastic effectiveness of nonideal strands in different loops and of different topological distances from the loops must

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be calculated. For primary and secondary loops ($l \le 2$), $\varepsilon_{l,f}^{m}$ can be calculated exactly (figs. S10 to S14), which results in

$$\varepsilon_{l=1,f}^{m=0} = 0 \quad (2)$$

$$\varepsilon_{l=2,f}^{m=0} = (f-2)/f \quad (3)$$

and

$$\varepsilon_{l=1,f}^{m\ge1} = 1 - \frac{2}{(f-2)(f-1)^{2m-1}} \quad (4)$$

$$\varepsilon_{l=2,f}^{m\ge1} = 1 - \frac{2}{f(f-2)(f-1)^{2m-1}} \quad (5)$$

The key to obtaining Eqs. 4 and 5 is to develop and solve the recursive relation that describes the propagation of a topological defect in the phantom network (*22*).

For larger loops ($l \ge 3$), it is difficult to obtain an exact solution of $\varepsilon_{l,f}^{m}$ because of the complex correlation between loop junctions; an approximate treatment is provided. We consider the general case, a strand (red strand in Fig. 3B) in an lth-order loop in the f-functional polymer network. The end junctions α of this loop strand are directly linked to two types of junctions. The first type is the ideal junction β, which is connected to the network boundary through repetitive branches. The second type consists of two loop junctions γ; these two junctions, as well as the loop junctions between them ($l - 2$ junctions in total), are simultaneously connected to the boundary through repetitive branches. The length of the equivalent phantom chain connected to junctions γ can be derived from the convolutional features of the Gaussian chain. Neglecting long-range correlations between junctions α through these $l - 2$ loop junctions, the elastic effectiveness of the targeted polymer strand (red strand in Fig. 3B) can be obtained by combining the contributions from junctions β and γ, which yields

$$\varepsilon_{l\ge3,f}^{m=0} = 1 - \frac{2}{(f-1)^{2(l-1)}+1} \quad (6)$$

The elastic effectiveness of the strand adjacent to the larger loops is similarly obtained as

$$\varepsilon_{l\ge3,f}^{m\ge1} = 1 - \frac{2}{(f-1)^{2(l+m-1)}+(f-1)^{2m-1}} \quad (7)$$

Equations 4 to 7 show that the effect of loops in reducing the elastic effectiveness of strands decreases rapidly with increasing loop order or distance from the loop (Fig. 3, C and D). The elastic effectiveness of small loops is much lower than 1, which implies that these strands will have a non-negligible negative impact on the gel modulus. In contrast, $\varepsilon(l \ge 4, m = 0, f) \approx 1$, which indicates that strands in larger loops ($l \ge 4$) behave nearly the same as ideal phantom chains (Fig. 3C). Furthermore, ε increases monotonically with f; the loop effect is more pronounced in networks with small junction functionality. Finally, the loop effect persists within only a few nearest-neighbor strands, as shown in Fig. 3D; strands far away from the loop ($m \ge 4$) behave as ideal phantom chains.

Substituting Eqs. 4 to 7 into Eq. 1 and rewriting the density of different nonideal strands $\nu_{l,f}^{m}$ in terms of the average number of loops per junction, $n_{l,f}$, an analytical expression can be produced for the shear modulus of a polymer network:

$$\frac{G'}{\nu_0 kT} = \frac{f-2}{f}\left(1 - A_f n_{1,f} - B_f n_{2,f} - \sum_{l=3}^{\infty} C_{l,f} n_{l,f}\right) \quad (8)$$

with

$$A_f = \begin{cases} 5/2 & \text{for } f=3 \\ 4/3 & \text{for } f=4 \\ 2/(f-2) & \text{for } f\ge5 \end{cases} \quad (9)$$

$$B_f = \frac{4(f-1)}{f^2(f-2)} \quad (10)$$

and

$$C_{l,f} = \frac{4}{f}\left[\frac{1}{(f-1)^{2l-2}+1} + \frac{1}{(f-1)^{2l-1}-1}\right] \quad (11)$$

where ν_0 is the total number density of polymer strands in the gel (loop or nonloop).

From Eqs. 8 to 11, to fully describe the effects of topological defects on the modulus, it is necessary to know the density of loops of order l in the given network. As described above, SILDaS can precisely measure the primary loop fraction, $n_{1,f}$. We have previously shown that that there is a one-to-one correspondence between the density of higher-order loops and the primary loop density (*21*). Therefore, $n_{2,f}$ and $n_{3,f}$ can be calculated once $n_{1,f}$ is measured [see (*22*) for Monte Carlo simulation algorithm] as shown in Fig. 3E.

With knowledge of G' and the densities of primary, secondary, and ternary loops, we applied RENT to calculate the effect of topological defects on G'; excellent agreement between the experimental $G'/\nu_0 kT$ (Fig. 4, black circles) and RENT theory (Fig. 4, black lines) for low $n_{1,f}$ values ($n_{1,3} < 0.20$ and $n_{1,4} < 0.28$) was observed. Although primary loops play the dominant role in reducing $G'/\nu_0 kT$ because of their zero elastic effectiveness (see Fig. 3C), Fig. 4 shows that consideration of primary loops alone in RENT (blue lines, Fig. 4) does not give complete agreement with experiment; higher-order loops should be included to give the best agreement. RENT also shows good agreement with simulation and experimental data from other research groups (figs. S15 and S16) (*25*–*27*).

Although RENT and experimental data show excellent agreement at low $n_{1,f}$ values, there is a deviation between experiment and theory as $n_{1,f}$ becomes large. This deviation is due to our assumption that loops can be treated as independent within an "ideal loop gas." As the fraction of loops in the networks becomes large, loops will become close to each other. In this case, the rules for elastic effectiveness as a function of m and l (as given in Fig. 3) break down; cooperative effects between loops that are close in space may have a greater negative impact on elasticity than a linear combination of isolated loops in an "ideal loop gas." Notably, the deviation between RENT and experiment for both $A_2 + B_3$ and $A_2 + B_4$ networks occurs at an A_2 concentration of 12 to 13 mM, which is very close to the estimated polymer overlap concentration c^* for our PEG macromers ($c^* \approx$ 13 mM) (*12*). We note that in most applications of gels, synthetic conditions are used (e.g., concentration > c^*; semidilute regime) that would avoid large numbers of loops; RENT as derived here should apply, given also that contributions from entanglements are minimal. Nonetheless,

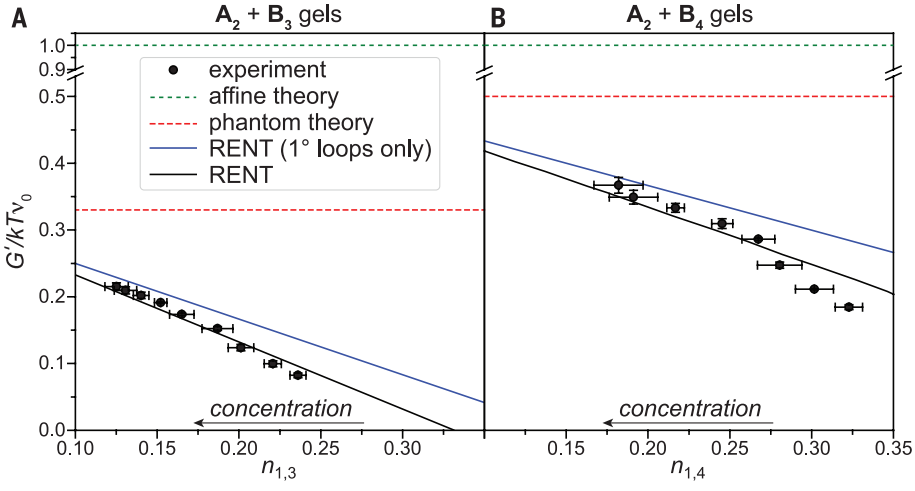

Fig. 4. Elastic moduli predicted by RENT. Comparison of experimentally determined $G'/kT\nu_0$ (black circles) to that predicted by affine theory (green dashed line), phantom theory (green dashed line), RENT with only a correction for primary loops (blue line), and RENT accounting for primary, secondary, and tertiary loops (black line). (**A**) $f = 3$ networks. (**B**) $f = 4$ networks. Error bars represent SD of nine measurements.

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this work provides an important advance in quantitative network theory and a foundation for further development of theories that account for interacting network defects and entanglements.

Combining RENT with primary and higher-order loop measurements provides quantitative agreement with measured elastic moduli for networks with compositions and structures that are relevant to common applications. In other words, RENT can predict the bulk mechanical properties of polymer networks on the basis of molecular information. We anticipate that RENT and loop-counting methods will be applicable to a wide range of polymer networks.

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NANOMATERIALS

1D nanocrystals with precisely controlled dimensions, compositions, and architectures

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The ability to synthesize a diverse spectrum of one-dimensional (1D) nanocrystals presents an enticing prospect for exploring nanoscale size- and shape-dependent properties. Here we report a general strategy to craft a variety of plain nanorods, core-shell nanorods, and nanotubes with precisely controlled dimensions and compositions by capitalizing on functional bottlebrush-like block copolymers with well-defined structures and narrow molecular weight distributions as nanoreactors. These cylindrical unimolecular nanoreactors enable a high degree of control over the size, shape, architecture, surface chemistry, and properties of 1D nanocrystals. We demonstrate the synthesis of metallic, ferroelectric, upconversion, semiconducting, and thermoelectric 1D nanocrystals, among others, as well as combinations thereof.

The synthesis of isotropic nanomaterials has provided access to an array of nanoparticles with controlled sizes, shapes, and functionalities. Going beyond zero-dimensional nanoparticles (*1*, *2*), one-dimensional (1D) nanocrystals such as nanorods (*3*), nanotubes (*4*), nanowires (*5*), and shish kebab–like heterostructures (*6*, *7*) exhibit a range of properties [e.g., optical (*8*), electrical (*9*), magnetic (*10*), and catalytic (*11*)] depending on their size and shape. Emerging approaches, including template-assisted synthesis (*12*–*14*), chemical vapor deposition (*15*), and colloidal synthesis (*16*), enable the preparation of intriguing 1D nanocrystals with controlled dimensions. However, some of these procedures require tedious multistep reactions and purification processes and rigorous experimental conditions; in addition, they are difficult to generalize.

In contrast to previously reported polymer brushes for the synthesis of nanowires (*12*), our bottlebrush-like block copolymers (BBCPs) are unimolecular cylindrical polymer brushes that have a cellulose backbone with densely grafted functional block copolymers as side chains (arms), which consist of multiple compartments (inner and intermediate blocks for templating nanocrystal growth and outer blocks for solubility). Cellulose forms a rigid backbone because of intramolecular hydrogen bridges between hydroxyl groups and oxygen atoms (*17*). In addition, the three substitutable hydroxyl groups on cellulose allow dense polymer side chains to be grafted from the cellulose backbone. We used these advantages inherent in cellulose to synthesize our straight cylindrical BBCPs (figs. S1 to S8 and supplementary text, sections I to III). Each family of BBCPs was synthesized by sequential atom transfer radical polymerization (ATRP) or ATRP followed by a click reaction (Fig. 1 and figs. S9 to S11).

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Plain nanorods of varied diameters and compositions were synthesized using amphiphilic cellulose-*g*-(PAA-*b*-PS) {cellulose-*graft*-[poly(acrylic acid)-*block*-polystyrene]} BBCP nanoreactors (Fig. 1A; table S2; and supplementary text, sections III and IV). Because each unit in the cellulose backbone enables the growth of three P*t*BA-*b*-PS diblocks (upper left panel in Fig. 1A; supplementary text, sections I and II), the densely grafted P*t*BA-*b*-PS arms in conjunction with the rigidity of the backbone force the cellulose-*g*-(P*t*BA-*b*-PS) BBCP to adopt a straight, rigid, cylindrical conformation (upper right panel in Fig. 1A) [P*t*BA, poly(*tert*-butyl acrylate)]. Subsequent hydrolysis of the P*t*BA blocks yields amphiphilic cellulose-*g*-(PAA-*b*-PS) with inner hydrophilic PAA blocks and outer hydrophobic PS blocks (lower right panel in Fig. 1A and fig. S47). When cellulose-*g*-(PAA-*b*-PS) BBCPs are dispersed in dimethylformamide (DMF) polar solvent (*18*), the resulting unimolecular micelles can be used as nanoreactors. The interaction between highly polar DMF and the inner PAA blocks is stronger than that between DMF and the outer PS blocks. Thus, there is a greater repulsion between PAA chains in DMF than between PS chains (*18*). As a result, the PAA chains are greatly stretched and form a large compartment. On addition and dispersion of inorganic precursors into the solution, the cylindrical compartment containing PAA blocks can accommodate a large volume of precursors. In addition to the solvent polarity effect noted above, the precursors are also preferentially partitioned in the cylindrical PAA block compartment as a result of the strong coordination interaction between the metal moieties of precursors and the carboxylic acid groups of PAA, which creates a high enough concentration of precursors to initiate the nucleation and growth of inorganic nanorods (*19*) (figs. S38 to S46 and S71; see supplementary text, section IV, for the proposed formation mechanisms). The in situ capping of PS chains on the nanorods facilitates dispersion and solubility in various organic solvents (lower left panel in Fig. 1A).

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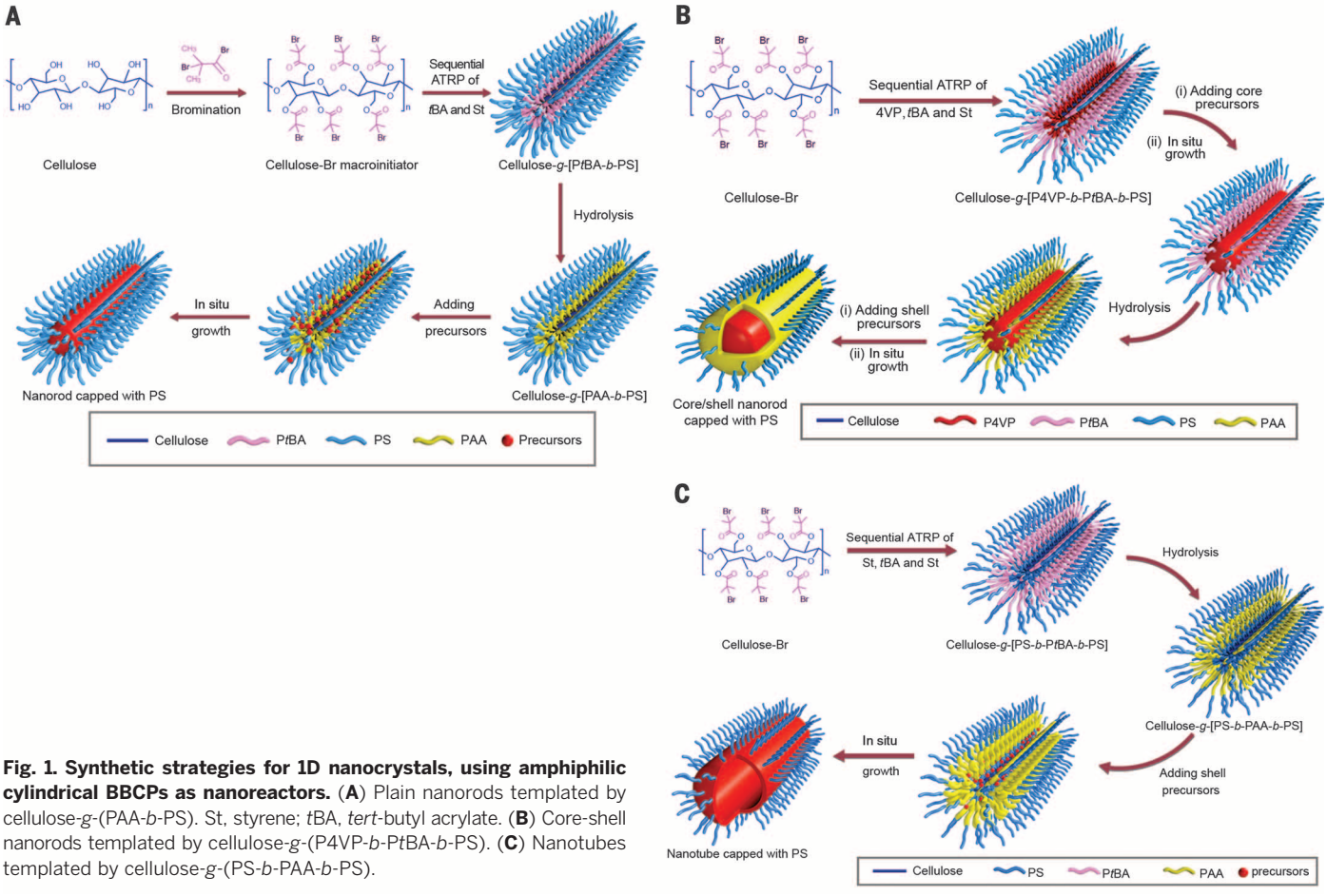

Fig. 1. Synthetic strategies for 1D nanocrystals, using amphiphilic cylindrical BBCPs as nanoreactors. (**A**) Plain nanorods templated by cellulose-*g*-(PAA-*b*-PS). St, styrene; *t*BA, *tert*-butyl acrylate. (**B**) Core-shell nanorods templated by cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PS). (**C**) Nanotubes templated by cellulose-*g*-(PS-*b*-PAA-*b*-PS).

As a proof of concept, we synthesized upconversion $NaYF_4$:Yb/Er nanorods. Transmission electron microscopy (TEM) images at different magnifications (Fig. 2A) demonstrate the formation of uniform $NaYF_4$:Yb/Er nanorods with an average diameter D of 9.8 ± 0.5 nm and length L of 97 ± 9 nm. These nanorods are highly crystalline and have a hexagonal phase, as established by high-resolution TEM (HRTEM; Fig. 2A) and x-ray powder diffraction (XRD) measurements (fig. S60). Energy-dispersive x-ray spectroscopy (EDS) confirmed the composition of the $NaYF_4$:Yb/Er nanorods (fig. S63). Thermogravimetric analysis (TGA) showed that the volume percentage of PAA blocks encapsulated by $NaYF_4$:Yb/Er nanorods was ~11.2% (fig. S54).

As a living free-radical polymerization technique, ATRP affords excellent molecular weight (MW) control (*20*), enabling the precise design of BBCPs with a tunable MW and narrow MW distribution for each constituent block (table S2 to S10). Consequently, the diameter of 1D plain nanorods is dictated by the length of the hydrophilic inner block in BBCPs, which can be regulated by tuning the polymerization time of the different blocks during ATRP. Moreover, the solubility of 1D nanocrystals (organic solvent–soluble or water-soluble) is rendered by the outer blocks of the BBCP brushes, which are covalently bonded to the inner blocks. Lastly, the length of 1D nanocrystals can be adjusted by varying the length of the macroinitiator (denoted cellulose-Br; supplementary text, section I).

To obtain a specific length of cellulose-Br macroinitiator, natural cellulose modified with 2-bromoisobutyryl bromide in a mixed solvent of ionic liquid, anhydrous 1-methyl-2-pyrrolidione (NMP), and DMF was purified by fractional precipitation (figs. S1 to S3; table S1; and supplementary text, section I). Taking noble metallic Au nanorods as an example, facile control over the nanorod length and diameter was achieved by deliberately tuning the length of the cellulose-Br macroinitiator and the MW of the PAA blocks, respectively, in cellulose-*g*-(PAA-*b*-PS) (Fig. 2B). As the MW of cellulose-Br increased from 11.2 × 10^3 to 79.6 × 10^3 g/mol, the length of the Au nanorods increased from 51 ± 4 to 414 ± 39 nm. By increasing the MW of the PAA block from 5.2 × 10^3 to 11.2 × 10^3 g/mol, the diameter of the Au nanorods increased from 10.4 ± 0.6 to 21.2 ± 1.5 nm.

Conceptually, because many appropriate precursors are amenable to the cylindrical unimolecular nanoreactor strategy, a variety of uniform nanorods can be created. Figure 3 shows noble metallic Au (figs. S12, S55, and S66) and Pt (fig. S13), ferroelectric $BaTiO_3$ (fig. S18), upconversion $NaYF_4$:Yb/Er (figs. S14, S15, and S58) and $NaYF_4$:Yb/Tm (figs. S16, S17, and S59), semiconducting CdSe (figs. S19 and S57), thermoelectric PbTe (figs. S20 and S67), and magnetic Fe_3O_4 (fig. S21) nanorods synthesized by our cylindrical cellulose-*g*-(PAA-*b*-PS) nanoreactor approach. The possible mechanisms for the growth of these plain nanorods are shown in figs. S38 to S42 (supplementary text, section IV). Their crystalline lattices are shown as insets in Fig. 3 and fig. S52, with their crystal structures and compositions substantiated by XRD (fig. S60) and EDS measurements (fig. S63) (supplementary text, section IX). Because the nanorods are capped with PS chains that prevent their aggregation (figs. S48 to S50), they are homogeneously soluble in a wide range of organic solvents (e.g, toluene and chloroform) (fig. S53). The excess precursors that are present outside the cylindrical BBCP nanoreactor can easily form large, irregular, inorganic materials because of the lack of surface capping by PS chains, and thus they readily precipitate from organic solvents when subjected to low-speed centrifugation. Alternatively, the upper solution containing the 1D nanocrystals can also be directly retrieved. Our nanoreactor strategy is effective in producing relatively pure 1D nanocrystals (figs. S51 and S70 and supplementary text, section V).

A double-hydrophilic cylindrical cellulose-*g*-(PAA-*b*-PEG) BBCP was synthesized by a combination of ATRP and a click reaction (fig. S9; table S3; and supplementary text, section II) [PEG, poly(ethylene glycol)]. Using this as a nanoreactor, we created a series of water-soluble PEG-capped

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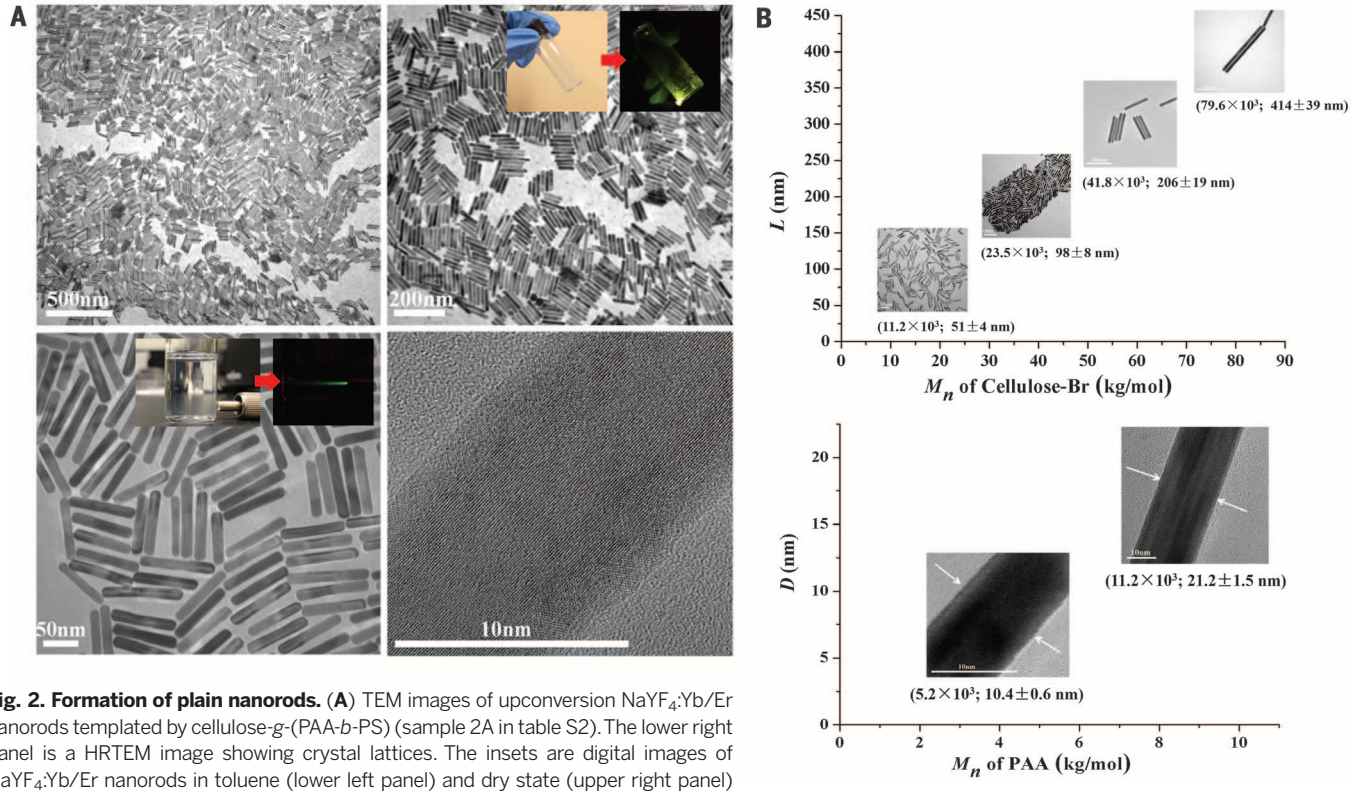

Fig. 2. Formation of plain nanorods. (**A**) TEM images of upconversion $NaYF_4$:Yb/Er nanorods templated by cellulose-*g*-(PAA-*b*-PS) (sample 2A in table S2). The lower right panel is a HRTEM image showing crystal lattices. The insets are digital images of $NaYF_4$:Yb/Er nanorods in toluene (lower left panel) and dry state (upper right panel) before (left) and after (right) exposure to a 980-nm near-infrared laser. (**B**) The dimensional tunability of 1D Au nanorods is shown as an example. The upper panel shows the dependence of the length *L* of Au nanorods on the molecular weight M_n of cellulose-Br macroinitiator (table S1). The lower panel shows the dependence of the diameter *D* of Au nanorods (marked by arrows in the insets) on the molecular weight of PAA block in BBCP (table S2).

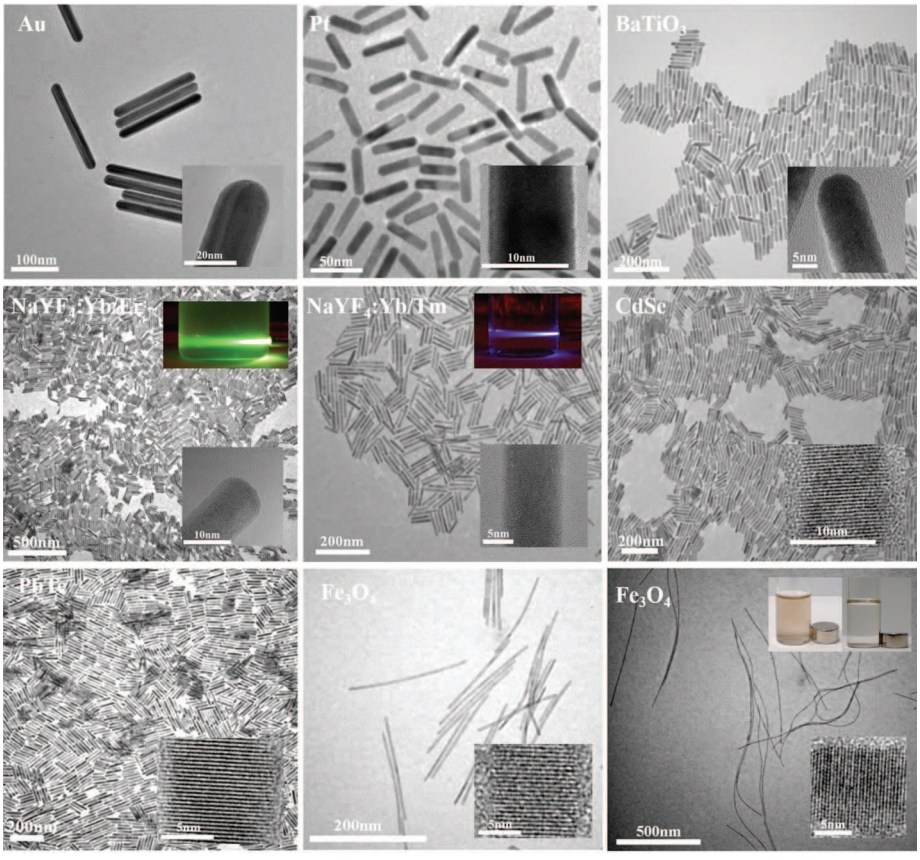

Fig. 3. TEM images of a variety of plain nanorods templated by cellulose-*g*-(PAA-*b*-PS). The dimensions of these nanorods are as follows: noble metallic Au, L = 206 ± 19 nm and D = 21.2 ± 1.5 nm from sample 3B; noble metallic Pt, L = 48 ± 5 nm and D = 10.2 ± 0.6 nm from sample 1A; ferroelectric $BaTiO_3$, L = 101 ± 8 nm and D = 10.6 ± 0.8 nm from sample 2A; upconversion $NaYF_4$:Yb/Er (green-emitting), L = 99 ± 10 nm and D = 9.6 ± 0.4 nm from sample 2A; upconversion $NaYF_4$:Yb/Tm (blue-emitting), L = 103 ± 7 nm and D = 10.4 ± 0.5 nm from sample 2A; semiconducting CdSe, L = 98 ± 9 nm and D = 10.1 ± 0.7 nm from sample 2A; thermoelectric PbTe, L = 102 ± 10 nm and D = 9.9 ± 0.6 nm from sample 2A; magnetic Fe_3O_4, L = 203 ± 16 nm and D = 10.2 ± 0.8 nm from sample 3A and L = 916 ± 87 nm and D =10.3 ± 0.5 nm from sample 5A. Insets at the bottom of each panel are HRTEM images showing the crystal lattice of the sample. The upper insets in the middle-left and center panels are digital images of green-emitting $NaYF_4$:Yb/Er and blue-emitting $NaYF_4$:Yb/Tm nanorods, respectively, under near-infrared laser illumination (980 nm at 2 W). The upper insets in the bottom right panel are digital images demonstrating the magnetic properties of Fe_3O_4 nanorods as they were deposited on the wall of vial (right) by a magnetic bar. Further details about the samples are given in table S2.

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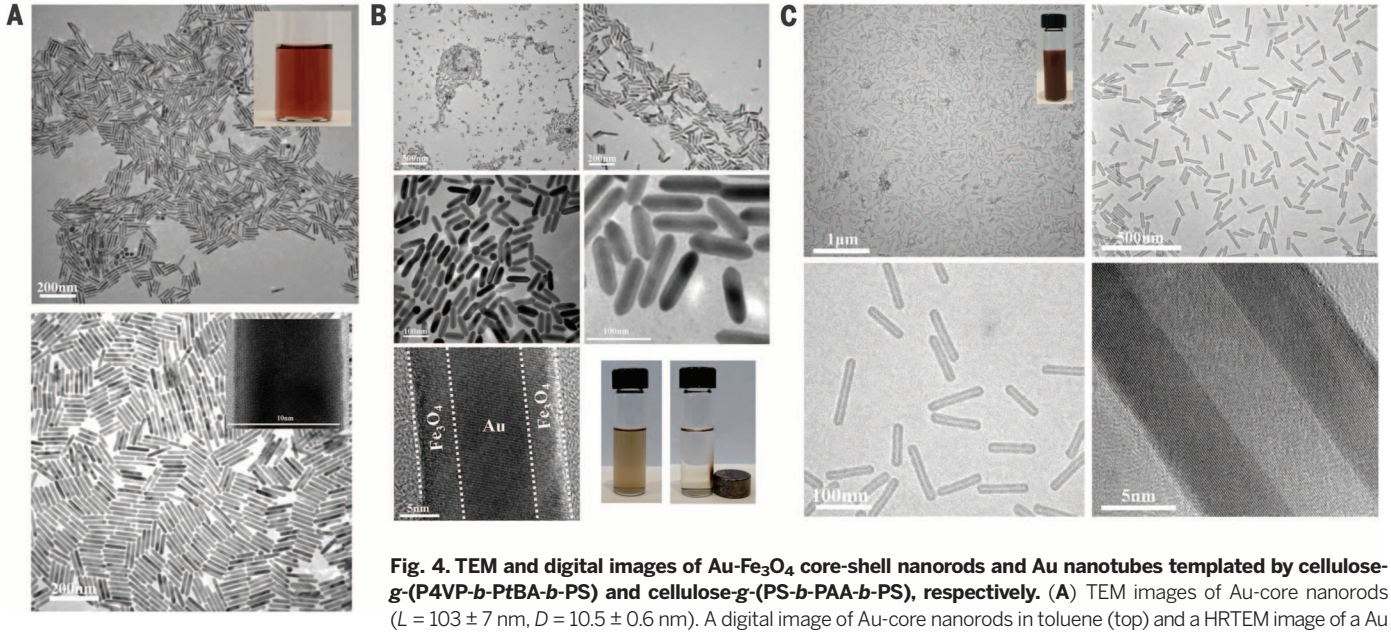

Fig. 4. TEM and digital images of Au-Fe_3O_4 core-shell nanorods and Au nanotubes templated by cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PS) and cellulose-*g*-(PS-*b*-PAA-*b*-PS), respectively. (**A**) TEM images of Au-core nanorods (L = 103 ± 7 nm, D = 10.5 ± 0.6 nm). A digital image of Au-core nanorods in toluene (top) and a HRTEM image of a Au nanorod (bottom) are shown as insets. (**B**) TEM images of Au-Fe_3O_4 nanorods (Fe_3O_4 shell thickness t = 4.6 ± 0.4 nm) (sample 2B; table S4). The HRTEM image (bottom left) shows the crystal lattice of the Au core and Fe_3O_4 shell (white dashed lines for guidance). The bottom right panel shows digital images demonstrating the magnetic properties of Au-Fe_3O_4 nanorods. (**C**) TEM images of Au nanotubes at different magnifications (L = 103 ± 12 nm, t = 5.1 ± 0.5 nm, hollow interior D = 5.3 ± 0.4 nm) (sample 2A; table S6). The inset is a digital image of Au nanotubes in toluene.

plain Au (figs. S22, S23, and S56), $NaYF_4$:Yb/Er (fig. S24), and Fe_3O_4 (fig. S25) nanorods (supplementary text, section III). These water-soluble 1D nanocrystals are pertinent to a number of scientific areas, including self-assembly, bioimaging, and biosensors.

Our cylindrical BBCP nanoreactor strategy also affords a platform to synthesize high-quality core-shell nanorods composed of two different materials that have large lattice mismatches. For the synthesis of high-quality core-shell nanoparticles, a moderate lattice mismatch (<2%) between two dissimilar materials is necessary for successful epitaxial growth (*21*). This greatly limits the core and shell material choices for sequential epitaxial growth (*22*). Furthermore, in comparison with core-shell nanoparticles, the effective methods to produce core-shell nanorods are few and limited in scope (*23*).

The creation of core-shell nanorods with controlled dimensions (core diameter, shell thickness, and length), compositions, and solubility is enabled by using a class of cylindrical BBCPs comprising densely grafted amphiphilic triblock copolymer side chains. Specifically, cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PS) and cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PEG) can serve as organic-soluble and water-soluble nanoreactors, respectively (supplementary text, section III) [PV4P, poly(4-vinylpyridine)]. Similar to the synthesis of plain nanorods, in polar DMF solvent, precursors are selectively partitioned in the corresponding template compartments of the triblock copolymer–containing BBCPs by means of a favorable coordination interaction between the functional groups of the template blocks and the metal moieties of the precursors. In succession, the core and shell components of the nanorod can be grown.

We describe the synthesis of nanorods with a noble metallic Au core and a magnetic Fe_3O_4 shell as an example to demonstrate the effectiveness of cylindrical cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PS) BBCPs (upper right panel in Fig. 1B) as nanoreactors in producing organic solvent–soluble core-shell nanorods (supplementary text, section III). The Au-core nanorod with L = 103 ± 7 nm and D = 10.5 ± 0.6 nm (Fig. 4A and fig. S68) was synthesized in DMF through preferential partitioning of Au precursors in the compartment of the inner P4VP blocks, whose pyridyl groups imparted and coordinated with a large population of precursors (central panel in Fig. 1B). The possible formation mechanism is illustrated in fig. S43. Subsequently, the P*t*BA blocks of the P*t*BA-*b*-PS capping on the surface of the Au-core nanorods were hydrolyzed into PAA blocks (supplementary text, section III), thus templating the formation of the Fe_3O_4 shell in DMF in a similar fashion to that of the Au core (fig. S44). The resulting Au-Fe_3O_4 core-shell nanorods capped by PS exhibited uniform dimensions. The HRTEM image clearly shows the dark crystalline Au core surrounded by a shell of relatively lighter Fe_3O_4 (4.6 ± 0.4 nm thick) (Fig. 4B). The XRD and EDS measurements further verified the crystal structure and composition of Fe_3O_4 and Au, respectively (figs. S61 and S64). It is important to note that despite a lattice mismatch of more than 50% between Fe_3O_4 and Au (*24*), Au-Fe_3O_4 core-shell nanorods were successfully created by capitalizing on the cylindrical cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PS) BBCP nanoreactor. Many other material combinations can also be prepared to produce organic solvent–soluble core-shell nanorods (e.g., magnetic-metallic Fe_3O_4-Au, shown in fig. S26, and metallic-semiconducting Au-TiO_2, shown in figs. S27, S28, and S45).

Similarly, the use of the cylindrical cellulose-*g*-(P4VP-*b*-P*t*BA-*b*-PEG) BBCP (supplementary text, section III; upper right panel in fig. S10) as a nanoreactor yielded water-soluble core-shell nanorods (lower left panel in fig. S10), such as metallic-semiconducting Au-TiO_2 (figs. S29 and S30) and metallic-upconversion Au-$NaYF_4$:Yb/Er (fig. S31), each with hydrophilic PEG blocks directly tethered to their surface (table S5 and supplementary text, section III). Likewise, the crystal structures and compositions of these nanorods were confirmed by XRD and EDS measurements (supplementary text, section IX).

The core diameter and shell thickness of nanorods can be precisely tuned by varying the MW of the inner P4VP block and the intermediate P*t*BA block. These are controlled by mediating the sequential ATRP polymerization times. A uniform nanorod length is attained by controlling the length of the cellulose-Br macroinitiator through fractional precipitation. Because the core and shell materials can be grown independently in their respective templates, the lattice structure of the synthesized shell material can be completely independent of the core material. This cylindrical BBCP nanoreactor strategy can virtually eliminate the restriction on lattice matching requirements (*21*). Thus, our cylindrical BBCP composed of amphiphilic triblock copolymer arms can be used to create a new class of exotic core-shell nanorods that would otherwise be challenging to obtain. Through the precise tailoring of various core and shell combinations, it is possible to explore potentially new coupled size- and shape-dependent properties.

The amphiphilic cylindrical BBCP nanoreactor strategy can also be used to synthesize uniform nanotubes (hollow nanorods) by selectively

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restricting the appropriate precursors to the intermediate B block of an A-*b*-B-*b*-A BBCP triblock copolymer nanoreactor. The inside and outside surfaces of the nanotubes are capped by inner and outer A blocks. As an example, organic solvent–soluble Au nanotubes (table S6 and supplementary text, section III) were synthesized by using amphiphilic cellulose-*g*-(PS-*b*-PAA-*b*-PS) as a nanoreactor (upper right panel in Fig. 1C and fig. S69). The Au precursors were sequestered in the compartment containing the intermediate PAA blocks and ultimately formed PS-capped Au nanotubes (lower left panel in Fig. 1C). In Fig. 4C, the center of the nanotubes appears brighter, signifying that they are hollow. The HRTEM image (lower right panel in Fig. 4C) and XRD pattern (fig. S62) suggest that the nanotubes are highly crystalline. Moreover, EDS measurements further corroborate the successful formation of Au nanotubes (fig. S65). The diameter of the hollow interior and the thickness of the nanotube can be controlled by tailoring the MWs of the inner PS block and intermediate P*t*BA block during sequential ATRP. Thus, an assortment of nanotubes with different sizes and compositions can be produced, including upconversion $NaYF_4$:Yb/Er nanotubes (figs. S32 and S33) and semiconducting TiO_2 nanotubes (figs. S34 and S46). Despite the uniform diameter and thickness, there was a distribution of Au nanotube lengths (Fig. 4C) because of the different lengths of the individual cellulose-Br macroinitiators used to prepare the cellulose-*g*-(PS-*b*-PAA-*b*-PS) nanoreactors. However, cellulose-Br macroinitiators with uniform lengths can be realized by fractional precipitation. Likewise, by using cellulose-*g*-(PS-*b*-PAA-*b*-PEG) nanoreactors (fig. S11), water-soluble nanotubes (e.g., Au, TiO_2, and $NaYF_4$:Yb/Er nanotubes in figs. S35, S36, and S37, respectively) can also be synthesized (table S7 and supplementary text, section III).

All of the 1D nanocrystals that we produced (Figs. 2 to 4) had round ends. This is not surprising, given that each cellulose backbone was heavily grafted with diblock or triblock copolymer arms. These arms can stretch out at the two ends of the cylindrical BBCPs because of the available space there. Moreover, the two ends of the cellulose backbone have two hydroxyl groups, which allows for the growth of a diblock or triblock copolymer arm at each end. Together, the brushes on the ends of the cylindrical BBCPs have a hemispherical chain conformation. This leads to the formation of hemisphere-shaped nanocrystals situated at both ends of the 1D nanocrystals. The reaction temperature for the synthesis of nanorods was lower than the degradation temperature T_d of nanoreactors measured by TGA [e.g., T_d = 210°C for cellulose-g-(PAA-*b*-PS)] (fig. S54). Thus, the polymer templates are likely encased by 1D nanocrystals.

We have developed a general and robust strategy for the synthesis of a variety of 1D nanocrystals in a way that allows high-level control over dimension, anisotropy, composition, surface chemistry, and architecture. Central to this effective strategy is the rational design and synthesis of functional BBCPs—composed of a cellulose backbone densely grafted with diblock or triblock copolymers of precisely tunable lengths—that serve as nanoreactors. All of these 1D nanocrystals can be used as building blocks in the bottom-up assembly of nanostructured materials and devices with desirable characteristics (enabled by the properties of individual nanocrystals and their proper spatial arrangement) for use in optics, electronics, optoelectronics, magnetic technologies, sensors, and catalysis, among other applications. They can also serve as model systems for fundamental research in self-assembly, phase behavior, and crystallization kinetics of nanocrystals (*25*).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S71
Tables S1 to S10
References (*26*–*42*)

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MARINE MICROBIOME

Decoupling function and taxonomy in the global ocean microbiome

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Microbial metabolism powers biogeochemical cycling in Earth's ecosystems. The taxonomic composition of microbial communities varies substantially between environments, but the ecological causes of this variation remain largely unknown. We analyzed taxonomic and functional community profiles to determine the factors that shape marine bacterial and archaeal communities across the global ocean. By classifying >30,000 marine microorganisms into metabolic functional groups, we were able to disentangle functional from taxonomic community variation. We find that environmental conditions strongly influence the distribution of functional groups in marine microbial communities by shaping metabolic niches, but only weakly influence taxonomic composition within individual functional groups. Hence, functional structure and composition within functional groups constitute complementary and roughly independent "axes of variation" shaped by markedly different processes.

Microbial communities drive global biogeochemical cycling (*1*). Bacteria and archaea, for example, strongly influence marine carbon, nitrogen, and sulfur fluxes, thereby modulating global ocean productivity and climate (*2*, *3*). Elucidating the processes that shape microbial communities over space and time is important for predicting how biogeochemical cycles will change with changing environmental conditions.

Taxonomic microbial community profiling can reveal intriguing, but often unexplained, variation

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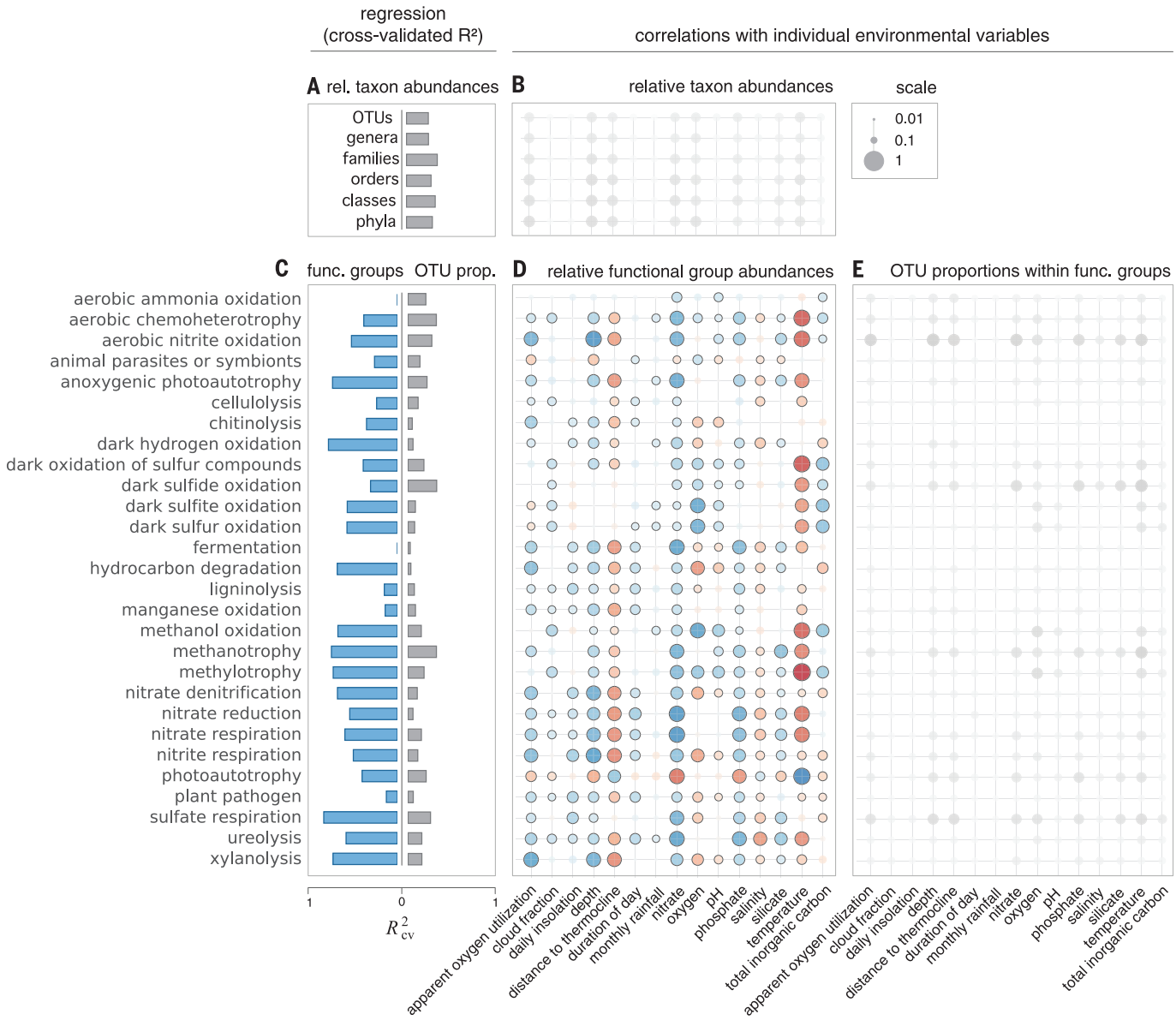

Fig. 1. Functional versus taxonomic predictability. (**A**) Cross-validated coefficients of determination (R^2_{cv}) for relative taxon abundances, achieved by regression based on environmental predictor variables, averaged across taxa. (**B**) Absolute rank correlations between environmental variables and relative taxon abundances, at various taxonomic levels (one circle per variable and per taxonomic level). Circle surface area and color saturation are proportional to the absolute correlation. (**C**) R^2_{cv} achieved by regression for relative functional group abundances (blue bars) as well as for OTU proportions within each functional group (gray bars, averaged across OTUs in each group). (**D**) Rank correlations between environmental variables and relative functional group abundances. Blue and red colors indicate positive and negative correlations, respectively. Significant correlations ($P < 0.05$) are indicated by a black perimeter. (**E**) Absolute rank correlations between environmental variables and OTU proportions within each group, averaged across OTUs. For full distributions of R^2_{cv} across taxonomic levels, see figs. S2 and S3. For full distributions of correlations across taxonomic levels, see figs. S24 to S28.

between environments (*4*). Differences in metabolic function among organisms are thought to underlie much of this variation as a result of selection for specific metabolic pathways based on physicochemical conditions ("metabolic niche effects" or "environmental filtering"). However, other factors such as biotic interactions (*5*–*7*), limits to spatial dispersal (*4*), and neutral demographic drift (*8*) could also affect community composition. Moreover, distantly related microbes can often perform similar metabolic functions, further complicating a mechanistic interpretation of taxonomic community variation (*9*). Direct estimates of functional potential in terms of community gene content using environmental shotgun sequencing—or metagenomics—have revealed correlations between the distribution of particular metabolic pathways and environmental conditions (*2*, *10*). However, it is not known how metabolic niche effects interact with other community assembly mechanisms, nor how variation in taxonomic composition relates to ecosystem function. Ideally, one would hope to split community variation into distinct components ("axes of variation"), each of which relates to different ecological processes.

Here, we combine taxonomic and functional profiling of more than 100 bacterial and archaeal communities across the global ocean to elucidate the role of environmental filtering, global functional redundancy, and dispersal limitation in shaping marine microbial communities. We generated taxonomic profiles based on shotgun DNA sequences of the 16*S* ribosomal gene, a standard marker gene in microbial ecology (*11*). Functional profiles were generated by associating individual organisms with metabolic functions of particular ecological relevance, such as photoautotrophy and nitrate respiration, using an annotation database

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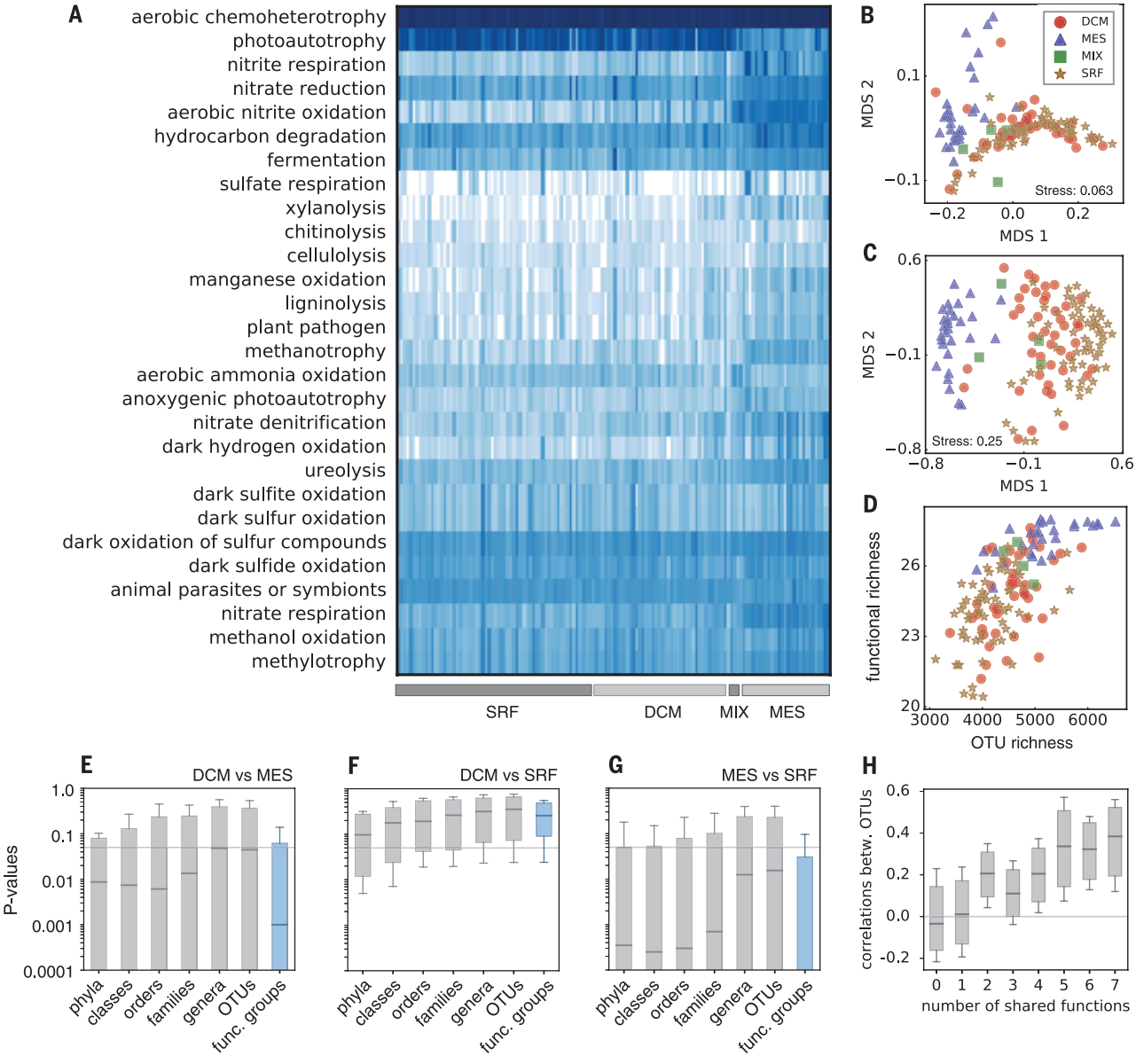

Fig. 2. Environmental filtering of microbial communities in the global ocean. (**A**) Functional community profiles, with samples ordered according to water column zone. SRF, surface water; DCM, deep chlorophyll maximum; MIX, mixed layer; MES, mesopelagic (*11*). Darker colors correspond to higher relative abundances. (**B** and **C**) Metric multidimensional scaling (MDS) of microbial communities (one point per sample) based on Bray-Curtis dissimilarities in terms of functional groups (B) and OTUs (C). Points in greater proximity correspond to similar communities. (**D**) Community richness in terms of functional groups and OTUs (one point per sample) after rarefaction. (**E** to **G**) Box plots of statistical significances of the segregation of taxa or functional groups between water column zones: DCM versus MES (E), DCM versus SRF (F), and MES versus SRF (G). The horizontal line at 0.05 is shown for reference. (**H**) Box plots of pairwise rank correlations between relative OTU abundances, depending on the number of shared functions. In (E) to (H), whisker bars extend between the 17th and 83th percentiles.

that we created on the basis of experimental literature. We then explored taxonomic composition, functional potential, and taxonomic composition within individual functional groups in relation to environmental conditions and geographical location.

We performed regression modeling of the relative abundances of functional groups, as well as the proportions of various operational taxonomic units (OTUs) within each functional group, using 15 key abiotic environmental variables such as dissolved oxygen, salinity, temperature, and depth (table S1). For each OTU or functional group, we used a stepwise model selection algorithm to choose the appropriate subset of environmental variables that maximized predictive power while avoiding unrelated variables. In general, environmental conditions strongly predicted the functional profiles of microbial communities but weakly predicted the taxonomic composition within each functional group, as measured by the cross-validated coefficient of determination of the models (R^2_{cv}). In particular, the R^2_{cv} for the relative abundances of almost all functional groups [mean R^2_{cv} = 0.48 ± 0.27 (SD) over all functions] exceeded the average R^2_{cv} achieved for the OTU proportions within those groups (mean R^2_{cv} = 0.15 ± 0.09 over all functions; Fig. 1C). To further explore the relative importance of individual environmental variables, we performed correlation analyses between each functional group or OTU and each environmental variable. Consistent with our regression modeling, most environmental variables either correlated more strongly with relative functional group abundances than with OTU proportions within functional groups, or did not correlate strongly with either of them (Fig. 1, D and E). These patterns persisted at higher taxonomic levels (e.g., genus, family, or order; figs. S1 and S2).

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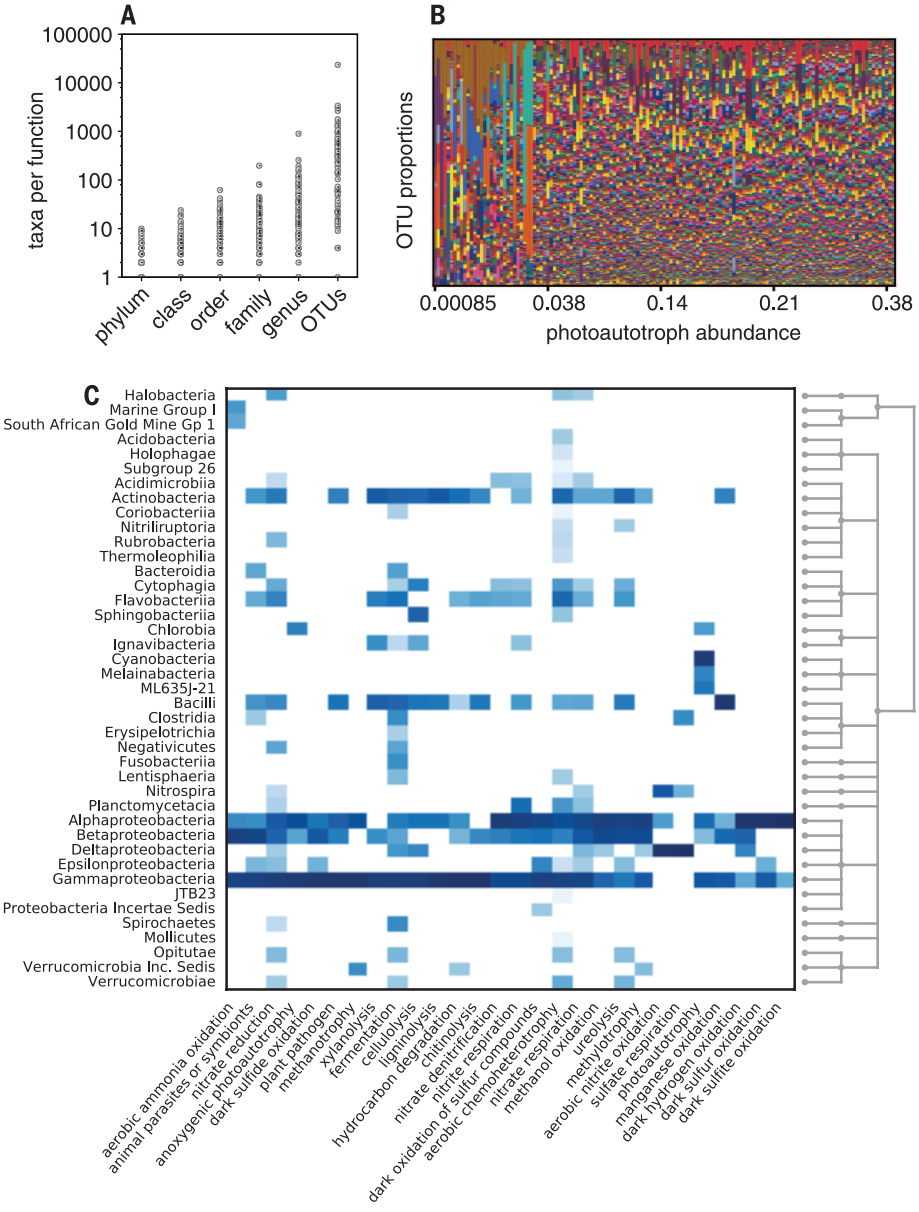

Fig. 3. Functional redundancy in the global ocean. (**A**) Number of bacterial and archaeal taxa represented within each functional group (one point per group) at various taxonomic levels. At the OTU and genus level, aerobic chemoheterotrophs represent the most diverse group. (**B**) OTU proportions within the group of aerobic ammonia oxidizers (one color per OTU). Samples are sorted according to the relative abundance of the entire functional group. For OTU proportions within other functional groups, see fig. S6. (**C**) Association of functional groups (columns) with members of microbial classes (rows). A darker color corresponds to a higher relative contribution of a class (in terms of the number of OTUs) to a functional group. Rows are clustered by taxonomic relationships [based on the SILVA database, release 119 (*28*), cladogram shown on the right]; columns are hierarchically clustered by similarity.

Hence, our results are not caused by suboptimal choice of taxonomic resolution, but reflect a general lack of environmental effects on the composition within functional groups.

The interaction between environmental conditions and function is only partly reflected in overall taxonomic community structure. Regression models of relative taxon abundances against environmental variables at the whole-community level and at any taxonomic resolution had a lower R^2_{cv} (Fig. 1A and fig. S3) than achieved for most functional groups (blue bars in Fig. 1C), but comparable to or higher than the mean R^2_{cv} achieved for the taxon proportions within functional groups (gray bars in Fig. 1C and fig. S2). Similarly, environmental variables were generally less correlated to relative taxon abundances at the community level than to relative functional group abundances (Fig. 1B versus Fig. 1D). Further, the distinction between water column zones based on function is comparable in strength to a distinction based on taxonomy at the whole-community level (Fig. 2, B and C, and fig. S4). Indeed, permutational multivariate analysis of variance (PERMANOVA) tests (*11*) of dissimilarities in community composition yielded similar pseudo-F statistics (23.8 and 24.4, respectively; $P < 0.001$ in both cases). Similarly, the segregation of functional groups between water column zones (e.g., mesopelagic versus surface) was comparable to or higher than the segregation of taxa (as determined by permutation tests; Fig. 2, E to G). Together, these results support the interpretation that overall community structure results from the superposition of functional structure, which is strongly related to environmental conditions, and composition within functional groups, which is only weakly related to environmental conditions. In particular, an organism's metabolic potential appears to be the main trait selected for by environmental conditions across the global ocean. In support of this interpretation, we found that OTUs sharing a higher number of functions tend to correlate with each other more positively (Fig. 2H), consistent with a strong role for metabolic niche effects (*11*).

Our results also suggest that energetic and stoichiometric constraints, not the identity of the microbes, drive ocean microbial metabolic function. Indeed, we found that the proportions of individual OTUs within a functional group were generally only weakly correlated to the group's overall abundance (figs. S5 and S6). Similar smaller-scale observations have been reported previously. In a wastewater treatment plant, the ratio of aerobic ammonia-oxidizing bacteria and heterotrophic bacteria remained constant over time, while highly variable taxonomic composition within each functional group was only weakly explained by environmental factors (*8*). In another study, metatranscriptomics in two distinct ocean regions revealed strongly conserved diurnal succession patterns of community gene expression, despite largely nonoverlapping taxonomic affiliations of transcripts between the two regions (*12*).

If environmental conditions interact with functional community structure, what drives the taxonomic variation within individual functional groups? The high taxonomic richness and variability within functional groups indicate a high global functional redundancy (Fig. 3 and fig. S6), which may have emerged partly as a result of horizontal gene transfer (*1*). The variation within functional groups that is not explained by our regression models could be due to limited granularity of our environmental characterizations and functional assignments. For example, unknown physicochemical variables and phenotypic differences within functional groups could drive location-dependent growth differences between competing clades. This may explain why, in some cases, functional groups were dominated by a few OTUs in multiple samples despite a high OTU richness (fig. S6). However, the variables considered here are known to be important predictors for marine microbial community composition (*13*, *14*), and it is unlikely that unconsidered environmental variables alone could explain the apparent randomness seen within so many functional groups (fig. S6)

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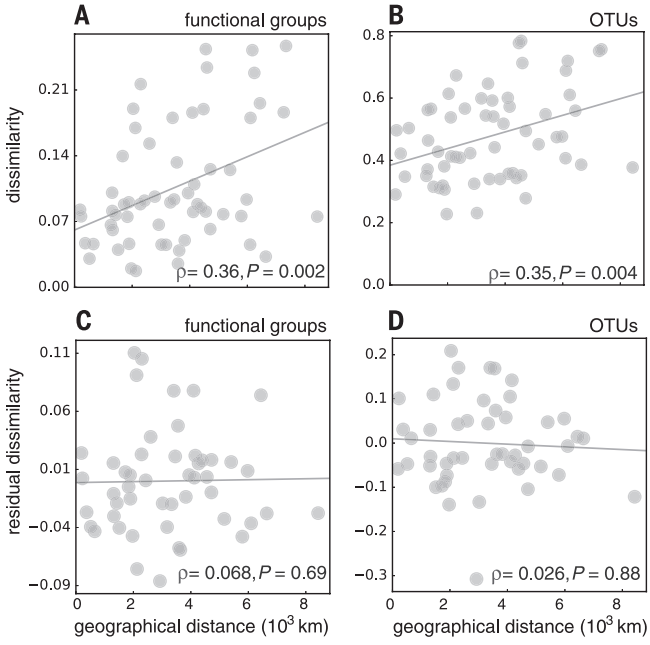

Fig. 4. Community dissimilarities versus geographical distances in the mesopelagic zone. (**A** and **B**) Bray-Curtis dissimilarities between microbial communities, compared with geographical distances (one point per sample pair). Community dissimilarities are calculated in terms of relative functional group abundances (A) and relative OTU abundances (B). Spearman rank correlations (ρ) and statistical significances (*P*) are shown. Only sample pairs in the mesopelagic zone and within the same ocean region are considered. Least-squares regression lines are shown for reference. (**C** and **D**) Similar to (A) and (B), but showing residual Bray-Curtis dissimilarities after accounting for environmental effects. For other taxonomic resolutions, see fig. S8. For dissimilarities within functional groups, see figs. S9 and S11. For surface water samples, see fig. S7.

when compared to the much better predictability of functional community structure (Fig. 1).

Alternatively, spatially limited dispersal may cause distribution patterns not attributable to environmental conditions, although its importance for microorganisms is generally thought to be low and to depend strongly on the type of environment [e.g., lakes versus open ocean (*4*)]. Marine microorganisms, in particular, can be dispersed at global scales by large ocean currents and were previously found to be recruited from a global marine seed bank (*15*). To test the effects of dispersal limitation, we compared geographical sample distances with dissimilarities in functional and taxonomic composition at the community level as well as within individual functional groups. To avoid comparisons spanning continental land masses, we only considered sample pairs within the same ocean region. When environmental effects were not taken into account, Mantel correlation tests revealed significant positive correlations between geographical distances and community dissimilarities ($P < 0.05$; Fig. 4, A and B, fig. S7, A and B, and fig. S8, A to C), consistent with previous findings at similar scales (*14*). We also observed weaker distance decay in similarity within functional groups (figs. S9 and S10). When we accounted for environmental effects, residual dissimilarities showed no significant correlations with geographical distance, neither at the community level (Fig. 4, C and D, fig. S7, C and D, and fig. S8, D to F) nor within functional groups (figs. S11 and S12). This suggests that the distance decay in similarity is driven by environmental variation affecting community function (and, to a lesser extent, composition within functional groups) and that dispersal limitation in the open ocean is of limited importance relative to other community assembly mechanisms.

Unexplained variation may be driven by community-level processes such as metabolic interdependencies, chemical signaling and defense, or predation by viruses and eukaryotes (*5*–*7*, *16*). For example, predation by bacteriophages specialized on specific hosts can cause variation in host community composition that cannot be attributed to environmental variation (*16*). Functional community structure and taxonomic composition within functional groups thus appear to constitute roughly complementary axes of variation, with the former being affected more strongly by environmental conditions and the latter shaped greatly by community-level processes (*5*). Other mechanisms such as priority effects (*17*) or demographic drift (*8*) may also explain some of the variation within functional groups.

Marker gene sequencing can yield detailed microbial taxonomic community profiles and reveal intriguing variation—for example, down the ocean water column (*14*). The high dimensionality of these profiles, however, poses a challenge to their mechanistic interpretation. Generic statistical techniques such as principal components analysis (*18*) are often used to identify important axes of variation, but these axes typically lack an ecological interpretation. Alternatively, detected species may be combined at higher taxonomic levels that resemble the depth at which traits vary across lineages; however, the optimal taxonomic level is highly trait-dependent (*9*). Further, many metabolic phenotypes are dispersed irregularly across microbial clades (*19*). Similar functions may be performed in distant clades (Fig. 3, A to C, and fig. S13) and, conversely, members of the same clade can fill separate metabolic niches (*9*, *19*). For example, none of the 28 metabolic functions considered here appear to be monophyletic (Fig. 3C and fig. S13). Irregular trait distributions are caused by diverse evolutionary processes, including adaptive loss of function (*20*) and metabolic convergence accelerated by frequent horizontal gene transfer (*1*). As a result, taxonomic profiles, at any level, often obscure the relationship between community structure and biogeochemical processes.

Here we have shown that binning organisms into functional groups enables the integration of functional, taxonomic, and environmental information to elucidate community assembly processes. Comparison of functional profiles with environmental variables (Fig. 1D) yields insight into the processes driving variation in community composition along geochemical gradients and, reciprocally, gives information about the effects of that variation on ecosystem processes, although specific causal mechanisms need to be experimentally validated. For example, our functional profiles reveal that groups capable of fermentation or nitrate respiration are overrepresented in deeper zones, where oxygen is often limiting and nitrate serves as an alternative electron acceptor for respiration (Fig. 2A). The prevalence of alternative metabolic modes at depth leads to increased richness of both functional groups and OTUs, especially in the mesopelagic zone (Fig. 2D). Although an increase of taxonomic richness with depth has been noted before (*14*), our analysis shows that this richness gradient is related to the number of available metabolic niches.

The zonation of functional groups with depth (Fig. 2A) is reflected in metagenomic profiles of the same samples (fig. S14) (*14*) and in previous metagenomic studies (*2*). However, metagenomic profiles do not directly translate into functional potential, because the same or similar genes can be involved in different—in some cases reversed—metabolic pathways, depending on the microorganism (*21*). For example, variants of the dissimilatory sulfite reductase genes (*dsrAB*), abundant in the mesopelagic zone (fig. S14), could be involved in either respiratory sulfur reduction or chemolithotrophic sulfur oxidation (*22*). Such ambiguities are inherent to metagenomics (*21*) and apply equally to methods that estimate community gene content by projecting marker genes to sequenced genomes (fig. S14) (*23*). On the other hand, our phenotype profiles suggest that sulfide and sulfite oxidizers are generally more abundant than sulfate respirers (Fig. 2A), indicating that detected *dsrAB* genes are mainly involved in sulfur

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oxidation. Translation of taxonomic information into phenotype profiles based on experimental evidence can thus facilitate the ecological interpretation of metagenomes. The full potential of phenotype profiling remains underused because of our current inability to associate many known taxa with any function. For example, a large fraction of the ubiquitous but poorly studied phylum Thaumarchaeota is potentially involved in ammonia oxidation (*24*) but had to be excluded from our functional annotations (*11*). Similarly, microeukaryotes likely contribute to several metabolic functions, such as photosynthesis or cellulolysis. Future functional profiling should thus include eukaryotic microorganisms and incorporate putative metabolic potential for uncultured clades revealed by single-cell genomics (*25*).

The bulk of global biogeochemical fluxes is driven by a core set of metabolic pathways that evolved in response to past geochemical conditions (*1*). Through time, these pathways have spread across microbial clades that compete within metabolic niches, resulting in an enormous microbial diversity characterized by high functional redundancy. As shown here, splitting variation of microbial community composition into variation of functional structure and taxonomic variation within functional groups reveals an intriguing pattern: The functional component in itself captures most of the variation predicted by environmental conditions, whereas the residual component (i.e., variation within functional groups) only weakly relates to environmental conditions. This has implications for the interpretation of differences in community structure across environments and time. Differences in taxonomic composition that do not affect functional composition may have little relevance to ecosystem biochemistry; conversely, physicochemically similar environments could host taxonomically distinct communities (*26*). Functional (rather than purely taxonomic) descriptions of microbial communities should therefore constitute the baseline for microbial biogeography, particularly across transects where geochemical gradients shape microbial niche distribution (*27*). The residual variation within functional groups can then be analyzed separately to elucidate additional community assembly mechanisms such as biotic interactions, dispersal limitation, or demographic drift. An incorporation of global microbial functional profiles, and their response to potentially changing environmental conditions, into future biogeochemical models will greatly benefit reconstructive and predictive modeling of Earth's elemental cycles.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6305/1272/suppl/DC1
Materials and Methods
Tables S1 to S4
Figs. S1 to S28
References (*29*–*62*)
Additional data table S1

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SENSORY BIOLOGY

Bats perceptually weight prey cues across sensory systems when hunting in noise

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Anthropogenic noise can interfere with environmental information processing and thereby reduce survival and reproduction. Receivers of signals and cues in particular depend on perceptual strategies to adjust to noisy conditions. We found that predators that hunt using prey sounds can reduce the negative impact of noise by making use of prey cues conveyed through additional sensory systems. In the presence of masking noise, but not in its absence, frog-eating bats preferred and were faster in attacking a robotic frog emitting multiple sensory cues. The behavioral changes induced by masking noise were accompanied by an increase in active localization through echolocation. Our findings help to reveal how animals can adapt to anthropogenic noise and have implications for the role of sensory ecology in driving species interactions.

Anthropogenic noise is a globally rising environmental pollutant that has been linked to lower survival and reduced reproductive success of many animal taxa (*1*–*3*). Noise can mask environmental cues, making it difficult to hear moving prey or approaching predators, and can interfere with the perception of acoustic communication signals (*3*–*6*). Signal producers may be able to reduce the masking impact—for example, by calling louder (*7*–*9*)—but such signaling strategy is unavailable to receivers. Some receivers can depend on perceptual strategies to maintain cue detection and thereby adapt to noisy environments (*10*, *11*).

Predators such as bats and owls are highly specialized to hunt prey by ear (*12*); thus, noise that masks prey sounds severely hampers their foraging success (*4*, *5*). However, predators may be able to adapt to masking levels of anthropogenic noise by actively shifting their attention or emphasis placed on processing cues from different sensory modalities from the same prey (*13*–*16*). We refer to this as cross-modal perceptual weighting (*17*).

We studied the effect of masking noise on the attack behavior of the fringe-lipped bat (*Trachops cirrhosus*), a neotropical species that is specialized

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to find frogs by eavesdropping on their mating displays (*18*, *19*). Bats can passively locate their prey using only prey-generated sounds, but their performance is severely hampered when exposed to noise (*20*). A male frog, however, provides additional, multimodal cues to hunting bats, as it inflates and deflates a vocal sac while calling (*14*, *16*). Bats can detect the frog's vocal sac with their echolocation (i.e., processing the ultrasonic echoes that return from their prey), but only when the sac is dynamically inflated; bats do not detect a static vocal sac (*21*). Echolocation

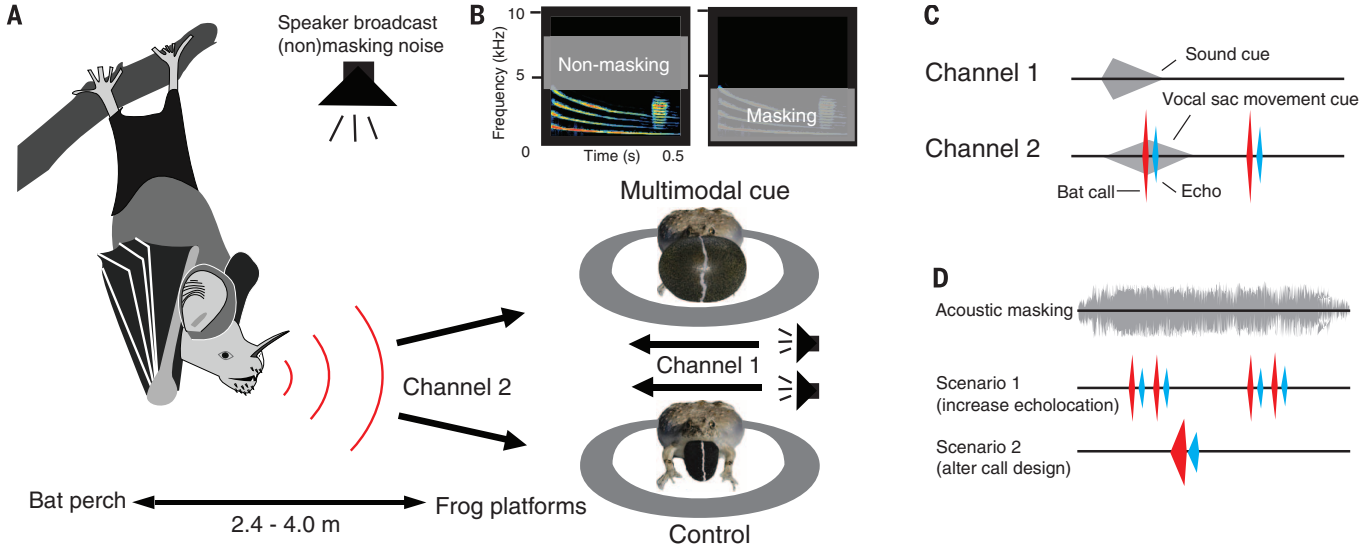

Fig. 1. Perceptual strategies to deal with prey signal masking. (**A**) Graphic representation of our experimental design. Bats can passively listen to frog sounds (channel 1) broadcast from speakers underneath the robofrog models; they can also actively use their echolocation (channel 2) to detect the dynamic vocal sac. (**B**) Bats were tested under nonmasking noise, masking noise, and control conditions (no noise) broadcast from a speaker placed above the frog models. Shown are two spectrograms of a frog call with frequency regions of noise treatments superimposed on it (4.0 to 8.0 kHz. 0.1 to 4.0 kHz). (**C**) Bats can rely on passive listening to their prey's mating sound (channel 1) as well as on active listening by processing multiple echoes returning from the frog's moving vocal sac (channel 2). Shown are the typical frog call amplitude profile (channel 1), the inflation and deflation of the frog's vocal sac (channel 2, gray symbol), and bat calls (red symbols) and echoes (blue symbols) overlapping as well as nonoverlapping in time with the vocal sac cue. (**D**) When noise masks the prey call, bats may increase their echolocation effort (scenario 1) or alter call design (scenario 2) to maintain target localization.

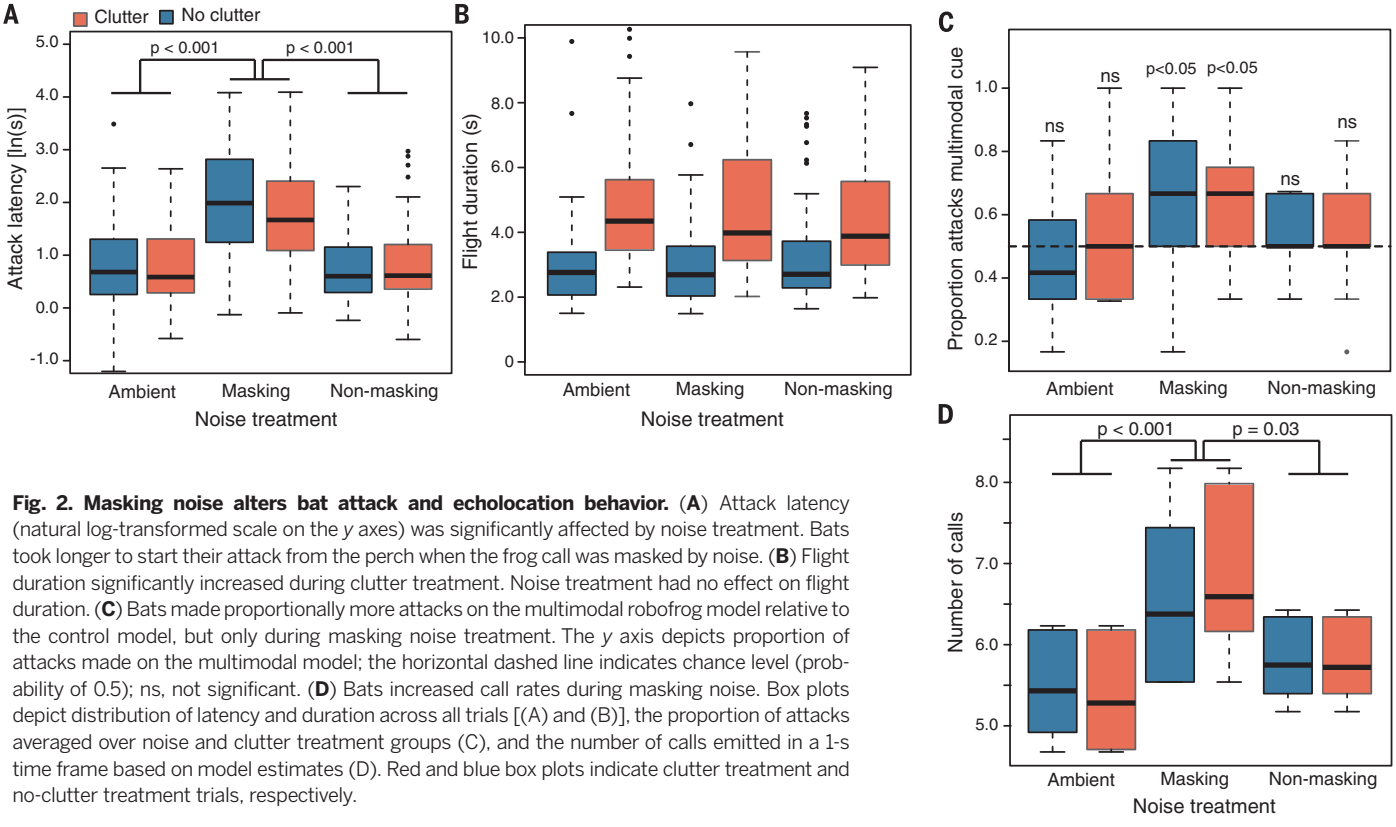

Fig. 2. Masking noise alters bat attack and echolocation behavior. (**A**) Attack latency (natural log-transformed scale on the *y* axes) was significantly affected by noise treatment. Bats took longer to start their attack from the perch when the frog call was masked by noise. (**B**) Flight duration significantly increased during clutter treatment. Noise treatment had no effect on flight duration. (**C**) Bats made proportionally more attacks on the multimodal robofrog model relative to the control model, but only during masking noise treatment. The *y* axis depicts proportion of attacks made on the multimodal model; the horizontal dashed line indicates chance level (probability of 0.5); ns, not significant. (**D**) Bats increased call rates during masking noise. Box plots depict distribution of latency and duration across all trials [(A) and (B)], the proportion of attacks averaged over noise and clutter treatment groups (C), and the number of calls emitted in a 1-s time frame based on model estimates (D). Red and blue box plots indicate clutter treatment and no-clutter treatment trials, respectively.

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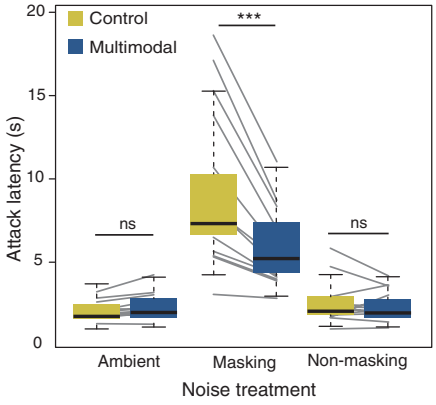

Fig. 3. Multimodal cues reduce attack latency under masking noise levels. Attack latency showed a significant interaction between noise treatment and multimodal cue use. Bats were faster in attacking the multimodal model relative to the control model only during masking noise. Box plots depict model estimates per noise treatment group and attack choice (on either the control or the multimodal model). Gray lines depict fitted slopes per individual per noise treatment. ***$P < 0.001$.

can provide highly accurate spatial information about target stimuli, and we therefore expected that these bats are capable of adapting to masking interference through a change in their perceptual weighting of sonic cues versus ultrasonic cues (*22*).

We used robotic frogs that emitted either multimodal cues (sound plus moving vocal sac) or control cues (sound plus static vocal sac) (Fig. 1A). Individual bats were given a choice to attack one of two models under three different noise treatments: (i) a masking noise overlapping the main frequency range of the frog call, (ii) a nonmasking noise, and (iii) an ambient control noise condition (Fig. 1B and supplementary materials). Although the signal-to-noise ratio of the frog call was strongly reduced during masking noise, the signal remained audible to the perched bat (Fig. 1B). We predicted that bats rely more on echolocation when presented with masking noise and would consequently make more attacks on the multimodal frog model and alter their echolocation behavior [see scenarios in Fig. 1, C and D, or (*23*)]. Objects surrounding a target also return echoes, and this so-called background clutter is known to interfere with detection and processing of echolocation target cues (*4*, *24*). We thus tested bats additionally on their attack behavior when dried leaves were added around the frog models (clutter treatment; fig. S1).

We trained 12 wild-caught bats to attack our robotic models in an outdoor flightcage (*21*). Bats always started their attack flight toward one of the frog models from their perch and only in response to stimulus playback. Attack latency was strongly influenced by our noise treatment (generalized linear mixed models, $N_{bats} = 12$, $N_{trials} = 432$, $\chi^2 = 22.07$, $P < 0.001$; Fig. 2A and table S1). Post hoc independent contrasts revealed that bats were slower in making their attacks under masking noise relative to nonmasking noise (z value = 5.57, $P < 0.001$) and ambient conditions (z value = 7.56, $P < 0.001$). We did not find a significant effect of the clutter treatment on attack latencies ($\chi^2 = 0.09$, $P = 0.76$). On the other hand, the time between leaving the perch and attack on the model (hereafter, flight duration) increased during the clutter treatment ($\chi^2 = 17.04$, $P < 0.001$; Fig. 2B) but was not affected by the noise treatment ($\chi^2 = 0.24$, $P = 0.89$). The number of attacks on the multimodal frog model relative to the control frog model was significantly affected by the noise treatment ($\chi^2 = 7.63$, $P = 0.022$; Fig. 2C). Post hoc binomial tests revealed that bats had a clear preference for the frog model displaying multimodal cues under masking noise (z value = 2.34, $P = 0.019$), but not under nonmasking noise (z value = −0.94, $P = 0.35$) or ambient conditions (z value = 1.17, $P = 0.24$). Clutter treatment had no significant effect on the probability of bat attack on either model ($\chi^2 = 0.60$, $P = 0.43$; Fig. 2C), nor did we find any significant interaction effects between noise and clutter treatment (all response variables $P > 0.5$).

We obtained ultrasonic recordings for a subset of six individuals and analyzed all calls made on the perch between stimulus onset and start of the attack flight. Bats made on average 7.69 ± 2.78 calls on their perch, the majority (77%) during the last second. We selected three calls from a 1-s portion of the recording (shortly before bats had taken flight) to test for an effect of experimental treatment on echolocation behavior. We found the number of calls produced at the perch, as well as the call rate, to be significantly affected by noise treatment (number of perch calls, $N_{bats} = 6$, $N_{trials} = 146$, $\chi^2 = 6.44$, $P = 0.039$; call rate during last second, $\chi^2 = 7.78$, $P = 0.022$; Fig. 2D). Bats increased their use of echolocation during masking noise relative to nonmasking noise (5 of 6 bats on average increased call rate; z value = 2.34, $P = 0.032$) and relative to ambient noise conditions (6 of 6 bats increased call rate; z value = 3.46, $P < 0.001$). We did not find any differences in call peak frequency ($\chi^2 = 0.27$, $P = 0.87$) or call duration ($\chi^2 = 2.85$, $P = 0.24$) between noise treatment groups. Clutter treatment had no significant effect on the number of calls emitted from the perch ($\chi^2 = 1.53$, $P = 0.22$) or on call rate ($\chi^2 = 1.26$, $P = 0.26$), call peak frequency ($\chi^2 = 1.53$, $P = 0.22$), or call duration ($\chi^2 = 0.84$, $P = 0.36$).

Masking noise increased attack latencies during our experiment, but bats could reduce this effect when using multimodal cues. We reanalyzed the attack latency data and added robofrog choice (control or multimodal) to our statistical model as an additional factor. We found a significant interaction effect between noise treatment and robofrog choice ($N_{bats} = 12$, $N_{trials} = 432$, df = 2, $\chi^2 = 11.82$, $P = 0.003$; Fig. 3 and table S1). Bats were faster in attacking the multimodal model relative to the control model, but only under masking noise levels (z value = −3.78, $P < 0.001$). Robofrog choice had no effect on attack latencies under nonmasking noise (z value = 0.86, $P = 0.78$) and ambient noise conditions (z value = −0.26, $P = 0.99$).

Anthropogenic noise can affect predator-prey dynamics through masking of acoustic cues or by distracting or disturbing individuals (*2*, *25*, *26*). Our results confirm a masking impact of noise on bat attack latencies, thereby giving frogs more time to escape predation. More important, the results show that bats can actively compensate the masking impact by making more use of cues available to them in an additional, less noisy sensory channel. We also found a factor of 2 increase in attack preferences on the multimodal versus the unimodal model, which suggests that noise can drive selection pressures acting on sexual signals.

A previous study on a bat species that echolocates silent prey showed that the negative impact of noise on hunting success is enhanced when individuals are tested in a highly cluttered environment (*4*). Clutter treatment in our experiment affected flight duration but surprisingly had no effect on attack choice or latency. Fringe-lipped bats can detect and localize the frog's vocal sac at distances up to 6 m from their perch (*21*, *22*), and it is likely that its movement allows bats to discriminate target echoes from the stationary background (*27*), such as the dried leaves we placed around our models during clutter treatment.

In conclusion, we showed that bats preferred multimodal displays to unimodal displays, but only under masking noise conditions. Such cross-modal perceptual weighting reduces the masking impact of noise and could be a general receiver strategy (*11*, *28*). A shift in the use of signals and cues across sensory systems will also alter selection pressures acting on sexual displays (*29*, *30*). Thus, in noisy human-impacted areas such as in cities or along highways, we would expect to find a change in the multimodal content of communication signals (*11*). We may also expect a shift in species composition in noisier areas based on perceptual as well as communicative traits. Species that can rapidly alter their perceptual mechanisms will likely do better in noise-impacted areas, and this in turn has consequences for their predator and prey species that emit different signals and cues. Human-induced changes to the sensory ecology of particular habitats can thus be an important factor in driving species interactions and ultimately determining community assemblages.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6305/1277/suppl/DC1
Materials and Methods
Fig. S1
Table S1
References (*31*–*33*)

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BRAIN MICROCIRCUITS

Awake hippocampal reactivations project onto orthogonal neuronal assemblies

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The chained activation of neuronal assemblies is thought to support major cognitive processes, including memory. In the hippocampus, this is observed during population bursts often associated with sharp-wave ripples, in the form of an ordered reactivation of neurons. However, the organization and lifetime of these assemblies remain unknown. We used calcium imaging to map patterns of synchronous neuronal activation in the CA1 region of awake mice during runs on a treadmill. The patterns were composed of the recurring activation of anatomically intermingled, but functionally orthogonal, assemblies. These assemblies reactivated discrete temporal segments of neuronal sequences observed during runs and could be stable across consecutive days. A binding of these assemblies into longer chains revealed temporally ordered replay. These modules may represent the default building blocks for encoding or retrieving experience.

The concept of "cell assembly" refers to a group of neurons that are coactivated repeatedly for a given brain operation (*1*). Cell assemblies thus represent a distinct cognitive entity embedded within neuronal networks (*2*). However, both their basic structural and functional organization, when outside world influences are minimal, as well as their long-term dynamics, remain unknown owing to the experimental difficulty of circumscribing them. In principle, the chained coordinated activation of such neuronal assemblies combines into sequences of neuronal activation supporting complex cognitive processes (*3*). Therefore, sequences of neuronal activation can represent a remarkable motif for revealing the activation of underlying neuronal assemblies. In the hippocampus, sequences occur at multiple time scales in the CA1 region—e.g., at the time frame of behavior—or compressed within the period of fast network oscillations (*2*, *4*). They can integrate time and/or distance, as well as any contextual information. Of particular interest are the coordinated patterns of neuronal activation that occur during awake immobility and that are related to sharp wave–associated ripples (SWRs), because these are produced when bodily or environmental control over hippocampal dynamics is minimal. Even though these coherent population events include sequential place cell reactivation representing past or future spatial experience, they are indeed also critically shaped by the internal functional organization of local circuits (*5*–*7*). Sequential neuronal reactivation can be split into separate chunks of current or remote experience (*8*–*11*), but their spatiotemporal organization into different cell assemblies remains unknown. So far, the dissection of hippocampal sequences into discrete reactivation patterns has been achieved by mapping them onto an external spatiotemporal template, such as an experienced behavior (*8*–*12*). It is important to minimize external sensory inputs to reveal the default organization of hippocampal dynamics into cell assemblies because local inputs are known to bias the content of both local and remote replay (*10*). We recently described a paradigm for revealing internally driven spatiotemporal sequences that occur during run behavior, which is particularly well suited to address this issue (*13*). However, monitoring large-scale multineuronal activity at high cellular density to identify cell assemblies represents a major technical challenge. This is particularly critical in the case of hippocampal population bursts, as they involve local microcircuits within the densely packed pyramidal layer (*7*, *14*). In vivo imaging of hippocampal dynamics is ideally suited to circumvent this limitation.

We used chronic two-photon calcium imaging of awake head-restrained mice allowed to self-regulate their motion in the dark on a nonmotorized treadmill (*13*). To map neuronal activity across consecutive days, we used a viral vector (AAV2/1.syn-GCaMP5G, -6m, and -6f; see table S1) (*15*) that allows for the detection of sparse firing through a glass window on the hippocampus (*16*), as described in (*17*). Additionally, mice were chronically implanted with an extracellular field electrode placed in the CA1 stratum pyramidale on the contralateral side to monitor the occurrence of network oscillations in the contralateral local field potential (LFP) during awake immobility periods. Particular attention was given to fast frequency domains (100 to 200 Hz) because most, but not all, CA1 population bursts that occur bilaterally during immobility are associated with SWRs. Because a fraction of awake SWRs is coherently observed in both hippocampi, supported by their anatomical interconnections (*18*), the contralateral LFP can be used as a reference to identify whether specific calcium transients are associated with the occurrence of SWRs. During each daily imaging session, mice spontaneously alternated between run and immobility epochs (Fig. 1A). Sequences of neuronal activation integrating spatiotemporal information (*13*) recurred during spontaneous run periods (RUN sequences) (Fig. 1A) (n = 3 out of 4 mice, table S1). During immobility periods, significant peaks of synchronous neuronal activity were observed (Fig. 1A)

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($P < 0.01$, supplementary methods) that appeared as transient "flashes" in the fluorescence movies (movie S1). These synchronous calcium events (SCEs) recruited a sparse population of neurons (Fig. 1B). Cell participation was log-normally distributed (fig. S1).

Given that SWRs are known as local synchronous events in the CA1 pyramidal layer (*19*), we next asked whether these synchronous calcium activities could be associated with SWRs. Electrophysiological recordings revealed that SWRs occurred during immobility (Fig. 1C) (see methods), at a rate of 0.12 Hz ($n = 3$ mice, fig. S1), which is comparable to previous reports (*5*, *20*, *21*). The rate of synchronous calcium events was not significantly different from that of SWRs (0.1 Hz, $n = 4$ mice, fig. S1, $P > 0.1$). In 15 out of 21 sessions, we found that only a frequency band of the contralateral LFP between 122 and 204 Hz ($1/e$ width) was significantly time-locked to the SCEs (fig. S1). In the remaining six sessions, no frequency band was prominent. This supports the association of SCEs with ripple events rather than other oscillatory events. Accordingly, using two arbitrary detection thresholds for SWRs and SCEs (see methods), we found that 23% of SWRs were time-locked to the synchronous calcium events imaged in the ipsilateral hemisphere (Fig. 1D, $n = 3$ mice, table S1, movie S1). Conversely, 20% of the synchronous activities detected with optical approaches during immobility co-occurred with an electrophysiological SWR (Fig. 1D). This rate of co-occurrence can reach values (up to 58%) similar to those obtained with the previously reported fraction of bilateral SWRs (*18*) and was comparable to the co-occurrence rates of SWRs measured with bilateral electrophysiological recordings in our particular conditions (fig. S10). Overall, the fraction of active neurons

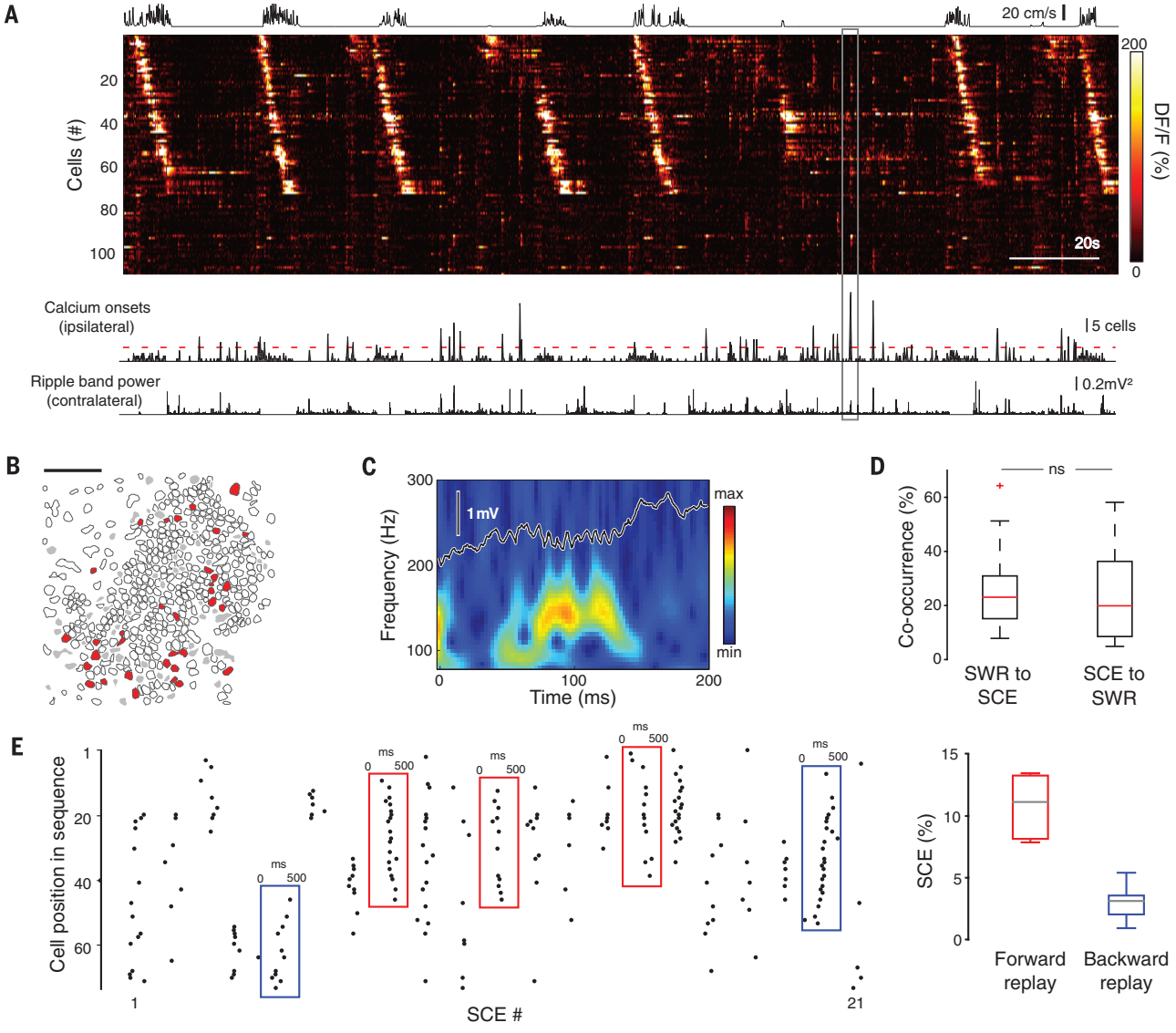

Fig. 1. Imaging hippocampal dynamics in the CA1 region of awake mice alternating between running and rest. (**A**) Recurring sequences of neuronal activation during mouse runs. Mouse speed (top row), heat map raster plot of single cell fluorescence signal (middle), and summed activity of cells (over a 200-ms window). Red dashed line indicates significance threshold for synchronous activity detection (five cells in this example); ripple band power (120 to 220 Hz, bottom row) is displayed over time. (**B**) Contour map of the neurons imaged in (A) during immobility. Cells shown in red were active during one synchronous calcium event [SCE, gray box from (A)]; all other active cells are shown in gray. Scale bar: 100 μm. (**C**) Sharp wave–associated ripple (SWR) oscillation co-occurring with the event outlined in (A) (black: raw LFP trace, power spectrogram). (**D**) Co-occurrence rate of contralateral ripples with SCE and vice versa measured during immobility. (**E**) Raster plot (left) of cell activation during 21 successive SCEs. Neurons were ordered according to their activation onset during the RUN sequence. Identified replay events (ordered reactivation of neurons) are indicated within a 500-ms window (red: forward replay; blue: backward replay). Right: Fraction of SCEs identified as forward or backward replay across all imaging sessions.

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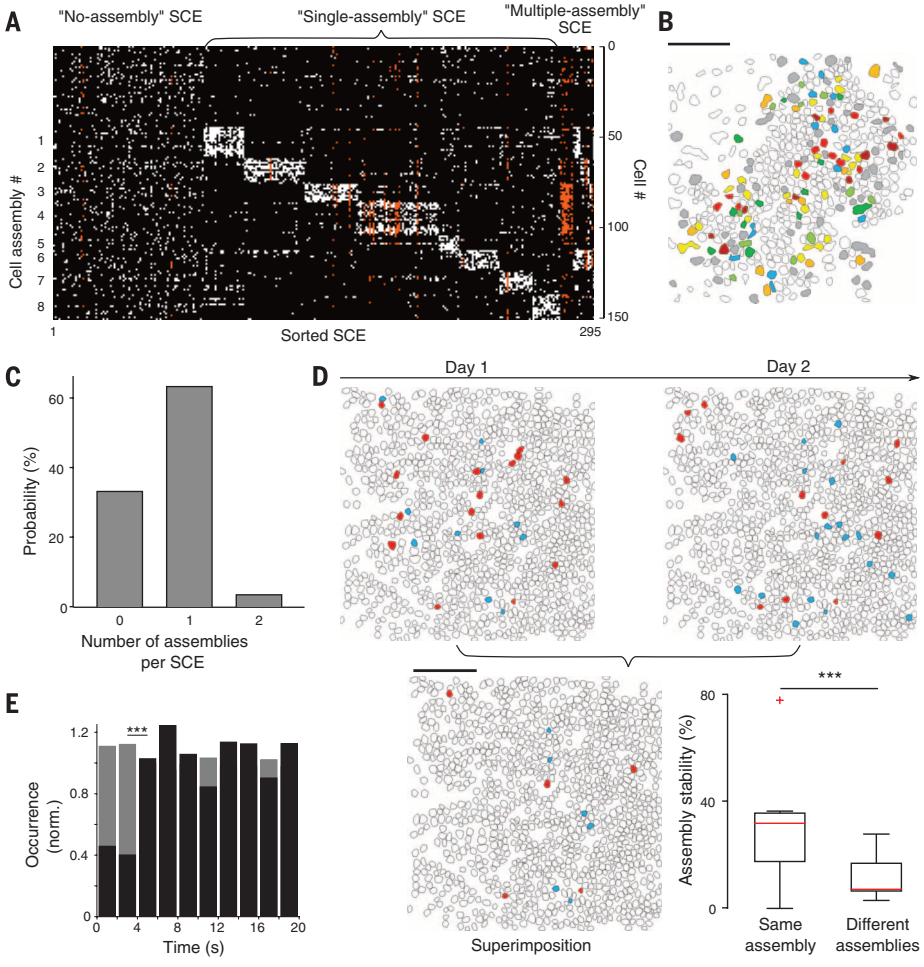

Fig. 2. SCEs segregated into functional cell assemblies. (**A**) Raster plot of all SCEs, within one representative imaging session, sorted by cell assemblies. SCEs displaying ordered sequential replay are indicated in red. (**B**) Contour map of the imaged cells; scale bar: 100 μm. Filled colored contours indicate different cell assemblies; filled gray contours indicate other active neurons. (**C**) Number of cell assemblies recruited during each SCE, pooled across all sessions where RUN sequences could be observed. (**D**) Top: Representative contour maps depicting two assemblies (filled in red and blue, respectively) from one day to the next. Bottom left: Contour map of the cells that remained in the same assembly across consecutive days days; scale bar: 100 μm. Bottom right: Box plot depicting the probability that a given cell pair is active on two consecutive days for cell pairs that do or do not belong to the same assembly on the first day. (**E**) Temporal dynamics of cell assemblies. Shortly after the activation of an assembly (t = 0), the probability that it was reactivated within a 4-s time interval (black) was lower than for a different assembly (gray); occurrences are normalized; the expected value for a random activation is 1.

participating in a SCE was significantly correlated with the ripple power ($P < 0.001$, Spearman's $r = 0.16$; fig. S1, top row, middle panel); bilaterally coherent SCEs and SWRs recruited a slightly higher fraction of active neurons (fig. S1). We conclude that the SCEs detected in this study are often associated with SWRs.

We next investigated the temporal order of cell activation within SCEs. Indeed, a fraction of CA1 population bursts are expected to reactivate, in forward or reverse order, previously experienced behavioral trajectories (*22*–*24*). We tested for temporally ordered RUN sequence replay in those mice for which recurring sequences reached a significant probability of detection [$n = 3$ mice; see (*13*)]. We computed the average activation time for each neuron within a peak of synchronous calcium activity and correlated this value with its average activation order within the RUN sequences (supplementary methods). In this way, we estimated that 14% of the SCEs were replaying RUN sequences ($n = 3$ mice, $P < 0.05$, supplementary methods, Fig. 1E). Of these, 24% were identified as backward replay and 76% as forward replay (Fig. 1E). These results are similar to the proportion of SWRs identified as significant replays in previous reports (*8*, *10*, *22*–*25*). In addition, they show that internally generated spatiotemporal sequences can be reactivated in a compressed form (*6*). Sequential replay could be detected independently from the occurrence of a contralateral electrophysiological ripple. The proportion of sequential replay that co-occurred with a ripple (21.3%) was not different from nonreplay (21.8%), further indicating that SCEs are not different, whether they are detected with a contralateral ripple or not. Overall, these results indicate that SCEs occurring during awake immobility can occur with ripples. We next further analyzed the microstructure and dynamics of SCEs.

One SCE recruited around 5 to 10% of active cells (interquartile range, log-normal distribution, fig. S1) (*5*, *6*, *19*). The small minority of neurons contributing to most SCEs (90th percentile) were more likely to remain active across consecutive days (fig. S2), as estimated previously (*19*). Given that SCEs seemed to be variably synchronizing active neurons, we asked whether single SCEs could be preferentially recruiting discrete neuronal assemblies. We used a clustering algorithm that allowed different types of SCEs to be sorted and single cells to be assigned to putative assemblies (see supplementary methods). In 21 out of 26 sessions (table S1), this analysis revealed several functional cell assemblies that were mostly independent from each other. Indeed, the activity of 88% of cells in a given assembly was significantly correlated with that assembly only (Fig. 2A and supplementary methods). To determine how cell assemblies related to reactivation events, we discarded the mouse without RUN sequences from the subsequent analysis (table S1). SCEs comprised three main categories of events (Fig. 2A): The majority of SCEs (63%) repeatedly recruited a single neuronal assembly ("single-assembly SCE"), 3.4% of them combined two distinct neuronal assemblies ("multiple-assembly SCE"), and 33% did not significantly involve any defined set of neurons ("no-assembly SCE"). This segregation of SCEs into three main categories was also observed when only SCEs co-occurring with electrophysiological ripples were included in the analysis (fig. S3). Half of the "multiple-assembly SCE" (50%) were associated with temporally ordered replay. Therefore, the majority of SCEs can be mathematically best described by an orthogonal basis of cell assemblies (fig. S4). These assemblies are bound together into "multiple-assembly" events, often associated with temporally ordered replay. Cell assemblies displayed notable short-term dynamics as they were less likely to repeat within the subsequent 4 s (Fig. 2E).

We also examined the topological distribution of cell assemblies and found that these were spatially intermingled (73 out of 85 assemblies; Fig. 2B and supplementary methods). Because the same network could be imaged over consecutive days, we asked whether these assemblies were transient or rather displayed some stability. Assembly membership was analyzed for cell pairs across consecutive days (Fig. 2D). The probability that a given cell pair was part of the same assembly on one day was significantly higher ($n = 3$ mice, supplementary methods) if the two cells already belonged to the same

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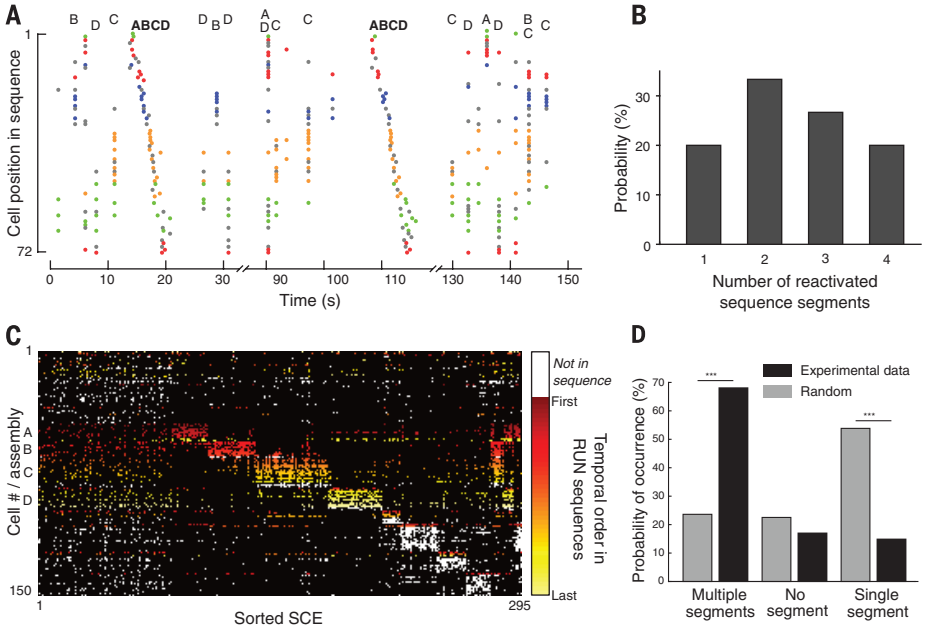

Fig. 3. Discrete segments of RUN sequences were reactivated during SCEs. (**A**) Raster plot displaying neuronal activation over time for a representative imaging session. Onsets of calcium transients were color-coded according to their cell assembly affiliation (labeled A to D). SCEs significantly recruiting one or two assemblies were labeled with the respective letter. RUN sequences were labeled ABCD. (**B**) Probability distribution pooled across sessions of the number of RUN sequence segments reactivated within SCEs. (**C**) Raster plot of all SCEs sorted by cell assemblies. Cells were color-coded according to their activation order within RUN sequences. (**D**) Probability distribution for "multiple-assembly SCE": (i) to reactivate RUN sequence segments (multiple segments); (ii) to activate "nonreplay" assemblies (no segment); or (iii) a combination of a RUN sequence segment and a "nonreplay" assembly (single segment). Experimental probabilities were tested against random data.

assembly on the previous day (32%) than if they did not (7%). We conclude that most SCEs either reliably reactivated a fixed preconfigured neuronal assembly ("single-assembly SCE") or recurrently linked together these assemblies ("multiple-assembly SCE").

In a final step, we asked how the different cell assemblies were recruited during RUN sequences. In the large majority of the imaging sessions (80%), RUN sequences activated successively two to four neuronal assemblies (Fig. 3), whereas only 50% of the cell assemblies were active during RUN sequences (median value, fig. S5). Thus, half of the "single assembly SCE" reactivated non-overlapping segments of RUN sequences (Fig. 3C, colored cells). The remaining events were not reactivating any part of the RUN sequence (Fig. 3C, white), in agreement with previous reports of "nonreplay" SWRs (*9*, *10*, *24*). Multiple-assembly SCEs were twice as likely to recruit multiple RUN sequence segments than expected by chance (49 versus 23%, Fig. 3D), indicating that these events preferentially linked together assemblies reiterating ongoing experience (i.e., RUN sequences). Conversely, the coactivation of a sequence segment with an assembly that was not part of the RUN sequences was less likely than expected by chance (32 versus 55%). In addition, even though cell assemblies were successively recruited within RUN sequences, these assemblies could not be inferred by RUN sequence activity alone (supplementary methods and fig. S9).

This study sheds light on the basic organization of awake CA1 synchronous population bursts that include ripples. These events fall into three distinct categories. Most of them recruit neurons sampled among a finite repertoire of preconfigured cell assemblies; these are mutually exclusive and therefore mathematically "orthogonal." Multiple-assembly SCEs preferentially bind these assemblies into the replay of ongoing internal dynamics on a compressed time scale, as reported for the extended replay phenomenon (*26*). This finding was enabled by the use of large-scale chronic calcium imaging combined with the mapping of ripples onto default recurring spatiotemporal sequences (*13*). Unfortunately, the temporal resolution of calcium imaging does not allow an exact determination of the time interval within which coactivation occurs for cells in the same assembly or across different assemblies. Multiple-assembly SCEs may indeed still bind together orthogonal assemblies, spreading across different gamma cycles (*11*). We hypothesize that orthogonal assemblies could be the calcium counterpart of the recently described attractors within trajectory replay, and that their combination would form the discontinuous representation of space in single awake ripples (*11*). The awake replay of memory-related information during SWRs has been shown to support encoding, consolidation, and retrieval of event memories and to guide memory-guided decision-making (*5*). The neuronal assemblies revealed here might indeed constitute separate attractor states, forming the building blocks onto which experience is encoded.

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SUPPLEMENTARY MATERIALS

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EXTINCTION

Ecological selectivity of the emerging mass extinction in the oceans

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Matthew L. Knope,[3] Douglas J. McCauley[4]

To better predict the ecological and evolutionary effects of the emerging biodiversity crisis in the modern oceans, we compared the association between extinction threat and ecological traits in modern marine animals to associations observed during past extinction events using a database of 2497 marine vertebrate and mollusc genera. We find that extinction threat in the modern oceans is strongly associated with large body size, whereas past extinction events were either nonselective or preferentially removed smaller-bodied taxa. Pelagic animals were victimized more than benthic animals during previous mass extinctions but are not preferentially threatened in the modern ocean. The differential importance of large-bodied animals to ecosystem function portends greater future ecological disruption than that caused by similar levels of taxonomic loss in past mass extinction events.

Terrestrial biodiversity is declining rapidly (*1*), and the oceans are poised to follow suit without intervention (*2*). This "sixth mass extinction" may approach or exceed the magnitude of the five major extinctions of the past 550 million years (My) if current loss rates persist (*3*). Because the effects of massive diversity loss are difficult to scale upward from laboratory experiments or local ecosystem disruptions (*4*), ancient extinction events provide critical information for forecasting the structure and function of the future biosphere. Previous attempts to contextualize present threats using past extinctions focused mainly on extinction intensity (rate and magnitude of taxonomic loss) [e.g., (*3*, *5*)]. However, the distribution of losses across ecological functional groups (e.g., predators versus nonpredators) also strongly affects postextinction ecosystem function. Indeed, the preferential loss of dominant functional groups characterizes the two most important, era-bounding mass extinctions (*6*): the end-Permian event [252 million years ago (Ma)] exterminated all reef-building animals, and the end-Cretaceous (66 Ma) eliminated the nonavian dinosaurs.

The current distribution of extinction threat among functional groups has yet to be compared quantitatively to past extinctions, leaving unknown which events, if any, provide useful analogs for future ecosystems. The rich fossil record of marine animals provides an excellent opportunity to compare current trajectories of change with ancient patterns. Here, we use a database of 2497 extinct and living marine mollusc and vertebrate genera (fig. S1) to compare the projected intensity and selectivity of future extinctions with previous mass extinction events and with background intervals. To ensure maximum correspondence between fossil and modern analyses, we conduct comparisons at the genus level and, for the modern oceans, include only extant genera also known as fossils, which ameliorates differences imposed by preservation. We classify extinction threat for modern taxa using assessments from the International Union for Conservation of Nature (IUCN). We calculate extinction intensity as the percentage of genera that did not survive from one time interval to the next. We evaluate extinction selectivity via multiple logistic regression using four ecologically important predictors: body size (maximum length), habitat zone (pelagic or benthic), motility (motile or nonmotile), and feeding mode (predator or nonpredator) (*7*).

Predicted extinction intensity varies owing to the incomplete assessment of extinction threat for living species. In optimistic projections, we consider genera that lack IUCN-assessed species or are data deficient to be nonthreatened. Under these assumptions, projections for molluscs and vertebrates diverge, with vertebrates facing greater losses, because a large fraction of molluscs lack assessment whereas the vast majority of vertebrate genera in the data set contain at least one assessed species (Fig. 1). Pessimistic scenarios, in which only IUCN-assessed species are analyzed and all genera containing species categorized as vulnerable or worse are lost, predict the extinction of 24 to 40% of genera overall (table S1), with similar loss levels in vertebrates and molluscs (Fig. 1 and fig. S2). The lower value is twice the background rate, and the higher is comparable to the end-Cretaceous mass extinction (Fig. 1).

Extinction threat to living genera is most strongly associated with body size (Fig. 2). The odds [i.e., $q/(1-q)$, where q is the probability of

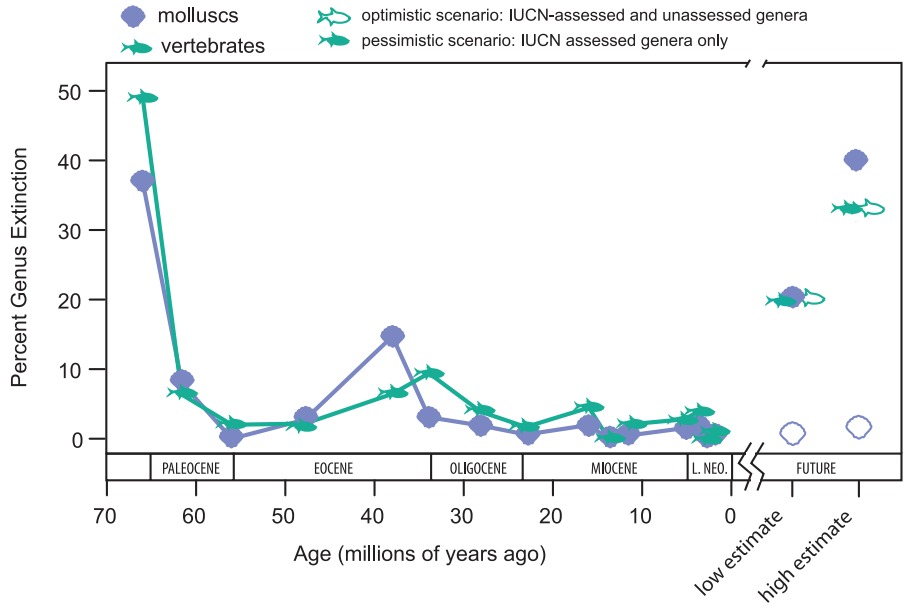

Fig. 1. Historic genus extinction intensity and modern predictions for marine molluscs and vertebrates. Extinction intensity has generally not exceeded 10% for either molluscs or vertebrates since the end-Cretaceous extinction. The proportion of modern genera with near-threatened species exceeds background extinction rates in most data treatments. The only exception is in molluscs under the assumption that genera lacking assessed species are entirely at low threat of extinction. Upper estimates approach the magnitude of the end-Cretaceous mass extinction. For future predictions, closed symbols indicate results from a pessimistic scenario based only on genera containing IUCN-assessed species; open symbols indicate results from an optimistic scenario in which genera lacking assessed species were included and assumed to be nonthreatened. Both scenarios include only living genera with fossil records to maximize comparability with extinction intensity for ancient events. Genera were classified on the basis of either the least- or most-threatened member species (low and high estimate, respectively). L. Neo., Late Neogene.

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being threatened with extinction] increase by a factor of 13 for each order of magnitude increase in body length (calculated by exponentiation of the coefficient in Fig. 2A) under our primary data treatment, in which genera lacking assessed species are assumed not to be threatened. This strong size bias holds in a wide range of data treatments, such as the exclusion of genera lacking assessed species (fig. S3), variation in the threat level used to distinguish threatened versus non-threatened genera (fig. S4), and the inclusion of living genera lacking fossil records (fig. S5). The size bias is present within both vertebrates and molluscs and most constituent classes (figs. S6 and S7). Motility is positively associated with extinction threat (Fig. 2C), although this result is sensitive to data treatment (figs. S3 and S5). Habitat zone and feeding mode are not correlated with threat (Fig. 2, B and D).

The ecological distribution of present-day extinction threat has no precedent in the fossil record. During the past 66 My, background extinction was generally associated moderately and inversely with body size and was independent of other predictors (Fig. 2). During previous mass extinctions, body size was inversely associated or not associated with extinction probability (Fig. 3). This result is robust to the exclusion of all genera smaller than 2 cm in maximum length and to the restriction of the analysis to narrower taxonomic groupings (figs. S8 to S10). Our finding that ancient extinctions were rarely selective with respect to body size is also largely consistent with previous analyses of fossil marine animals, although previous studies were taxonomically and temporally more restricted and generally did not adjust for other ecological predictors (*7*). Motility was inversely associated with extinction during the end-Cretaceous extinction but is generally positively associated with threat in the modern oceans (Fig. 3). Pelagic genera were preferentially lost in all previous mass extinctions, whereas modern threat is not preferentially associated with the pelagic habitat zone (Figs. 2 and 3). These results are also robust to alternative data treatments, such as the exclusion of genera smaller than 2 cm in maximum length (fig. S10).

A principal component analysis of the regression coefficients shown in Figs. 2 and 3 illustrates the unique selectivity of the emerging mass extinction (Fig. 4). None of the previous mass extinctions or background intervals plot near the modern extinction threat, reflecting differences in the coefficients of association for body size and habitat zone. The extreme bias against large-bodied animals distinguishes the modern diversity crisis from all potential deep-time analogs.

The association between body size and modern extinction threat is reflected in descriptions elsewhere of elevated threats to large-bodied marine animals and overfishing-induced population declines (*8*, *9*). It is consistent with the tendency for fisheries to first exploit species at higher trophic levels and subsequently move down the food web (*10*), as animals at higher trophic levels are typically larger than their counterparts at lower trophic levels (*11*). Size-biased vulnerabilities operate within species as well (*12*, *13*), reflecting a unique human propensity to cull the largest members of a population (*14*). Humans also heavily influence small-bodied species (*15*), but these impacts have yet to register in global threat profiles. These pressures on large-bodied marine animals are mirrored in patterns of terrestrial defaunation (*16*), which are also distinct from background patterns spanning the past 66 My (*17*).

Although climate and ocean chemical responses to anthropogenic CO_2 emissions can be predicted by historical episodes of climate warming and ocean acidification [reviewed in (*18*)], climate change does not appear to be the dominant driver of extinction threat for the taxa examined here. Rather, human fishing and hunting define the dominant threat to modern marine fauna (*2*, *19*, *20*). Although we observed no contemporary association between threat and habitat zone in these taxa, others have documented preferential and widespread modern declines in benthic marine microfauna resulting from nutrient pollution and oxygen deprivation in bottom waters (*21*). The sustained proliferation of dead zones associated with nutrient pollution (*22*) may eventually leave a similar, global mark on the macrofaunal groups that we examined. If climate change ultimately surpasses fishing in shaping extinction threats, patterns of selectivity would likely converge with earlier mass extinctions.

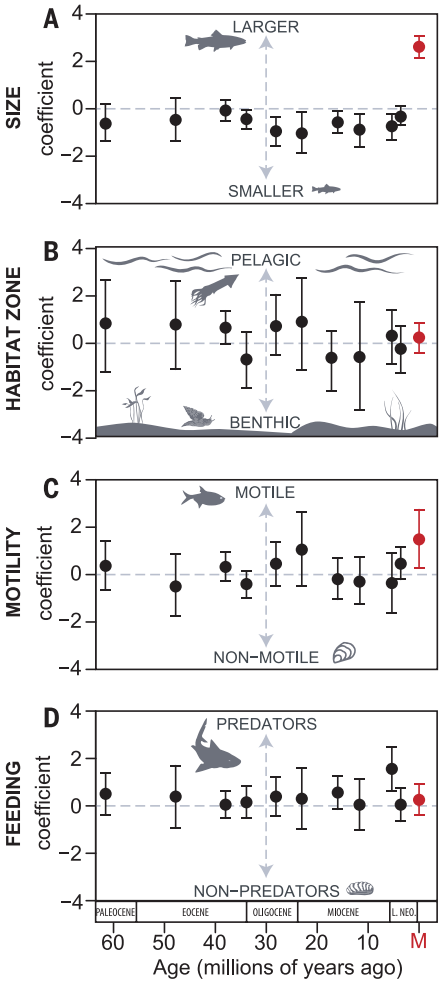

Fig. 2. Extinction threat in modern oceans is uniquely biased against larger-bodied animals. Ecological selectivity of extinction risk in the modern oceans (red symbols) and background extinction selectivity in the Cenozoic Era (66 Ma to present) based on the fossil record (black symbols). The vertical axis represents the coefficient associated with the predictor averaged across all subsets of the full multiple regression model. Error bars represent 95% confidence intervals on estimated coefficients.

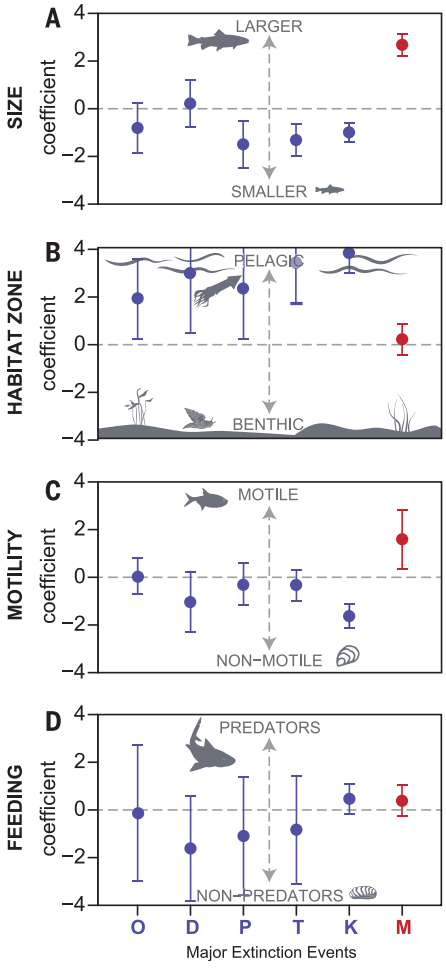

Fig. 3. Ecological selectivity of extinction threat in the modern oceans is unlike any previous mass extinction. Previous mass extinction events (blue symbols) preferentially eliminated pelagic genera and, sometimes, smaller genera, whereas the modern extinction threat (red symbols) is strongly associated with larger body size and moderately associated with motility. Error bars represent 95% confidence intervals on estimated coefficients. O, Late Ordovician; D, Late Devonian (Frasnian/Famennian); P, end-Permian; T, end-Triassic; K, end-Cretaceous; M, modern extinction threat.

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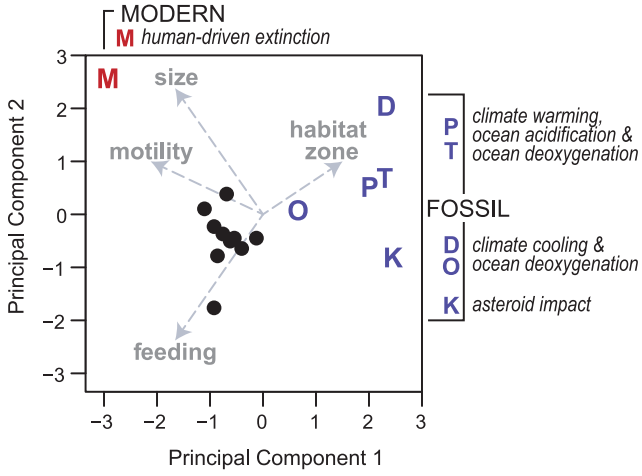

Fig. 4. Unique ecological signature of the emerging marine mass extinction. The first two principal components from a principal component analysis of the logistic regression coefficients presented in Figs. 2 and 3, illustrating the distinctive selectivity pattern of the modern extinction threat relative to the past 66 My of background extinction and the five main mass extinctions in the fossil record (letter and color scheme as in Fig. 3). Black dots represent Cenozoic background intervals. The first two principal components explain 79% of the variance.

The preferential threat to large-bodied marine animals poses a danger to ecosystems disproportionate to the percentage of threatened species. Large-bodied animals are critical to ecosystem function because of their preferential position at the top of food webs (*11*, *23*) and importance to nutrient cycling (*24*) and bioturbation of sediments (*25*). Removal of large-bodied predators can also trigger trophic cascades affecting many other species [e.g., (*26*)]. Although previous mass extinctions did not preferentially remove the largest taxa, the sheer magnitude of loss across the body-size spectrum entailed the extinction of many of the largest species, and surviving genera may have become smaller as well (*27*). The loss of large taxa may have caused more ecological disruption than the loss of comparable numbers of smaller taxa; indeed, loss of large animals may explain in part the multimillion-year delays in ecosystem recovery following these catastrophes. The preferential removal of the largest animals from the modern oceans, unprecedented in the history of animal life, may disrupt ecosystems for millions of years even at levels of taxonomic loss far below those of previous mass extinctions. And, unfortunately, the lack of correlation between the proportion of species assessed within higher taxa (phyla, classes, and orders) and the proportion considered threatened for marine animals (*28*) suggests that the pessimistic projection of future genus losses (Fig. 1) may more closely approximate the true threat level than the optimistic projection. Without a dramatic shift in the business-as-usual course for marine management, our analysis suggests that the oceans will endure a mass extinction of sufficient intensity and ecological selectivity to rank among the major extinctions of the Phanerozoic (541 Ma to present). Such an event would usher the world not only into a new geological epoch (Anthropocene) but also into a new period (Anthropogene) or even a new era (Anthropozoic) (*29*, *30*).

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SUPPLEMENTARY MATERIALS

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Development of Major Enterprises

In 2015, there were approximately 21.1 thousand industrial enterprises in this region. Among them, 1630 have reached considerable scales, including one 100-billion-giant enterprise, three 10-billion-giant enterpises, and 397 100-million-giant erterprises. Many are famous nationwide namely Media, Galanz and Country Garden, etc. Currently, there are 18 public listed enterprises, and 27 listed in new tertiary board.

Major Economic Indicators

In 2015, the GDP of Shunde has reached 258.7billion yuan, increased by 8.5%. The industrial output value of enterprises with 20-million-turnover has reached 629.71 billion yuan, increased by 8.0%. The total import and export value has reached 25.77 billion USD and the foreign investment in actual use has been 930 million USD.

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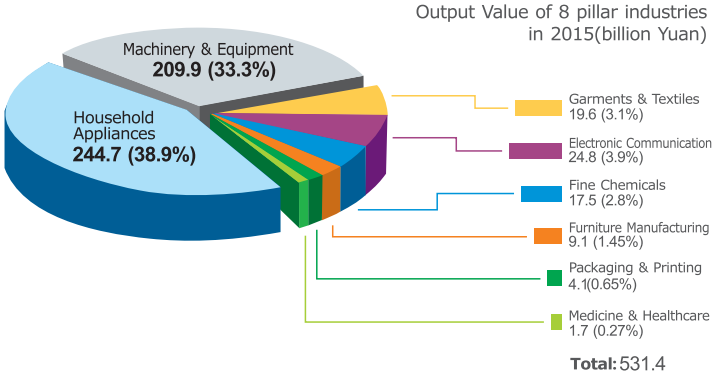
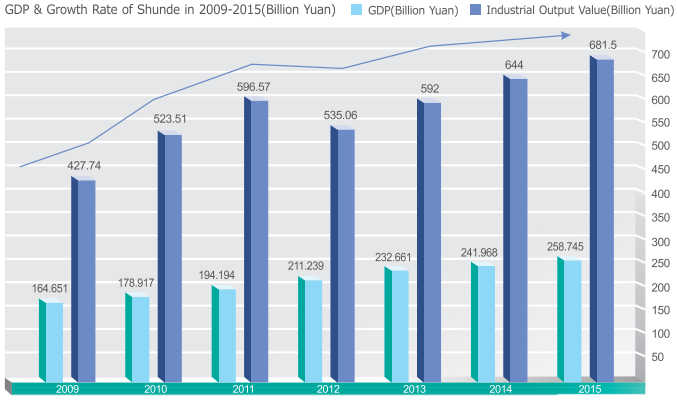
Located in the Pearl River Delta, Shunde is close to Guangzhou, Shenzhen, Hongkong and Macau. With a total area of 806km², She is one of the most famous manufacturing hub in that region. Shunde is also considered as one of the "Four Tigers in Guangdong". She was ranked No. 1 in "Top 100 cities in China" for 4 consecutive years from 2000 to 2003, and No. 1 in "Top 100 Districts in China" for 4 consecutive years from 2012 to 2015, attracting investments all over the world. Currently, there are 2029 foreign-invested companies and 97 representative offices in Shunde. Over 46 independent enterprises were established by 29 enterprises of the Fortune 500. In 2016, Media Group is lised in the Fortune 500 for the first time, making it the first Foshan-originated enterprise stepping in the Global 500, as well as the first Chinese-originated enterprise specilizing in household appliances on the Global 500 list.





Development of Major Industries

In 2015, the ratio of primary, secondary and tertiary industries in Shunde is 1.5:58.5:40.0. The major industries in Shunde include household appliance, machinery equipment, electronics, IT, textile, garment, advanced chemical engineering, packaging, printing, furniture manufacturing, automotive accessories, pharmacy, and healthcare. The two dominating industries in our district are household appliances and mechanical equipment, with annual production of 224.7 billion RMB and 209.921 billion RMB respectively, growing at a rate more than 9% per annum. Nowadays, industrial robots are the most important component of intelligent equipment industry. In recent years, the development of industrial robots in Shunde market has increased by leaps and bounds, and many robotic enterprises have gathered in Shunde, KUKA Robotics China (Shanghai) Ltd has constructed the Robotic Application Engineering Center as well as more than 6 supporting robotics enterprises in Sino-European Services Center of Foshan New City.

With a comprehensive approach to the robotic equipment industry, the Media Corporate and Yaskawa Japan founded a robotics company in March this year. This company will focus on the manufacturing of industrial robots and service robots, and it is expected to be in production in October. Apart from holding 94.5% stock of KUKA Robotics Company, Media has become the second largest shareholder of Anhui Efort Robotics Company.

After introducing Fanuc Robotics into Sino-European Services Center, the Fanuc Robotics Engineering Application Center in Shunde was established. Moreover, LXD Robot System Company Ltd, ABB and COMAU have signed the strategic cooperation agreement and carried out collaborations in research and development as well as constructions.

In addition, LS Robotics Company cooperated with Kawasaki Heavy Industries Ltd to build the Application Engineering Center for White Appliances and Industrial Robots, as well as Training Center of Kawasaki Robot in South China. They have become the largest executive agent and cooperative partner of Kawasaki in South China. Anhui Efort Intelligent Equipment Company Ltd is also looking forwards to establishing an integrated plant for robotic system in Shunde and entering the Huayin Robotic Industrial Park.

ADVERTISEMENT

Individual cell analysis for protein expression and cell quantitative alternative to standard bulk detection me

Detection and quantitation of proteins in biological samples is an essential task routinely performed in countless disciplines and in laboratories all over the world, for activities ranging from basic protein characterization to clinical diagnostic testing and drug development. Common methods for protein detection include enzyme-linked immunosorbent assay (ELISA), dot blot, and Western blot (also called protein immunoblot).

While these standard assays continue to present a reliable means of cell analysis for thousands of targets, recent advances in instrumentation offer significant improvements in time savings and convenience for common markers. An additional limitation of bulk assays is the need to homogenize cells or tissue, resulting in a loss of information from individual cells in a population. Techniques that detect and report signal from individual cells can provide quantitative data from large populations about protein target or other marker levels, from cells of varying phenotype, developmental state, or health status.

Quantitative data from large populations with single-cell precision

Flow cytometry addresses this need for quantitative data from significant cell populations by interrogating individual cells for the presence and relative strength of signal from fluorescent reagents or antibodies. However, traditional flow cytometers require extensive operator training and expertise, and sheath fluid-based systems are characterized by extensive setup and shutdown—as well as considerable cost to purchase, operate and maintain.

The Muse® Cell Analyzer was developed to give researchers simple, affordable access to the quantitative data that flow cytometry provides for measuring markers of viability, mitochondrial health, protease activity, and more. Built on flow cytometry principles, Muse® uses microcapillary fluidics and pre-optimized reagents to create an inexpensive, compact, portable system that requires little setup and no expertise to operate. These attributes present a rapid, simplified alternative to more time-consuming methods like Western blot (that may also demand considerable technical expertise) for routine analysis of cell culture health, and to assess the effects of compounds for toxicology and drug discovery screening.

Assay	Key materials and equipment needed	Hands-on time	Typical total assay time: From cells to data
ELISA	Coated multiwell plates Capture antibody Detection antibody conjugate Enzyme substrate Stop solution Plate washer ELISA reader	1.5 hours	8 hours – 2.5 days
Dot blot	Blot cassette Blocking buffer Nitrocellulose membrane Protein standard Primary antibody Secondary antibody Detection reagent	1.25 hours	5.5 hours
Traditional Western blot protocols **	Lysis buffer protein quantification kit Protein standards SDS page gels Electrophoresis chamber Loading buffer Running buffer Transfer buffer Protein transfer chamber Membranes Filter paper Blocking buffer Primary antibody Secondary antibody Gel/blot imager	2 – 3 hrs	10 hrs – 2.5 days*
Muse®	Muse® Cell Analyzer Relevant Muse® assay	10-15 mins	10 mins – 4 hrs.

Table 1. Workflow comparison among protein detection methods. For proteins not already in solution, bulk methods require lysis of samples to render proteins accessible to detection. Reagents, supplies and equipment for separation, transfer, probing, detection and imaging may be needed, depending on technique. Lengthy primary antibody incubations and serial washes following each binding step can result in significant hands-on and total elapsed time for routine protocols. The Muse® Cell Analyzer uses preoptimized reagent cocktails to minimize variation and instrument setup, resulting in appreciable reductions in time spent on the bench and total start-to-result time.

*Significant time savings can be achieved by use of a vacuum-driven method such as the SNAP i.d.® 2.0 system for Western blotting.

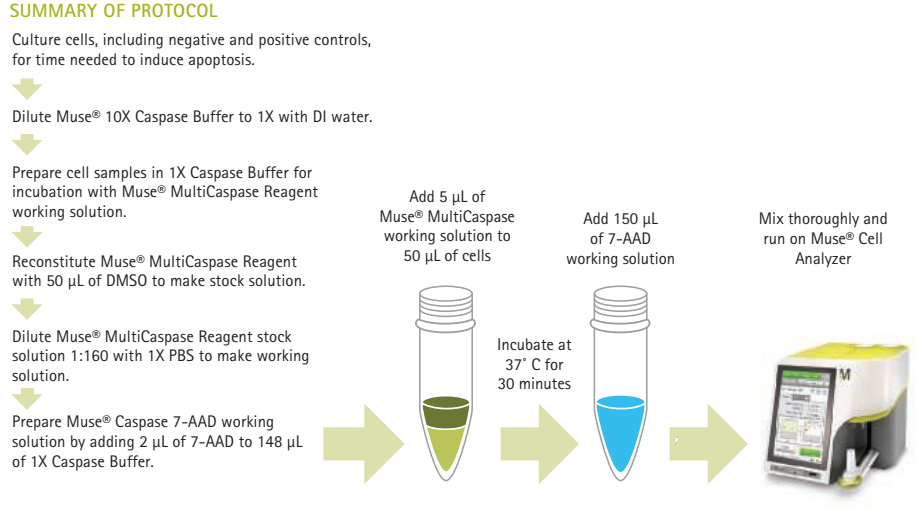


Figure 1. Typical Muse® assay experimental protocol summary. Steps shown are from the protocol for the Muse® MultiCaspase Assay kit (Cat. No. MCH100109) for detection of the activity of caspases 1, 3, 4, 5, 6, 7, 8, and 9. Results from this assay are available in approximately one hour, about 20 minutes of which requires hands-on activity.

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health markers provides a rapid, ethods for fundamental assays

Population means vs. individual cell quantitation

Data that can be quantified are increasingly important in the life sciences, as quantitative data are objective and therefore considered more reliable, as well as being subject to statistical analysis. Quantitative data are assumed to be more representative of populations than qualitative data, and therefore must be characterized both by significant sample size and by the capacity to measure individual events in a sample.

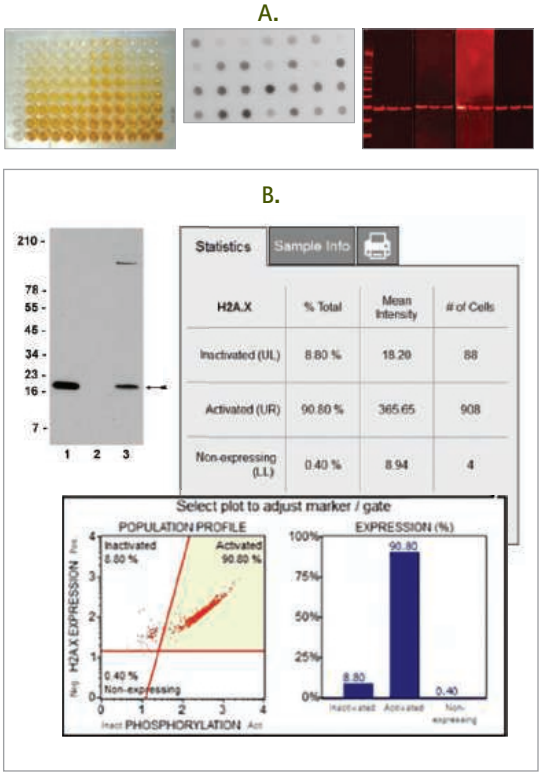

Figure 2. Common bulk immunodetection assay results contrasted with quantitative data. A. Enzyme-linked immunosorbent assay (ELISA), left panel, uses a colorimetric or a fluorescent detection reagent. Spectrophotometry can be used to transform signal intensity into numerical values, but signal intensity is a mean from all cells or cell products in a sample, as is the case with dot blot (middle panel). The right panel is an example of fluorescent detection of immunoblot (Western blot), showing the 'ladder', or molecular standard, in lane 1. B. Left panel, Western blot of recombinant histone H2A.X (lane 1), recombinant histone H2A (lane 2), and acid extracted proteins from HeLa cells (lane 3) were probed with anti-histone H2A.X. The right panel shows representative data from the Muse® H2A.X activation dual detection assay, which uses two directly conjugated antibodies against the unmodified and phosphyorylated histone target to map signal from every cell in the sample onto a scatter plot. Absolute numbers and percent of cells activated in the sample are automatically calculated and displayed on the 'Statistics' tab.

Although spectrophotometry and densitometry can be used to transform sample well color or the size of a blot or band into numerical values for comparison of relative signal intensity among samples, these methods rely on homogenization of all of the cells or tissue in a particular sample. Western blot relies on concurrent electrophoresis of a mixture of proteins of known weight to create a standard, or 'ladder' of bands on the blot, to which positive bands from sample lanes are compared for confirmation of the protein's identity (Figure 2A, right panel). Because no identity information can be gained from immunoblot without the standard for comparison, Western blot is considered 'semi-quantitative'.

Conclusions

Immunoblot and immunosorbent assays continue to be among the most popular methods for protein detection in the life sciences, as they are amenable to measuring virtually any target for which an epitope binder such as an antibody can be developed. These methods are constrained, however, by the inability to capture population variation due to the homogenization of sample. Standard bulk methods may also not be optimal for routine screening because of the time they consume in the lab and the expertise they require in order to optimize reagents and to obtain, interpret, and troubleshoot results.

Simplified flow cytometry-based analysis presents a rapid, uncomplicated, cell-based alternative to methods such as immunoblot, particularly for routine and frequent screening of cell cultures, or for response of cell models to compounds in development for chemotherapeutics, drug discovery, cosmetics and similar applications. In addition to detecting key protein targets, The Muse® system incorporates assays for detection using familiar cell status indicators that do not rely on antibody-protein interactions, such as fluorescent membrane integrity dyes and nucleic acid binders.

The Muse® software automatically returns cell-by-cell results from these reagents, unlike microscopy or other low-throughput, time-consuming or subjective techniques for measuring their signal. Muse® assays are selected to provide an efficient means for the most essential viability, cell health, and signaling screening, and reagents are pre-optimized to minimize variation and the need for complex setup adjustments that characterize traditional open-system cytometers.

Despite its small size and remarkably simplified operation, the Muse® system returns the same powerful single-cell data as larger, more costly and complex systems. The availability of rapid, quantitative cell analysis without the need for extensive investment in supplies or trained personnel has the potential for significant impact on compound screening and cell culture model paradigms in the pharmaceutical and life science research domains.

To learn more about the Muse® Cell Analyzer and see a complete list of Muse® assays, please visit: www.emdmillipore.com/muse

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ZHEJIANG UNIVERSITY CALLS FOR GLOBAL TALENTS

Brief Introduction of Zhejiang University

Located in the historical and picturesque city of Hangzhou, Zhejiang University is a prestigious institution of higher education with a long history. After a hundred years of construction and development, Zhejiang University has become a comprehensive research university with distinctive features and relatively great impact at home and abroad. Research at Zhejiang University spans 12 academic disciplines, covering philosophy, economics, law, education, literature, history, art, science, engineering, agriculture, medicine, management and etc. In all 22 disciplines of ESI, there are 18 disciplines leading to top 1% in the world. Zhejiang University has always been committed to cultivating talent with excellence, advancing science and technology development, serving for social well-being, and promoting advanced culture with the spirit best manifested by the university motto "Seeking the Truth and Pioneering New Trails".

Zhejiang University has long been holding the educational philosophy of putting people foremost, cultivating students in an all-round way, seeking the truth and pioneering new trails in search of excellence, and is committed to cultivating future leaders with an international perspective.

Brief Introduction of Hundred Talents Program

Zhejiang University (ZJU) is seeking faculty candidates for highly competitive, well funded, and newly launched Hundred Talents Program. This program covers all colleges and departments at ZJU. Successful candidates are expected to establish internationally competitive and independent research program in cutting-edge areas of relevant field. According to their qualification, candidates should have demonstrated commitment to excellence in teaching and research, which is comparable to the academic achievements of assistant professor or associate professor in world-renowned universities.

Know More of Our Talent Programs

Except for the Hundred Talents Program, there are a number of talent recruitment programs and open job opportunities for high-end talents worldwide, as for details please visit http://talent.zju.edu.cn/redir.php?catalog_id=20038&object_id=40799, or scan the QR code of recruitment programs of ZJU below.

Zhejiang University sincerely invites excellent talents to join us and together to create a glorious future for Zhejiang University and its people.

Contact: Talent Office, ZJU
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E-mail: tr@zju.edu.cn

• What We Offer

Successful candidate will be employed as ZJU100 Professor who is qualified to supervise doctoral student. ZJU will offer a decent compensation with the opportunity to purchase one of the apartments reserved for high-end talents, a good facilitate of office and laboratory space as well as internationally competitive startup packages will be provided.

• How to Apply

Qualified applicants are strongly encouraged to submit your application electronically to tr@zju.edu.cn with the following materials in PDF format: a comprehensive CV, a statement of research and teaching plan, certificates of academic degrees, 3 samples of major publications and a list of 3 to 5 references with detailed contact information.

• Qualifications and Requirements

Candidates are expected to be about 35 years old. Successful applicants should hold a doctoral degree of world-renowned university and postdoctoral experiences are preferred. Successful applicants are supposed to work full-time and deliver excellent research and teaching at ZJU. The spirits of "Seeking the Truth and Pioneering New Trails" are expected.

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For more information, please see website: **http://goo.gl/448XFS**. Enter 87650, 87352 or 87384 in search field and follow the instructions there on how to apply. Applications received by web-specified respective deadlines will receive full consideration. *Women and candidates from groups traditionally under-represented in engineering are strongly encouraged to apply.*

COLLEGE OF THE HOLY CROSS DEPARTMENT OF BIOLOGY TENURE-TRACK FACULTY POSITION IN NEUROBIOLOGY

The Department of Biology at the College of the Holy Cross invites applications for a **TENURE-TRACK ASSISTANT PROFESSOR** appointment in Neurobiology to begin August 2017. More detailed information is available at: http://www.holycross.edu/academics/programs/biology/neurobiosearch. The position carries a 3-2 teaching load with full salary and benefits, and a one-semester research leave prior to tenure review.

Candidates should demonstrate a commitment to undergraduate teaching at the introductory and advanced levels and be prepared to develop an active research program in which engaged undergraduate students may meaningfully participate. A Ph.D. is required; postdoctoral experience is preferred. The College and the Department maintain a core commitment to diversity and inclusion. Competitive applicants will excel at working with a diverse student body. For more information, please visit http://holycross.edu/diversity & http://holycross.edu/mission.

Please submit a cover letter, a CV, a statement on teaching expertise and specific teaching interests, a research plan, transcripts (undergraduate and graduate), and three confidential letters of recommendation. Please submit all application materials to website: **https://apply.interfolio.com/36761** by October 15, 2016. Contact Ann Sheehy, e-mail: asheehy@holycross.edu, with questions related to this search.

The College of the Holy Cross is a highly selective Catholic liberal arts college in the Jesuit tradition, enrolling about 2,900 students and located 45 miles west of Boston. The College supports resources for dual career couples, and embraces all Equal Employment Opportunity Employer and Affirmative Action laws in the workplace.

UNIVERSITY OF ROCHESTER

The Department of Chemistry invites applications in the areas of biophysical and organic chemistry (including bioorganic and organic materials) at the **ASSISTANT PROFESSOR** level starting in the summer of 2018, with the possibility of an earlier start date. Candidates are expected to establish an outstanding program of original research, and be effective teachers at the graduate and undergraduate levels. Application materials should be submitted online at website: **https://www.rochester.edu/faculty-recruiting**. Additional information about our department can be found at website: http://www.chem.rochester.edu/ and questions may be sent to email: **facrec@chem.rochester.edu**. Review of completed applications will begin on **October 1, 2016**.

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Position 1: Molecular geneticist using molecular approaches in their genetics research program, preferably incorporating quantitative and/or computational techniques. Successful applicants must provide evidence of the potential to teach genetics and molecular biology. Position begins January 2018.

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Reviews begin October 21, 2016. See full position announcement, including all required and preferred qualifications, at website **https://cse.wwu.edu/biology**. For application information and instructions, go to the WWU Employment website **http://www.wwu.edu/jobs.** *AA/EOE*

PRINCETON UNIVERSITY DEPARTMENT OF CHEMISTRY ASSISTANT PROFESSOR

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The Jackson Laboratory (JAX) is inviting applications for Assistant, Associate, and full Professors to join in expanding a major program in Microbiome Research and Microbial Genomics. We are seeking individuals who are taking innovative approaches to understand the role of microbiotas in human health to join our interactive culture of cooperation and program integration.

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- State of the art mouse genetic and veterinary facilities including a large repository of strains and mutants, expertise for microbiome and pathogen research, a germ-free/gnotobiotic facility, and an extensive phenotyping capability
- Advanced genetic techniques such as CRISPR/Cas9 genome editing technologies forinnovative approaches to investigating host-microbiome interactions

JAX is an independent, nonprofit biomedical research institution with a highly collaborative environment fostering multidisciplinary approaches to investigate complex biological questions. Faculty members are supported by outstanding scientific services, unparalleled mouse and genomic resources, postdoctoral and predoctoral training programs, and numerous courses and conferences.

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Reviewers rule: Strategies for faculty advancement

As you progress in your academic science career, it is vital to know how to not only write papers and grant proposals, but how to review them as well. Ensuring you develop skills in reviewing the currency of academia will provide both tangible and intangible professional benefits. **By Alaina G. Levine**

At the Fred Hutchinson Cancer Research Center (The Hutch) in Seattle, Washington, the Office of Scientific Career Development does a little extra in assisting postdocs and graduate students with their career planning. **Karen Peterson** directs this enterprise, and tucked into her repertoire of advice and resources for finding a job, landing a fellowship, and honing core competencies required of successful scientists, is a crucial opportunity: the chance to develop prowess in reviewing grant proposals.

The review process, and all of the critical thinking, analysis, organizational, and communications skills that are sharpened while pursuing it, is an essential element of academic science. And yet, while protégés are often taught how to write papers and proposals early in their careers, they usually have to figure out the reviewing part on their own. If they are fortunate, their advisors provide guidance.

But counselors like Peterson are looking to change that, by offering advice on how to review papers and grant proposals and how to effectively serve on grant panels and study sections. It's a good thing, too, considering that the capabilities developed from conducting reviews are what scientists need to advance in their academic careers. "Our currency is writing manuscripts and grants—that is what is most highly valued in academia," she says, "so it's incredibly important for your professional development and training as a scientist if you want to stay in academia."

Tracy L. Kijewski-Correa

When you review others' work, you gain insight into the cutting-edge issues of the field, as well as the skills needed to produce your own winning outputs. As Peterson puts it, "As a junior faculty, if you're lucky to get on a study section, it can take you to another level in your career and help you be more successful in your own grant writing. It's the best way to learn how to write grants."

Opt for the opportunities

Young investigators looking to boost their CVs may not realize it, but review opportunities are hidden around many corners. The best place to start is with your own principal investigator (PI). "Professors get asked to review papers all the time," says **Takashi ("TK") D. Y. Kozai,** assistant professor of bioengineering at the University of Pittsburgh in Pennsylvania. "If you let them know you are interested, they are often willing to refer you when they decline."

But there is more to leverage besides your advisor's pass-alongs. It is common practice in academia for scholars who are asked to review papers to share the task with their charges. This is referred to as an "undisclosed review," says **Tracy L. Kijewski-Correa,** Leo E. and Patti Ruth Linbeck Collegiate Chair and associate professor in the Department of Civil and Environmental Engineering and Earth Sciences at the University of Notre Dame. As a graduate student, her PI gave her the chance to earn her chops at reviewing by engaging in such a scenario. "The advisor accepted the review and fed it to me, and I conducted the review. He looked it over, massaged it, and we worked together to finalize it."

As long as you are discrete, you can assist your PI in reviewing the papers they are given to review, as **Rubina Kotak** attests. A reader (essentially an associate professor) in astrophysics at Queen's University Belfast in Northern Ireland, Kotak is also an associate editor for the journal *Astronomy & Astrophysics*. When she sends out papers for review, she has no problem if the advisor shares the responsibility with their protégé. She even suggests that early career investigators ask for these opportunities. "As an editor, I wouldn't mind this," says Kotak. "You can discuss the paper with colleagues as long as you maintain complete confidentiality."

Adriana S. Bankston, a postdoctoral research associate in the Department of Anatomical Sciences and Neurobiology at the University of Louisville School of Medicine in Kentucky, appreciates that her postdoc mentor invited her to assist with paper reviews. "We both read the paper independently and wrote our own critiques. We then came together and discussed them, and he submitted a response to the journal containing parts of both our responses," she says. "From this experience, I learned what kinds of things to pick out in terms of writing a good paper review."

While divvying out paper reviews is fairly routine, doing so with proposal reviews is quite another story—this is a definite no-no. In fact, scientists who are given grant cont.>

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FACULTY POSITIONS

"Learning to be very rigorous about analyzing strengths and weaknesses in reviews helps you in all aspects of your career, whenever you need to weigh different paths."

—Kanwal Singh

proposals to scrutinize are under strict dictate not to reveal the content of the proposals or share the load of reviewing them. While this can be disheartening for early career academics who wish to gain experience in proposal reviewing, there are other ways to learn and hone this craft. At The Hutch, Peterson leads a training module that consists of U.S. National Institutes of Health Career Development (K)-Award proposal-writing basics, a mock study section where trainees can learn by critiquing proposals, and an opportunity to look at examples of successful K-award proposals. Participants also get to hear firsthand from faculty who have sat on K-award study sections and reviewed many K-award proposals. This type of hands-on training is invaluable in building an academic career, she notes.

As a graduate student, Bankston participated in a grant-writing course that included a chance to review grant proposals in a simulated study section. "Overall, this was a great opportunity to learn about this process as part of a graduate course before going out into the real world to review grants as PIs do on actual study sections," she says.

Ask the gatekeepers

Good talent is hard to find, as are good reviewers. So it is not surprising that when you begin to build your academic brand and become known for being a good reviewer, you become sought after and can get asked to review—a lot.

Editors and granting agency program officers are always looking for new reviewers—they need fresh perspectives to provide high-quality reviews. Thus, early career scholars shouldn't feel hesitant to reach out to editors and ask about the opportunity or even suggest themselves as a reviewer, says Kijewski-Correa. After reviewing over 100 grant proposals and 5–10 journal articles per year for the last 12 years as a faculty member, she joined the editorial board of the *Journal of Structural Engineering*. "As an associate editor, I see that young faculty are emailing their expertise to editorial boards to ask about future review opportunities," she notes. "I welcome this kind of input. Editorial boards are overwhelmed. Getting someone who wants to do a quality review is getting increasingly hard, so if I know someone who will do that, I am glad. I would balance the less-experienced reviewers with those who are more experienced."

Reviewers are especially coveted in emerging and interdisciplinary fields. "If you have expertise in a specific area of science, particularly something new that nobody's done, you might be able to get in sooner," says **Carmen R. Cid,** dean of the School of Arts and Sciences at Eastern Connecticut State University in Willimantic.

Scrutinize the skills

When you review someone else's work, you grow your abilities in numerous areas that are strategically critical for would-be faculty leaders. First and foremost, as Bankston notes, "it teaches you to be a better writer and more critical about your own results."

Scott Franklin, a professor of physics and astronomy and director of the Center for Advancing STEM Teaching, Learning and Evaluation at Rochester Institute of Technology in New York, shares how much he has improved as a communicator by paying attention to seemingly inconsequential organizational, formatting, and design decisions in grant proposals and papers. From how much space the authors devote to background information and literature reviews, to their use of bullet points to describe their goals, these methods of presenting information "leave an impression on you," says Franklin. "You are seeing tricks, like a figure presented in a way you never saw before. It could be something small like putting the figure on the side and wrapping the text around it. But these are soft skills that you pick up and can apply when appropriate."

Reviewing grant proposals enables you to "learn how to get to the point quickly," says Peterson. "You realize that some proposals are read after 20 hours of no sleep, sucking back the coffee, and sitting on an airplane," she says with a quick laugh. In this environment, "you learn what makes the best proposal."

In addition to the external conditions that can impact reviews, the reviewers themselves don't necessarily have the same background and deep knowledge of a subfield as the authors, and yet all they have to evaluate the work on is what's written on the page. "Something that's really obvious to you because you've been looking at it for weeks on end may not be clear to others," says Kotak. So you have to ensure that anyone with a general education in your major field of study can grasp the importance of the concepts you are putting forward.

Kanwal Singh, dean of Sarah Lawrence College in Bronxville, New York, has served on review panels and offers another skill that is gained in the review process. "You learn to work like a jury to come to consensus," she says. "You have to function within constraints—it isn't realistic to recommend everything you think is good for support, so you have to be really meticulous about making decisions."

There's also the matter of acquiring a code of ethics as a scientist, yet another "skill set" that can be developed by conducting reviews. Early in her career, Bankston already recognizes "the great responsibility that scientists have to produce fair, constructive paper reviews." Given the gravity of the reviewer's position, "if I don't do this ethically and objectively, I could kill someone's career," admits Kijewski-Correa. This forces scientists to think critically about how they cont.>

PHOTO: DON HAMERMAN

EMBL

The European Molecular Biology Laboratory (EMBL) is searching for Group Leaders. EMBL offers a highly collaborative, uniquely international culture. It fosters top quality, interdisciplinary research by promoting a vibrant environment consisting of young, independent researchers with access to outstanding graduate students and postdoctoral fellows. EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

EMBL Group Leader Opportunities

at EMBL Heidelberg, Germany

GROUP LEADER

CRYO-ELECTRON MICROSCOPY

The Structural and Computational Biology (SCB) Unit at EMBL Heidelberg seeks to recruit an outstanding Group Leader in cryo-electron microscopy (single-particle analysis and/or electron tomography) to address fundamental questions in molecular or cellular biology.

Imaging from molecules to organisms across different scales and resolution by correlating different technologies is a strong research focus of EMBL with single-particle cryo-electron microscopy, electron tomography and correlative light and electron microscopy playing central roles. The successful candidate is expected to develop an exciting biological or methodological research programme, complementing the existing activities, but also to synergize with and build on other structural biology, computational, chemical and systems biology approaches that are utilised by the SCB Unit. The successful candidate should therefore demonstrate a strong motivation to work in the collaborative and multidisciplinary environment at EMBL.

The SCB Unit EMBL offers access to an outstanding integrated structural biology environment that includes a particularly well equipped cryo-electron microscopy facility housing several high-end transmission electron microscopes including by next year two Titan Krios, one Talos Arctica and one Polara microscopes with direct detectors, a full complement of electron microscopy preparation equipment including a focused-ion-beam milling and scanning electron microscope (FIB/SEM), and high performance computing clusters.

For further information about the position please contact **recruitment@embl.org**.

Interviews are planned for 14 to 16 November 2016.

GROUP LEADER (2 Positions)

DEVELOPMENTAL BIOLOGY

The Developmental Biology Unit studies the development of multicellular organisms. Research in the unit covers all levels, from the cellular to the whole organism, and is highly interdisciplinary, combining a wide range of approaches and innovative techniques, with special emphasis on quantitative and real-time imaging. Research in the unit is firmly embedded within the overall EMBL research environment, with extensive in-house collaborations and support from world-class services, including the gene core, transgenic, metabolomics and mass-spectrometry core facilities.

We are seeking outstanding candidates using model organisms to address fundamental principles of multicellular development across the entire spectrum of developmental biology. Complementary approaches, including simplified systems, such as organoid and stem cell systems, are also welcome. Candidates with strong background and research using theoretical approaches are also encouraged to apply.

The successful candidates should have a strong motivation to work in the multidisciplinary and collaborative environment of EMBL, grasping the opportunity to interact with many other research groups.

Further information about the positions can be obtained from the Head of Unit Anne Ephrussi (**anne.ephrussi@embl.de**).

Interviews are planned for 19 to 21 December 2016.

APPLICATION INSTRUCTIONS

Please apply online through **www.embl.org/jobs** and include a cover letter, CV and a concise description of research interests and future research plans. Please also arrange for 3 letters of recommendation to be emailed directly by your referees to **references@embl.de** at the latest by 6 October 2016.

Further information on Group Leader appointments can be found under **www.embl.org/gl_faq**.

For more information please visit:

www.embl.de/jobs/work-at-embl

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FACULTY POSITIONS

Featured Participants

Center for Advancing STEM Teaching, Learning and Evaluation, Rochester Institute of Technology
www.rit.edu/science/castle

Eastern Connecticut State University
www.easternct.edu

Office of Scientific Career Development, Fred Hutchinson Cancer Research Center
www.fredhutch.org/en/education-training/oscd.html

Queen's University Belfast
www.qub.ac.uk

Sarah Lawrence College
www.sarahlawrence.edu

University of Louisville School of Medicine
louisville.edu/medicine

University of Notre Dame
www.nd.edu

University of Pittsburgh
www.pitt.edu

Karen Peterson (right) talking with colleague Robson Dossa

judge others' work. In Kijewski-Correa's case, she purposely developed a three-point rubric for all papers she reviews, which ensures that she remains consistent and impartial, and provides a clear mechanism to tell the authors what they can do better.

Review the benefits

Paper and proposal reviewing is challenging, time-consuming, detail-oriented work that is woven into both the research and service commitment of faculty. And yet, "you don't get glory" from it, says Kijewski-Correa. "You get a few indirect benefits, but those indirect benefits are huge." And it is the indirect gains that early career scientists should carefully consider as they pursue review opportunities.

One of the most important career advancement assets young scientists receive from the reviewing process is that "you're getting a feel for your field and what's going on," says Kijewski-Correa. "There's a lot of literature. Reviewing is a way of keeping you abreast. It keeps you fresh. You can't help but be inspired by osmosis." Indeed, "You get a sneak preview of what science people are doing way before the papers come out," concurs Kotak.

Singh notes that "learning to be very rigorous about analyzing strengths and weaknesses in reviews helps you in all aspects of your career, whenever you need to weigh different paths." Moreover, it allows you to "figure out when to take a chance on something that is a little riskier than something else, but where the potential payoffs may be much bigger." Additionally, reviewing papers and grants throughout the years has "helped me mentor my junior colleagues," she adds. "I can talk about the process with them, perhaps demystify it, and encourage them to get involved." Adds Kotak: "You learn how to give good feedback, and that's a skill you can use down the line for your own grad students and postdocs."

One of the biggest benefits of serving on a panel is the face time gained with program officers (POs), says Franklin. "When you are on a panel, there's downtime, and you get to talk to the POs and can run ideas by them," he notes. "It's an opportunity to sit down with a PO in person, instead of a phone call, and have a discussion that can extend over hours or days, not just a few short minutes. These conversations can be very valuable; they've definitely had an influence on how I frame grant proposals."

Franklin estimates he has reviewed 40–50 grant proposals during his career, and shares that serving on panels has given him crucial insight into how to present quickly and to hook the audience, something he has applied to his own proposals. There are usually several proposals being reviewed by the panel, he notes, which consists of scientists with expertise in a variety of areas—and while the science in all proposals is sound, those that clearly communicate goals, objectives, and methods are the ones that grab the reader. "So we go around the room and discuss it," Franklin explains. "Your proposal has to have a real hook that makes it stand out and be memorable to the reader … it has to jump off the page."

The reviewing experience can have comprehensive career repercussions for faculty. "It has helped me in a broad, holistic way and provided me with some context," adds Franklin. "For example, recognizing the funding landscape tends to make you accept rejection more calmly, whether it is a grant or in university life, like hearing 'no' from your department head or dean."

"Get your training wheels"

As with any career-enhancing experience, it is always a good idea to get started early as a reviewer. Talk with your PI and see if you can embark on an undisclosed review. But even if your advisor can't get you involved, there are usually other mechanisms on your campus to help you gain expertise in this process. For example, Bankston suggests offering to do paper reviews within the journal club in your department. And "you can always get your training wheels by reviewing scientific posters on campus," says Kijewski-Correa. Offering to review and make suggestions for papers written by colleagues within your department is another way to acquire these skills.

Cid offers this valuable advice: Even as a graduate student, you may have the chance to organize a workshop on a technical topic of relevance to your community. When you do, "invite people to your workshop whom you want to review for. They could even give a talk, and that way you can get to know them," she suggests.

No matter how they gain the experience, faculty who have engaged in reviewing emphasize that it has benefited their growth as scientists. "Most people are too narrow in their focus. There is often not enough incentive or encouragement to venture outside your particular area of expertise," says Kotak. "So gaining breadth in scientific knowledge [from reviewing papers] makes someone a better scientist. You're able to place your own research in a wider context."

Alaina G. Levine is a freelance science writer based in Tucson, Arizona.

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PHOTO: BO JUNGMAYER/FRED HUTCH

EMBL

The European Molecular Biology Laboratory (EMBL) is searching for Team and Group Leaders. EMBL offers a highly collaborative, uniquely international culture. It fosters top quality, interdisciplinary research by promoting a vibrant environment consisting of young, independent researchers with access to outstanding graduate students and postdoctoral fellows. EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

Team and Group Leader Opportunities

at EMBL Heidelberg, Germany

TEAM / GROUP LEADER

CELL BIOLOGY AND BIOPHYSICS

The Cell Biology and Biophysics Unit is a vibrant interdisciplinary department where biologists, physicists and chemists work closely together. The Unit's highly collaborative set of research groups employs approaches ranging from advanced electron and light microscopy, chemical biology and biochemistry, genetics and functional genomics to bioimage informatics, modelling and computer simulations. The research focusses on comprehensive understanding of the molecular mechanism of essential cellular functions, currently including transport, signaling, migration, differentiation and division.

We are seeking to recruit outstanding group leaders who wish to carry out cutting-edge molecular cell biology research. We also welcome applications to establish research or technology development groups in the areas of chemical biology and fluorescent probes, bioimage informatics and computational modelling of complex dynamic biological processes. In addition to biologists, applicants with a strong background in (bio)informatics/mathematics, chemistry or physics, and a keen interest in cell biological applications are especially encouraged to apply.

The successful candidate should have a strong motivation to work in the multidisciplinary and collaborative environment of EMBL, grasping the opportunity to interact with many other research groups.

Further information about the position can be obtained from the Head of Unit Jan Ellenberg (**jan.ellenberg@embl.de**).

Interviews are planned for 22 to 24 November 2016.

GROUP LEADER

GENOME BIOLOGY

The Genome Biology Unit is looking for outstanding candidates performing exciting interdisciplinary research, which complements on-going work within the Unit and the institute as a whole. We particularly encourage candidates with interests in specific aspects of gene expression regulation (transcriptional, post-transcriptional or post-translational regulation), nucleotide modifications, metabolism and cellular heterogeneity. We also welcome strong proposals from candidates with demonstrated excellence in any area of genome biology that dissects and/or models complex processes going from genotype to phenotype. This group leader position provides a very competitive package to support an independent research group in an excellent scientific and highly collaborative environment.

The Genome Biology Unit is a highly interdisciplinary department, including groups with expertise in Genomics, Genetics, Proteomics, Computer science and Microfluidics, which provides a collaborative and supportive environment to perform cutting edge research. The research focusses on dissecting fundamental principles of how different layers of molecular information (DNA, RNA, Protein, Metabolites) are regulated and interconnected to give rise to diverse phenotypes, and how variation alters those processes.

Further information about the position can be obtained from the Head of Unit Eileen Furlong (**furlong@embl.de**).

Interviews are planned for 5 and 6 December 2016.

APPLICATION INSTRUCTIONS

Please apply online through **www.embl.org/jobs** and include a cover letter, CV and a concise description of research interests and future research plans. Please also arrange for 3 letters of recommendation to be emailed directly by your referees to **references@embl.de** at the latest by 6 October 2016.

Further information on Team/Group Leader appointments can be found under **www.embl.org/gl_faq**.

For more information please visit:

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FACULTY POSITIONS

NEW YORK UNIVERSITY

FACULTY POSITION

Center for Genomics
& Systems Biology
Department of Biology

ARTS AND SCIENCE

Center for Genomics and Systems Biology
New York University

New York University's Center for Genomics & Systems Biology (**http://cgsb.as.nyu.edu**) in the Department of Biology invites applicants to apply for a faculty position to begin September 1, 2017, or as negotiated pending budgetary and administrative approval. The position would ideally be at the level of Assistant Professor (tenure track) but more senior applicants will also be considered.

We are particularly interested in applicants who are developing novel computational and/or experimental techniques in genomics and systems biology, to address important biological questions, as well as those using systems approaches to study plants or microbes but other outstanding individuals are also encouraged to apply.

Candidates will be expected to have or develop active, externally funded, research programs and to participate in the department's undergraduate and graduate teaching. The Department of Biology (**http://biology.as.nyu.edu**) offers an outstanding, collegial and interdisciplinary research environment that supports ambitious research projects in genomics and systems biology, with access to high-throughput robotics, sequencing and high-performance computing. In addition, strong interactions exist with faculty in other divisions within NYU including the Courant Institute of Mathematical Sciences.

Application packages should include a cover letter, research statement, teaching statement, curriculum vitae and three letters of reference. Please apply online via the New York University Department of Biology website (**http://biology.as.nyu.edu**), using the "**Employment**" link. The cover letter should be addressed to: **Chair of the CGSB Search Committee, Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003**. *Selection will begin* ***November 15, 2016****; applications received prior to this date will be guaranteed full evaluation.*

The Faculty of Arts and Science at NYU is at the heart of a leading research university that spans the globe. We seek scholars of the highest caliber, who embody the diversity of the United States as well as the global society in which we live. We strongly encourage applications from women, racial and ethnic minorities, and other individuals who are under-represented in the profession, across color, creed, race, ethnic and national origin, physical ability, gender and sexual identity, or any other legally protected basis. NYU affirms the value of differing perspectives on the world as we strive to build the strongest possible university with the widest reach. To learn more about the FAS commitment to diversity, equality and inclusion, please read **http://as.nyu.edu/page/diversityinitiative**.

EOE/Affirmative Action/Minorities/Females/Vet/
Disabled/Sexual Orientation/Gender Identity.

UCSB

UNIVERSITY OF CALIFORNIA
SANTA BARBARA

The Department of Molecular, Cellular and Developmental Biology at the University of California, Santa Barbara invites applications for a tenure-track **Assistant Professor** faculty position in **Microbiology**. Competitive applicants will apply state-of-the-art experimental approaches to address fundamental questions in the molecular biology of microorganisms, including eubacteria, bacteriophages, archaea, fungi and protozoa. Candidates must hold postdoctoral (or equivalent) appointments and have outstanding records of research accomplishment. Preference will be given to candidates with broad scientific interests and a strong commitment to undergraduate and graduate teaching. The Department is especially interested in applicants who will contribute to the diversity and excellence of the academic community through research, teaching and service.

Applicants should submit a cover letter, a curriculum vitae, statement of past and future research interests, statement of teaching, and arrange for three letters of recommendation to be submitted directly to our website. Materials should be submitted electronically via **https://recruit.ap.ucsb.edu/apply/JPF00784**. Applications will be reviewed on a rolling basis and should be submitted by **November 1, 2016** for primary consideration. However applications will continue to be accepted until the position is filled. Inquiries should be addressed to **ap@mcdb.ucsb.edu**.

The University of California is an Equal Opportunity/Affirmative Action Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law.

THE UNIVERSITY OF TEXAS
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ASSOCIATE PROFESSOR, TERM TENURE TRACK – RADIATION ONCOLOGY

The Department of Radiation Oncology at The University of Texas MD Anderson Cancer Center is actively recruiting for a physician scientist faculty position at the Texas Medical Center. Candidates should have excellent clinical training and a strong publication and funding history in translational research. The position includes substantial, dedicated research time. Qualified candidates are required to be board eligible.

The Department of Radiation Oncology treats approximately 6,000 new cancer patients each year. The Department consists of its main facilities within the Texas Medical Center, the Proton Therapy Center and six satellite treatment centers. This is an exciting opportunity to practice in a state-of-the-art clinical, multidisciplinary environment with significant resources to support clinical and translational research. Applicants must have an M.D., but M.D., Ph.D. is preferred, and will be expected to develop an independent, extramurally funded research program. All faculty have the opportunity to teach in a leading residency and fellowship program. We offer a generous salary and benefits.

Please send a single pdf file containing: (1) cover letter, (2) curriculum vitaie, (3) short research summary (three page maximum); and (4) contact information for three to five references. This pdf file should be sent to: Stephen M. Hahn, M.D., Professor and and Division Head, Division of Radiation Oncology, The University of Texas MD Anderson Cancer Center; Email: RadOnc@mdanderson.org

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Executive Branch Fellow at the National Science Foundation
Current: Executive Director, ICUSP Inc. &, Associate Professor, The University of Texas at Austin

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Department Chair
Biomedical Sciences

Creighton University
School of Medicine

Creighton University School of Medicine invites applications and nominations for Professor and Chair of the Department of Biomedical Sciences. The ideal candidate must have a PhD, qualify for appointment as full professor, and possess outstanding interpersonal, leadership, teaching, and administrative skills and experience. The candidate should also have an internationally recognized research program supported by extramural funding, and express a strong commitment to mentoring department faculty and fostering collaborative, interdisciplinary research and education, both within the Department and with other basic and clinical science departments. Primary responsibilities of the Chair include developing and implementing strategies to support and enhance the research, training, and educational programs in the department, preparing and managing the department budget, mentoring and evaluating department faculty and staff, teaching in appropriate graduate and/or professional school courses, and engaging with various stakeholders to articulate and achieve the missions of the Department, School, and University. The position includes a competitive startup package and additional resources to significantly expand the faculty base.

The Department of Biomedical Sciences has a diverse portfolio of federally funded research investigators who also participate in graduate and professional education. The School of Medicine is one of nine schools and colleges at Creighton University including professional schools of Business, Law, Nursing, Dentistry, Pharmacy and Health Professions, and the Graduate School. Founded in 1878, Creighton University is a Catholic, Jesuit institution with an enrollment of approximately 7000 students. It is consistently ranked as one of the finest comprehensive universities in the nation by U.S. News & World Report and regularly appears in Best Buys in American Colleges.

Applications received before **November 30th, 2016** will receive full consideration. *Creighton University is an Equal Opportunity, Affirmative Action Educational Institution and Employer, title IX University. Creighton University particularly welcomes applications from minorities, women and persons with disabilities.*

A **Curriculum Vitae** and letter of application that includes a description of administrative and teaching experiences, current and future research activities, and the names and contact information of three references may be submitted by mail or e-mail to:

Chair, Search Committee
c/o Lurae McCloskey
Creighton University School of Medicine
2500 California Plaza, Omaha NE 68178
luraemcloskey@creighton.edu

UT Southwestern Medical Center

The Department of Molecular Biology and the Hamon Center for Regenerative Science and Medicine (CRSM) at the University of Texas Southwestern Medical Center invite applications for tenure track faculty position at the level of Assistant Professor. We are seeking interactive individuals with strong research programs focused on mechanistic aspects of gene regulation, cell growth and differentiation, and stem cell biology, including the use of cellular and animal models to study human disease. Attractive recruitment packages, state-of-the-art core facilities, and exceptional laboratory space are available. UT Southwestern has a vibrant graduate program and an atmosphere of collegiality and collaboration.

Applicants should submit a curriculum vitae containing a summary of past research accomplishments, a statement of future objectives, and names of three references via email to:

MolBioSearch@UTSouthwestern.edu
Department of Molecular Biology
Hamon Center for Regenerative Science and Medicine
University of Texas Southwestern Medical Center

UT Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women, minorities, veterans and individuals with disabilities are encouraged to apply.

TENURE-TRACK PROFESSOR
Harvard University
Faculty of Arts and Sciences
Cambridge, MA
Department of Chemistry and Chemical Biology

Position Description: Candidates are invited to apply for an open field tenure-track assistant professorship in the Department of Chemistry and Chemical Biology at Harvard University. The appointment is expected to begin on July 1, 2017. The tenure-track professor will be responsible for teaching at the undergraduate and graduate levels.

Basic Qualifications: Doctorate or terminal degree in chemistry or related discipline required by the time the appointment begins.

Additional Qualifications: Outstanding research record and a strong commitment to undergraduate and graduate teaching.

Special Instructions: Please submit the following materials through the ARIeS portal (**http://academicpositions.harvard.edu/postings/7065**). All applications and materials must be submitted no later than **October 15, 2016**.

1. Cover letter
2. Curriculum Vitae, including list of publications
3. Select Full Publications
4. Teaching statement (describing teaching approach and philosophy)
5. Future research plans
6. Names and contact information of 3-5 references (three letters of recommendation are required, and the application is complete only when all three letters have been submitted)

Contact Information: Helen Schwickrath, Search Administrator, Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford St., Cambridge, MA 02138, Phone: 617-496-8190; helen@chemistry.harvard.edu

Harvard is an Equal Opportunity Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law.

Molecular, Cellular, & Developmental Biology Faculty Search

University of Michigan

UNIVERSITY OF MICHIGAN FACULTY POSITIONS IN NEUROSCIENCE

The Department of Molecular, Cellular, and Developmental Biology (MCDB) in the College of Literature, Science and the Arts at the University of Michigan solicits applications from neuroscientists for faculty positions at the assistant professor level, but appointment at a more senior level is possible for applicants with suitable experience. We seek to fill an open position in the field of neuroscience broadly defined. We encourage applications from scientists who use cellular, genetic, molecular, and/or *in vivo* imaging approaches. The faculty position will be tenure track or tenured with a university year appointment starting September 1, 2017 or January 1, 2018. Successful candidates will be expected to establish a vigorous, extramurally funded research program and to be involved with instruction of both undergraduate and graduate students. For further information about research areas in MCDB please visit www.lsa.umich.edu/mcdb.

All applications must be submitted online at **http://labs.mcdb.lsa.umich.edu/search16**. You will be asked to upload the following materials: A cover letter, a *curriculum vitae*, a brief summary of recent research accomplishments and statement of future research plans, a statement of teaching philosophy and experience, and evidence of teaching excellence for those who have teaching experience. Candidates for appointment at the assistant professor level should provide names and contact information for at least three references, as instructed in the **online application form**. To ensure full consideration, all materials should be received by **October 14, 2016**.

Women and underrepresented minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

ScienceCareers online @sciencecareers.org
FACULTY POSITIONS

THE UNIVERSITY OF CHICAGO

INSTITUTE FOR MOLECULAR ENGINEERING (IME)

The Institute for Molecular Engineering (IME) is a unique interdisciplinary institute launched by the University of Chicago in 2011 with the aim of translating molecular-level science into technological solutions with potential societal impact in health care, energy, environmental sustainability and information technology. An independent unit within the university, IME is also affiliated with Argonne National Laboratory (ANL) and joint appointment is encouraged when appropriate. IME invites applications from outstanding candidates for the following tenure-track or tenured faculty positions. All candidates must have a doctoral degree in a relevant field of study and an outstanding research record. Successful candidates will be expected to establish and maintain a strong research program and teach at the graduate and undergraduate levels.

Quantum Information Engineering

IME invites applications for tenure-track and tenured faculty positions at the ranks of Assistant Professor, Associate Professor and Professor in the area of Quantum Information Engineering. Areas of interest include, but are not limited to quantum coherent devices and systems, optics and nanophotonics, optomechanics, quantum measurement, quantum materials, quantum information and communication. The appointment will be at IME with positions available both in experiment and theory. To apply, please visit the University of Chicago's Academic Career Opportunities website, **https://academiccareers.uchicago.edu/**. The review of the applications will start on **October 15, 2016** and continue until the position is filled. Applicants are required to upload a cover letter, curriculum vitae, a list of publications, and a statement of research interests. In addition, candidates are requested to provide the names and contact information for three references, who will be contacted separately for letters of recommendation. To be considered for a position at the rank of Assistant Professor, please apply to Requisition **#03104**. To be considered for a position at the rank of Associate Professor, please apply to Requisition **#03105**. To be considered for a position at the rank of Professor, please apply to Requisition **#03106**.

Materials Synthesis and Engineering

IME invites applications for tenure-track and tenured faculty positions at the ranks of Assistant Professor, Associate Professor and Professor in the area of experimental materials synthesis and integration. Areas of interest include, but are not limited to, materials and systems for new computing approaches, quantum materials, sensing, energy conversion, additive materials and additive manufacturing, engineered low-dimensional materials with novel properties, and biologically inspired materials. The candidate will be expected to establish a strong independent research program and be interested in a multidisciplinary, system level approach to materials science and engineering. The IME also has close ties with Argonne National Laboratory (several faculty members have joint appointments) and the position will enable close interaction with Argonne scientists. While we will consider exceptional candidates from all fields of materials science, we seek in particular expertise in inorganic materials based approaches to the areas noted above. To apply, please visit the University of Chicago's Academic Career Opportunities website, **https://academiccareers.uchicago.edu/**. The review of the applications will start on **October 15, 2016** and continue until the position is filled. Applicants are required to upload a cover letter, curriculum vitae, a list of publications, and a statement of research interests. In addition, candidates are requested to provide the names and contact information for three references, who will be contacted separately for letters of recommendation. To be considered for a position at the rank of Assistant Professor, please apply to Requisition **#03110**. To be considered for a position at the rank of Associate Professor, please apply to Requisition **#03111**. To be considered for a position at the rank of Professor, please apply to Requisition **#03112**.

Immunoengineering

IME invites applications for tenure-track and tenured faculty positions at the ranks of Assistant Professor, Associate Professor and Professor in the field of Immunoengineering to candidates working in translational immunoengineering, and to those applying computational and experimental engineering approaches addressed to basic questions in immunology. The position is offered in a rich environment of biomolecular and materials science, immunology, and cancer immunology and interactions with academic units that include the Committee on Immunology, the Institute for Genomics and Systems Biology and the Ben May Department for Cancer Research (benmay.ucicago.edu). To apply, please visit the University of Chicago's Academic Career Opportunities website, **https://academiccareers.uchicago.edu/**. The review of the applications will start on **November 1, 2016** and continue until the position is filled. Applicants are required to upload a cover letter, curriculum vitae, a list of publications, and a statement of research interests. In addition, candidates are requested to provide the names and contact information for three references, who will be contacted separately for letters of recommendation. To be considered for a position at the rank of Assistant Professor, please apply to Requisition **#03109**. To be considered for a position at the rank of Associate Professor, please apply to Requisition **#03108**. To be considered for a position at the rank of Professor, please apply to Requisition **#03107**.

The University of Chicago is an Affirmative Action/Equal Opportunity/Disabled/Veterans Employer and does not discriminate on the basis of race, color, religion, sex, sexual orientation, gender identity, national or ethnic origin, age, status as an individual with a disability, protected veteran status, genetic information, or other protected classes under the law. For additional information please see the University's Notice of Nondiscrimination at http://www.uchicago.edu/about/non_discrimination_statement/.
Job seekers in need of a reasonable accommodation to complete the application process should call 773-702-5671 or email ACOppAdministrator@uchicago.edu with their request.

Faculty Positions - Chemistry

The Department of Chemistry and Biochemistry at Auburn University invites applications for two tenure-track, 9-month positions at the level of Assistant Professor. Auburn University is an institution that is both highly research-active and committed to maintaining teaching excellence as one of the nation's premier land, sea, and space grant institutions. With these positions, the department is looking to fill research needs that will directly complement existing research programs and align with campus-wide research clusters. The successful candidates are expected to develop vigorous, externally funded research programs.

Of particular interest is research in the following two areas related to Chemical Biology:

1) Small-Molecule Synthesis. Synthesis of organic or inorganic small molecules that can be used to investigate biochemical processes and/or serve as pharmaceuticals.
2) Biochemistry or Biological Imaging. A focus on the development and application of probes (macromolecules, small molecules, bio-conjugates, etc.) or spectroscopic techniques to investigate biochemical processes is of particular interest, although other areas of biochemistry will be considered.

Duties also include teaching at both the undergraduate and graduate levels. A Ph.D. in chemistry or a related field and at least one year of postdoctoral experience are required. The candidate selected for this position must meet eligibility requirements to work in the United States on the date the appointment is scheduled to begin (August 2017) and must be able to continue working legally for the proposed term of employment. Excellent written and interpersonal communication skills are required.

Applicants should submit a curriculum vitae, a detailed statement of research plans, a 2-page statement of teaching philosophy, and the names and contact information of three professional references. For more information about the College of Sciences and Mathematics and the Department of Chemistry and Biochemistry, please refer to our website: http:// www.auburn.edu/chemistry. The review of applications will begin **October 31, 2016** and continue until the positions are filled.

To apply for the position in the area of **Small-Molecule Synthesis**, please visit: **http://aufacultypositions.peopleadmin.com/postings/1859**.

To apply for the position in **Biochemistry/Biological Imaging**, please visit: **http://aufacultypositions.peopleadmin.com/postings/1860**.

Auburn University is an EEO/Vet/Disability Employer.

Cleveland Clinic
Lerner Research Institute

FACULTY POSITION IN MOLECULAR CARDIOLOGY

Cleveland Clinic Lerner Research Institute invites applications for a new faculty position at **Assistant/Associate Professor/Staff** level to join the collaborative and vibrant research programs in the Department of Molecular Cardiology. Applicants should have a MD, PhD or an equivalent degree with robust post-doctoral training and exceptional promise for developing a rigorous research program focused on basic structural, cellular and molecular mechanisms underlying heart failure, ischemic heart disease and arrhythmogenesis, performing translational studies including but not limited to cardiac hypertrophic signaling/remodeling and hypertension, cardiac hetero-cellular interactions and cell renewal/turnover and metabolic syndrome. Successful candidate is expected to establish or have an established innovative, extramurally funded research program. Interactions with world class clinical programs are available and provide the potential to develop multi-disciplinary and translational research programs. Outstanding facilities and generous start-up funds are available.

Interested candidates should submit applications electronically as a single PDF file containing curriculum vitae, a statement of future research interests and cover letter addressed to Sathyamangla Prasad, PhD, Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, at following email: **crehore@ccf.org**. Reference letters will be requested following review of the applications.

Northwestern University

The **Department of Molecular Biosciences** seeks applications at the level of assistant professor for faculty candidates with research interests that complement our existing interdisciplinary strengths. Applicants with outstanding credentials from all fields will be considered, however, we are particularly interested in candidates whose research focuses on molecular evolution and/or using quantitative methods to address fundamental questions about biological mechanisms.

The department is a highly collaborative and dynamic research and training environment located on the main campus of Northwestern University in Evanston, IL.

Applicants should prepare a cover letter, curriculum vitae, a statement of previous research accomplishments and future research goals, and a statement of teaching experience and interests. For further instructions and to submit an application, please visit the Molecular Biocciences homepage at **http://www.molbiosci.northwestern.edu**. Applicants should arrange for at least three letters of recommendation to be submitted on their behalf. Questions should be sent to **molbioscisearch@northwestern.edu**.

To ensure full consideration, please submit all materials by **November 1, 2016**.

Affirmative Action Employer of all protected classes including veterans and individuals with disabilities. Women and minorities are particularly encouraged to apply. Hiring is contingent upon eligibility to work in the United States.

Sandia National Laboratories

Sandia National Laboratories is one of the country's largest research facilities employing nearly 8,500 people at major facilities in Albuquerque, New Mexico and Livermore, California. Please visit our website at www.sandia.gov.

We are searching for outstanding Ph.D. candidates to apply for the Harry S. Truman Fellowship in National Security Science and Engineering. The Truman Fellowship provides the opportunity for recipients to pursue independent research of their choosing that supports Sandia's national security mission. Candidates are expected to have solved a major scientific or engineering problem in their thesis work or will have provided a new approach or insight to a major problem, as evidenced by a recognized impact in their field.

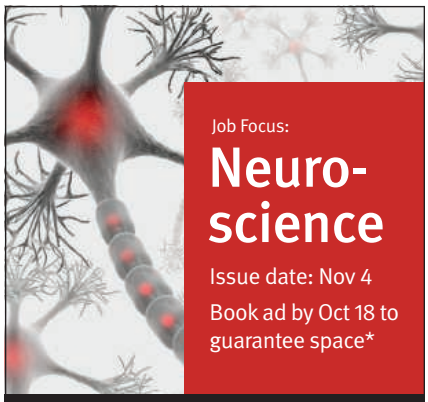
Candidates must have received a Ph.D. within the past 3 years, or will complete all Ph.D. requirements by commencement of appointment, with a broad-based background and extensive knowledge of research in one or more of the following focus areas: bioscience, computing and information science, engineering sciences, geosciences, materials science, nanodevices and microsystems, and radiation effects science and high energy density physics. Candidates must be seeking their first national laboratory appointment (pre postdoc internships excluded), have excellent academic (minimum 3.5 undergraduate and 3.7 graduate GPA preferred) and research qualifications, good communication skills, and enjoy working in a team-oriented, dynamic environment.

The Truman Fellowship is a three-year appointment normally beginning on October 1. The salary is $111,200 per year plus benefits and additional funding for the chosen research proposal. This position requires the ability to obtain and maintain a United States Department of Energy security clearance.

For complete application instructions, please visit: **http://www.sandia.gov/careers/students_postdocs/fellowships/truman_fellowship.html**

Please submit the complete package to: **Yolanda Moreno, Sandia National Laboratories, P.O. Box 5800, MS 0359, Albuquerque, New Mexico 87185-0359**, or email: **ymoreno@sandia.gov** (email preferred). Please reference: Job **#653576**. All materials and online application must be received by COB **November 1, 2016**.

Equal Opportunity Employer/Disability/Vet/GLBT.



Job Focus:

Neuro-science

Issue date: Nov 4

Book ad by Oct 18 to guarantee space*

There's only one *Science*.

Why choose this job focus for your advertisement?

- Relevant ads lead off the career section with special neuroscience banner
- Bonus distribution to Society for Neuroscience, Nov 12–16, San Diego, CA.

*Ads accepted until Oct 28 if space allows.

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RUTGERS
THE STATE UNIVERSITY OF NEW JERSEY

Faculty Position in Genetics

The Department of Genetics in the School of Arts and Sciences at Rutgers, The State University of New Jersey seeks an outstanding scientist to fill one of several new faculty positions in genetics. Appointment will be made at the Assistant Professor level. We are interested in individuals with research interests that will complement and expand our existing strengths, which include, but are not limited to: human genetics, developmental genetics, reproductive genetics, cellular genetics, population genetics, bioinformatics, statistical genetics, computational genetics, microbial genetics, epigenetics, cancer genetics, neurogenetics, and psychiatric genetics. Appropriate candidates will also be considered for appointment to the Human Genetics Institute of New Jersey. Core resources, startup funds, and laboratory space in the newly constructed Life Sciences Building will be provided.

The Department of Genetics is home to over 30 faculty members who use a broad range of approaches and experimental systems in numerous well-funded research programs. The department is part of a vibrant and interactive life sciences community that includes the School of Arts and Sciences Division of Life Sciences, the Waksman Institute, the Center for Advanced Biotechnology and Medicine, the Cancer Institute of New Jersey, the Institute for Quantitative Biomedicine, and the Robert Wood Johnson Medical School. A leading research university, Rutgers is a member of the AAU and the CIC and attracted over $638M in research grant and contract funding in FY2016. The New Brunswick/Piscataway campus is located in suburban central New Jersey, close to New York City, Philadelphia, beaches, and countryside. For more information on the Department, our other ongoing searches, and Rutgers University see: http://genetics.rutgers.edu/faculty/faculty-recruitment.

Candidates must have a Ph.D. and/or M.D., demonstrated record of significant research, the potential to make substantial contributions as an independent investigator, and have a commitment to teaching undergraduate and graduate students. Applicants should submit a CV, a detailed statement of research interests, a teaching statement, and full contact information for three individuals willing to provide letters of reference. Applications should be submitted electronically at **https://apply.interfolio.com/37129** and inquiries made to Ms. Mary Carmona, carmona@dls.rutgers.edu. Review of applications will begin **October 15, 2016** and continue until the position is filled.

Rutgers, the State University of New Jersey, is an Equal Opportunity/Affirmative Action Employer. Qualified applicants will be considered for employment without regard to race, creed, color, religion, sex, sexual orientation, gender identity or expression, national origin, disability status, genetic information, protected veteran status, military service or any other category protected by law. As an institution, we value diversity of background and opinion, and prohibit discrimination or harassment on the basis of any legally protected class in the areas of hiring, recruitment, promotion, transfer, demotion, training, compensation, pay, fringe benefits, layoff, termination or any other terms and conditions of employment.

Weill Cornell Medicine

Faculty Position
Department of Radiology
Faculty Position in
Molecular Imaging Innovations Institute

The Molecular Imaging Innovations Institute (MI^3) in the Department of Radiology at Weill Cornell Medical College is seeking highly motivated applicants to join our newly established institute, which is located in the state-of-the-art Belfer Research Building. MI^3 is committed to becoming an internationally recognized institute of excellence in the discovery and development of new molecular imaging agents, and strategies for use in basic and translational research as well as in clinical care. Scientists will have access to on-site imaging systems for animal and human studies, including MRI, PET, SPECT, CT, optical and ultrasound.

Experienced scientists with a PhD and/or MD in developing multimodality imaging strategies are encouraged to apply. Junior candidates must have at least three years of postdoctoral experience, publications in high impact journals, and the demonstrated ability or potential to acquire external research funding. Senior positions will be considered commensurate with experience and track record of extramural funding and publications.

Qualified applicants should respond by sending or emailing a letter of interest, including: research interests, CV, and three references to: **Dr. Ching Tung, Director of Molecular Imaging Innovations Institute, Department of Radiology, Weill Cornell Medical College, 525 East 68th Street, Box 141, New York, NY 10065-4870; Email at MI3@med.cornell.edu.**

Evaluation of applications will begin immediately and continue until the position is filled.

Weill Cornell Medicine is an employer and educator recognized for valuing AA/EOE/M/F/Protected Veterans, and Individuals with Disabilities.

University of Massachusetts UMASS Medical School

Tenure-Track Faculty Position
The Department of Microbiology and Physiological Systems

The Department of Microbiology and Physiological Systems at the University of Massachusetts Medical School (http://www.umassmed.edu/) invites applications for a tenure-track faculty position at the rank of ASSISTANT PROFESSOR. Depending on qualifications, candidates may be proposed for a more senior appointment at the rank of ASSOCIATE or FULL PROFESSOR. The Department is seeking candidates who use cross-disciplinary or systems-level approaches to solve problems in bacterial or viral infection including, but not limited to: molecular mechanisms of pathogenesis; cell biology of infection; analysis of complex microbial communities and their impact on the host; and host responses and adaptation to infection. Candidates will be expected to develop and maintain an innovative, externally funded research program. We offer generous support and a collaborative environment with opportunities for both basic and translational research. The position will be highly competitive with regard to start-up funds and salary. Applications will be reviewed on an ongoing basis and continue until the position is filled.

Applicants should submit a cover letter explaining their interest in the Department, a curriculum vitae that includes honors and publications, and a succinct research plan to https://academicjobsonline.org/ajo/jobs/7512. To expedite the review process, applicants should invite three individuals who are familiar with their work and potential for success to upload recommendation letters at the same web address.

UMass Medical School is committed to being an equal opportunity and affirmative action employer and recognizes the power of a diverse community. We encourage applications from protected veterans, individuals with disabilities and those with varied experiences, perspectives and backgrounds to consider UMass Medical School as their employer of choice.

Keck School of Medicine of USC

TENURE-TRACK FACULTY POSITIONS
Department of Molecular Microbiology and Immunology

The Department of Molecular Microbiology and Immunology at the Keck School of Medicine of the University of Southern California in Los Angeles, California, has an ongoing expansion to build upon existing strengths in Immunology and Microbiology.

The Department invites applicants for tenure-track **Assistant and/or Associate Professor** positions in the field of infectious diseases and immunology with a research emphasis on pathogens and immune responses. We are particularly seeking highly motivated individuals with demonstrated potential for creativity who are working on translational aspect of immune responses. Scientists with a record of achievement and commitment to excellence in both research and teaching are encouraged to apply. Successful candidates will receive generous start-up packages and laboratory space. The Keck School of Medicine has strong research programs in Cancer, Immunology, Stem Cells, Neurobiology, Public health and Virology and offer outstanding technical resources in many areas, including flow cytometry, imaging, proteomics and genomics. Also, go to link for list of supporting research core facilities: http://keck.usc.edu/research/research-resources/find-core-resources/

Applicants should submit a letter of application, Curriculum Vitae, a statement of current and future research plans, and three letters of recommendation. Please complete faculty application through the USC job website at **https://jobs.usc.edu/postings/55204** with position number **P01456930**.

USC values diversity and is committed to equal opportunity in employment. Women and men, and members of all racial and ethnic groups are encouraged to apply.

Faculty Positions, Vollum Institute
Oregon Health & Science University
Portland, Oregon

The Vollum Institute (**www.ohsu.edu/vollum**) is undergoing an exciting phase of reinvestment by OHSU and growth under a new Director, and announces multiple faculty openings for outstanding scientists. We are particularly interested in individuals whose research focuses on **molecular and cellular neuroscience, molecular genetics, development and/or mechanisms of signal transduction**.

Vollum appointments are full-time research positions within the Vollum Institute with minimal teaching or clinical requirements. Ample opportunities are available for collaboration with clinical units within the School of Medicine and additional research units at OHSU (**www.ohsu.edu**).

Applications will be accepted at junior and mid-career levels. Applicants should have a strong record of research success and an interest in training graduate students. We offer highly attractive start-up packages and the opportunity to work in an outstanding scientific environment that includes strong mentoring for junior scientists.

Candidates with a PhD and/or MD and at least several years of postdoctoral experience should apply by submitting their curriculum vitae, a description of research plans and goals, and three letters of reference to **https://academicjobsonline.org/ajo/jobs/7768**. Applications must be received by **December 1, 2016**.

For questions contact Gary Westbrook, Search Chair, at volljob@ohsu.edu

OHSU is an equal opportunity/affirmative action employer committed to maintaining diversity in its faculty and addressing faculty family issues including dual career couples and single parents.

OHSU

Assistant Professor Position
Department of Molecular and Cellular Biology, Faculty of Arts & Sciences
Harvard University

We seek applications for a tenure-track faculty position in the Department of Molecular and Cellular Biology. We are particularly interested in candidates who use quantitative or computational approaches to study the mechanisms underlying fundamental questions in biology. The department offers an interactive research community with interests in a broad range of topics, including cell biology, systems biology, biophysics, biochemistry, developmental biology, neurobiology, genetics, microbiology, computational biology, and structural biology. The appointment provides access to state of the art animal facilities and core facilities for imaging, proteomics, genomics and bioinformatics. The Department is also closely associated with science initiatives at Harvard such as the Center for Brain Science, the Center for Systems Biology, the Harvard Stem Cell Institute, the Broad Institute, and the Center for Nanoscale Systems, and provides opportunities for interactions with the broader Harvard community. We strongly encourage applications from women and minority candidates.

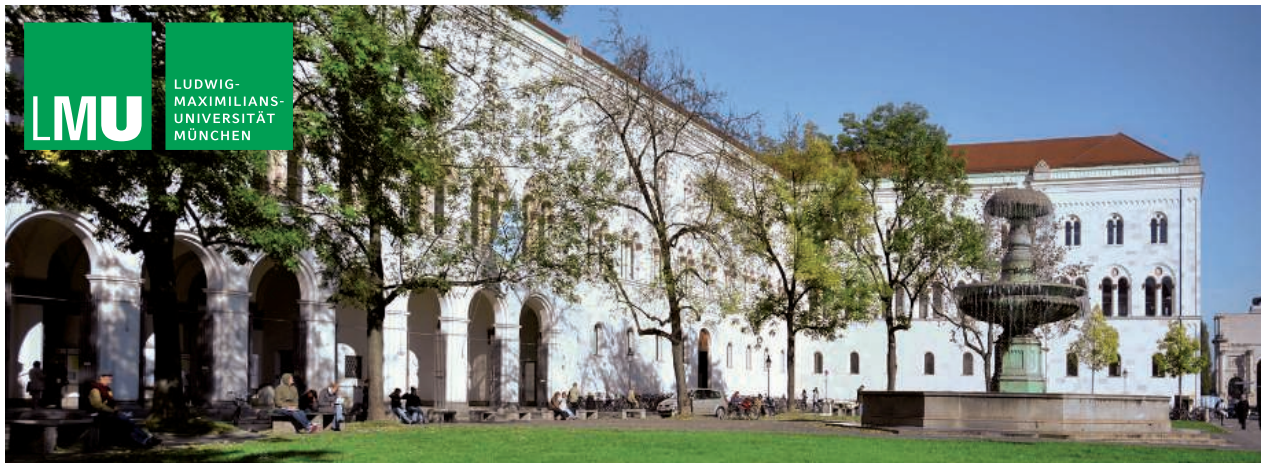
Doctorate or terminal degree in relevant field or related discipline required by the time the appointment begins. Applications should include: curriculum vitae, teaching statement (describing teaching approach and philosophy), a statement of present and future research plans (1-3 pages), three letters of recommendation, and reprints of publications. Complete applications, including letters of recommendation, should be received no later than November 1, 2016.

Submit applications to:
http://academicpositions.harvard.edu/postings/7067

For information contact Polina Kehayova | kehayova@fas.harvard.edu | **www.mcb.harvard.edu**

Harvard is an equal opportunity employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law.

HARVARD UNIVERSITY | DEPARTMENT OF
Molecular and Cellular Biology



Ludwig-Maximilians-Universität (LMU) in Munich is one of the leading research universities in Europe, with a more than 500-year tradition. LMU aims to create favorable conditions for world-class academics. To provide outstanding junior researchers in all subject areas with a long-term career perspective, LMU is offering

Tenure Track Professorships to Successful ERC Starting Grantees

in the current call of the European Research Council (for candidates 2-7 years after their PhD). If you would be interested in joining LMU's research community, please contact the appropriate faculty member in your field of research as soon as possible.

For further information regarding this offer, please see:
www.lmu.de/excellent/erc-tenuretrack

PHYSIOLOGY UCLA
Assistant or Associate Professors (2)

The Department of Physiology at the David Geffen School of Medicine at UCLA invites applications for tenure track faculty positions, preferably at the level of Assistant or Associate Professor.

We are especially interested in candidates studying fundamental mechanisms of physiological processes in neuroscience and the cardiovascular system. However, we will consider applicants in all areas of modern physiology. The Department of Physiology at UCLA has a strong tradition of excellence in molecular biophysics, neuroscience, cardiovascular research, and structural biology in a highly collaborative environment. For more information about the department, please visit our website: http://www.physiology.ucla.edu/

Candidates must have a PhD, M.D. or equivalent and are expected to have a strong track record of research achievements and a demonstrated interest in tackling outstanding biological problems using state-of-the-art approaches. The successful candidate will be expected to develop an independent research program and participate in the teaching mission of the Department for medical, dental, and graduate students. We welcome candidates whose experience in teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence.

Interested applicants should submit their curriculum vitae, a brief statement of research interests and career goals, a description of teaching experience, and the names of three references to **https://recruit.apo.ucla.edu/apply/JPF02411**. Applications will be accepted between **September 1 and November 30, 2016**.

The University of California is an Equal Opportunity/Affirmative Action Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability, age or protected veteran status. For the complete University of California nondiscrimination and affirmative action policy see: UC Nondiscrimination and Affirmative Action Policy.

University of Massachusetts Medical School
UMASS

RNA Therapeutics Institute

Tenure-Track Positions

The RNA Therapeutics Institute (RTI) at the University of Massachusetts Medical School, invites applications for tenure-track faculty positions. Successful candidates will conduct innovative research to understand normal physiology and development, disease mechanisms and/or to develop novel diagnostics or therapies. Candidates need not necessarily conduct research directly focused on RNA biology or development of RNA therapeutics, but should indicate how their research will synergize with ongoing research within the RTI and the greater UMass Medical School community.

Physician scientists and researchers with significant industrial experience who wish to return to academia are particularly encouraged to apply. Other specialties of interest include synthetic chemists, materials scientists, computational researchers, bioengineers, geneticists, cell and molecular biologists, and biochemists.

The RTI is housed in a new state-of-the-art research building, and is central to a campus-wide interdisciplinary initiative in RNA therapeutics, stem cell biology and gene therapy designed to facilitate collaboration among basic scientists, preclinical investigators and clinicians. As a recipient of a Clinical and Translational Science Award (CTSA) from the NIH, UMass Medical School seeks to build an environment in which basic, applied and clinical researchers collaborate to inform our understanding of human disease and accelerate development of novel diagnostics and therapies. To further encourage collaboration, all RTI investigators hold joint appointments within other departments on campus.

Applicants should submit a cover letter, curriculum vitae, a two-page statement of research interests, and contact information for three references to **https://academicjobsonline.org/ajo/jobs/7506**. Applications will be reviewed expeditiously, with interviews commencing in **October**. Inquiries (but not application materials) may be directed to Tiffanie Gardner at **Tiffanie.Gardner@umassmed.edu**. Applications at the Assistant, Associate and Full Professor levels will be considered. The position will remain open until filled.

As an Equal Opportunity and Affirmative Action Employer, UMW recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives and backgrounds.

MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

Assistant Professor

The Department of Biological Chemistry at the University of Michigan is recruiting a tenure-track Assistant Professor. We are interested in candidates applying molecular, cellular, genetic and biochemical approaches that address important problems in biology and complement existing areas of strength that include enzymology, structural biology, signal transduction, development, neurobiology and regulation of gene expression. Ideal candidates will be highly motivated and interactive and will be expected to establish an internationally recognized research program. Qualifications include a Ph.D. and/or M.D. and a strong record of research accomplishments. For further information, see http://www.biochem.med.umich.edu/.

Applications should be assembled into a single pdf and include a cover letter, a curriculum vitae and a 3-5 page statement of current and future research plans. All materials should be sent via email to **Amanda Howard (amanhowa@umich.edu)**. Candidates must also arrange to have 3 letters of recommendation sent as PDFs to the above email address. Applications and letters of recommendation will be considered up until **November 1, 2016**.

The University of Michigan is supportive of the needs of dual career couples and is an Affirmative Action/Equal Opportunity Employer.

BINGHAMTON
UNIVERSITY
STATE UNIVERSITY OF NEW YORK

Director for First-year Science Programs

Binghamton University seeks a director to head its science undergraduate teaching initiative, which supports innovative, evidence-based first-year undergraduate science education through academic programming, faculty development, and educational research. The director will collaborate in the design, implementation, and assessment of new and current faculty-driven first-year undergraduate science initiatives. This position supervises educators and administrative personnel associated with the University's Freshman Research Immersion (FRI), a three-course sequence of CUREs (course-based undergraduate research experience) that involves STEM departments and programs campus-wide (http://www.binghamton.edu/freshman-research-immersion/). This position collaborates with faculty to broaden the impact of biological research through first-year undergraduate education. The director must have a PhD and additional experience in an area of biological research that aligns synergistically with the research strengths of the Department of Biological Sciences; track record of biology education scholarship; and a record of success in obtaining grant funding and other external resources. The director is expected to actively seek grant funding to support science education programming. This tenure-track appointment is planned at the associate professor level; however, qualified applicants may be considered at other levels.

Application materials required: Applicants should submit a cover letter, curriculum vitae, selected reprints and preprints, statements of research and teaching interests, and list of at least three references to: **http://binghamton.interviewexchange.com**. Review of applications will begin on **1 December 2016** and will continue until the position is filled.

Additional Information: Binghamton University is one of the four doctoral granting universities in the State University of New York (SUNY) system, and is an Equal Opportunity/Affirmative Action Employer.

Portland State
UNIVERSITY

Hiring Tenured and Tenure-Track Positions in Environmental Extremes College of Liberal Arts and Sciences

pdx.edu/clas/faculty-job-openings

The College of Liberal Arts and Sciences at Portland State University is pleased to announce a multidisciplinary cluster hire of tenured and tenure-track faculty positions related to the research, discovery, and communication of environmental extremes.

This cluster hire initiative will expand Portland State University's multidisciplinary research capacity, creating an opportunity to identify the impacts, characterize the adaptations, and design solutions to manage and cope with environmental extremes of the 21st century. We are especially interested in candidates whose research involves laboratory and field approaches, modeling and theory and, based on academic preparation and research area, exemplifies dedication to creativity and excellence in teaching and scholarship in one of the following academic departments:

Biology: Tenured Associate or tenure-track Assistant Professor, with particular interest in a cell or molecular biologist whose research program investigates extremes in cellular environments.

Chemistry: Tenured Associate or tenure-track Assistant Professor, seeking candidates with a research emphasis on fundamental problems in physical and/or analytical chemistry relevant to environmental extremes, including novel spectroscopy and technologies for environmental sensing, and the physicochemical basis for adaptation to extreme environments.

Communication: Tenured Associate or tenure-track Assistant Professor with a promise of research productivity in the areas of environmental, risk, and science communication.

English: Tenured Associate or tenure-track Assistant Professor with expertise in professional and technical writing, especially science and/or environmental writing.

Environmental Science and Management: Tenured Associate or tenure-track Assistant Professor with research emphasis on examining the environmental and social effects of hydrologic extremes, and their potential management solutions.

Geography (2 positions): Tenured Associate or tenure-track Assistant Professor with particular interest in a political ecologist or hazard geographer whose research focuses on human impacts of, and community resilience to, environmental extremes. In addition, a water resource geographer whose research focuses on societal and institutional responses, and adaptations to, water-related environmental extremes.

Physics: Tenured Associate or tenure-track Assistant Professor with particular interest in candidates who focus on climate science of the Anthropocene, including forcings and mitigations; materials science and nanotechnology approaches for "green" solutions; and/or biophysics research, including experimental and computational approaches, for studying extremophile systems.

Opportunity awaits.

clusterhiring.ucr.edu

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FACULTY POSITIONS

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Faculty Careers

Issue date: October 7

Ads accepted until Sept 30 if space allows

For recruitment in science, there's only one *Science*.

Hiring Faculty? The October 7 feature covers business principles for researchers. Reach *Science* readers and share opportunities at your university.

What makes *Science* the best choice for recruiting?

- Read and respected by 400,000 readers around the globe
- 62% of our weekly readers work in academia and 65% are Ph.D.s. *Science* connects you with more scientists in academia
- Your ad dollars support AAAS and its programs, which strengthens the global scientific community.

Why choose this Faculty Feature for your advertisement?

- Relevant ads lead off this career section with a special Faculty banner
- October 7 issue will be distributed at the American Society of Human Genetics meeting, 18 –22 October, Vancouver.

Expand your exposure by posting your print ad online:

- Link on the job board homepage directly to Faculty jobs
- Dedicated landing page for faculty positions.

Produced by the *Science*/AAAS Custom Publishing Office.

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COLLEGE of PHARMACY
THE UNIVERSITY OF TEXAS AT AUSTIN

Assistant Professor
Division of Chemical Biology and Medicinal Chemistry

The Division of Chemical Biology and Medicinal Chemistry at The University of Texas at Austin College of Pharmacy invites applications for a full-time tenure-track faculty position at the Assistant Professor level effective August 2017. The division (http://sites.utexas.edu/medchem/) is a highly interdisciplinary and interactive group engaged in cutting edge research. The successful applicant will engage in productive interdisciplinary research, provide high-quality teaching, and actively participate in university service. The candidate will be expected to establish a vigorous and externally funded research program, focusing on chemical and molecular mechanisms of high biomedical significance. All qualified candidates will be considered, but those with research interests related to cancer or infectious diseases will be viewed more favorably. Teaching in the professional (Pharm.D.) program and development of graduate courses in the area of research expertise are expected. Qualified candidates must have a Ph.D. in a relevant field.

Applications received before **December 1, 2016** will receive first consideration, but applications will be accepted until the position is filled. The position carries a highly competitive salary, benefits, and start-up package. All highly qualified applicants will be considered. This institution is using Interfolio's ByCommittee to conduct this search. Applicants may access this position at **https://apply.interfolio.com/36240** and will be prompted to submit a cover letter, curriculum vitae, research plan, and a list of 3 references.

The University of Texas at Austin is an Equal Opportunity Employer with a commitment to diversity at all levels. All qualified applicants will receive consideration for employment without regard to race, color, religion, gender, national origin, age, disability, or veteran status. (Compliant with the new VEVRAA and Section 503 Rules). Security sensitive; conviction verification conducted on applicant selected. If hired, you will be required to complete the federal Employment Eligibility Verification form, I-9. You will be required to present acceptable and original documents to prove your identity and authorization to work in the United States. UT Austin is a tobacco-free campus.

GEORGE MASON UNIVERSITY

Chair and Professor of Bioengineering

The Department of Bioengineering invites applications for the position of chair and professor. The successful candidate will lead our dynamic and rapidly growing department in achieving continued excellence and national recognition. The department was established in 2010 and now consists of 13 full-time faculty. Current focus areas are biomedical imaging, neuroengineering and computational neuroscience, biomechanics, and nanoscale bioengineering. Faculty conduct research funded by a number of federal agencies, including the NIH, NSF, DoD, and the U.S. Department of Veterans Affairs, with over $16 million in active funding. The department has a thriving ABET-accredited undergraduate program with enrollment over 200 students, a Ph.D. program that started in 2015, and a new M.S. program launching in 2017. Continuing growth in student enrollment and the strategic recruitment of new faculty in emerging areas of bioengineering and healthcare will be priorities for the next department chair.

Candidates must have an earned doctorate in bioengineering or a related field and be eligible for tenured appointment at the rank of full professor. The successful applicant should have appropriate administrative experience, including fostering interdisciplinary research/education and mentoring students, faculty and staff. For the full job advertisement and further information about the department, please see http://bioengineering.gmu.edu/.

Applicants should apply for position number **F9261z** at **http://jobs.gmu.edu/**, complete and submit the online application, and upload a cover letter, CV, vision statement (attached as 'Other Doc'), and the names of three references with contact information. Review of applications will begin after **November 15, 2016**, and will continue until the position is filled.

UCLA

Department Chair/Professor of Ecology and Evolutionary Biology

The UCLA Department of Ecology and Evolutionary Biology (https://www.eeb.ucla.edu) is a top-20 program with an intellectual culture that fosters multi-disciplinary approaches and cross-campus collaborations to address major conceptual issues in ecology and evolution. Building on this foundation of success, the Division of Life Sciences in the UCLA College of Letters and Sciences seeks nominations and applications for an outstanding scientist and motivated leader to be Professor and Chair of the Department of Ecology and Evolutionary Biology. We seek candidates with appropriate administrative experience and dynamic research program who are ready to utilize upcoming faculty recruitments to raise the department to its next level of excellence. Candidates must have a PhD or equivalent degree within the field of biology and must be eligible for appointment at the full professor rank in any area of departmental expertise and have strong records of scholarly publications, research funding, teaching experience, and commitment to diversity, inclusion and excellence.

Please direct nominations and inquires to Professor Barney Schlinger (schlinge@lifesci.ucla.edu). Submit application packages online through https://recruit.apo.ucla.edu/apply/JPF02484 and include the following: (1) curriculum vitae; (2) statement of research interests; (3) statement of teaching expertise; (4) statement of administrative activities and leadership philosophy; (5) statement of formal and informal activities to promote diversity and inclusion among students, staff, and faculty; and (6) cover letter that explains interest in position, special qualifications for the chair position, and names of three referees. All items should be distinct documents. We encourage applications from women, minorities, and individuals with a history and commitment to the success of students from under-represented demographics in the sciences. Review of applications will begin on **15 October 2016**, and continue until position is filled.

The Department of Ecology and Evolutionary Biology has 28 faculty, a large graduate program, over 1800 undergraduates across three majors (Biology; Ecology, Behavior, and Evolution; Marine Biology), and two minors (Conservation Biology and Evolutionary Medicine. The department has close ties with the Institute of the Environment and Sustainability, and departments across the College, David Geffen School of Medicine, and the School of Public Health. EEB is associated with the UCLA La Kretz Center for California Conservation Sciences, UCLA Stunt Ranch University of California Reserve, the Mildred E. Mathias Botanical Garden, the Donald E. Dickey Collection of Birds and Mammals, the Center for Education and Innovation and Learning in the Sciences, and the Institute for Quantitative and Computational Biosciences.

The Division of Life Sciences (www.lifesciences.ucla.edu) includes over 200 faculty, 7000 undergraduates, 500 graduate students, 12 undergraduate majors, and more than 12 PhD programs. The UCLA College through its four academic divisions—Humanities, Life Sciences, Physical Sciences, and Social Sciences is the academic heart of UCLA, which is California's largest university with an enrollment of nearly 43,000 undergraduate and graduate students.

The University of California is an Equal Opportunity/Affirmative Action Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability, age or protected veteran status. For the complete University of California nondiscrimination and affirmative action policy, see: UC Nondiscrimination & Affirmative Action Policy. (http://policy.ucop.edu/doc/4000376/NondiscrimAffirmAct)

UT Southwestern Medical Center

Faculty Positions in Infectious Diseases

The Division of Infectious Diseases in the Department of Internal Medicine at the University of Texas Southwestern (UTSW) Medical Center at Dallas is seeking new faculty members at the Assistant Professor level. Successful applicants should have an M.D. and/or a Ph.D. degree with at least two years of postdoctoral experience and an exceptional publication record. Candidates will be expected to develop independent, externally-funded independent research programs that focus on understanding the biologic basis of infectious diseases and/or host defense. Our search is broad, and we are interested in applicants who are creatively pursuing "cutting-edge" research on medically important pathogens including those resistant to antimicrobials, emerging pathogens, novel pathogen discovery, and/or agents of potential biothreat. UTSW is an outstanding scientific environment with established strengths in structural biology, biochemistry, molecular biology, microbiology, immunology, genetics, and numerous other areas.

UTSW has a strong tradition of academic excellence and an exceptionally collegial and collaborative culture that cuts across departmental boundaries. Opportunities exist for collaborations with faculty members in Infectious Diseases, the Departments of Microbiology and Immunology and the Centers for Autophagy Research and Genetics of Host Defense.

We offer other substantial resources such as outstanding core facilities and research infrastructure. Candidates will be expected to contribute to the teaching and research training of Infectious Diseases fellows. The positions offer attractive start-up packages and laboratory space.

To apply, submit a C.V., three letters of reference, and a description of research interests to: **Dr. Trish M. Perl, Chief, Division of Infectious Diseases, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9113.** E-mail: **Cindy.Jozefiak@UTSouthwestern.edu**

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.

Penn Engineering

MULTIPLE FACULTY POSITIONS
Department of Electrical and Systems Engineering

The School of Engineering and Applied Science at the University of Pennsylvania is growing its faculty by 33% over the next five years. As part of this initiative, the **Department of Electrical and Systems Engineering** is engaged in an aggressive, multi-year hiring effort for multiple tenure-track positions at all levels. Candidates must hold a Ph.D. in Electrical Engineering, Systems Engineering, or related area. The department seeks individuals with exceptional promise for, or proven record of, research achievement, who will take a position of international leadership in defining their field of study, and excel in undergraduate and graduate education. Leadership in cross-disciplinary and multi-disciplinary collaborations is of particular interest. We are interested in candidates in all areas that enhance our research strengths in:

1. **Nanodevices and nanosystems** (nanoelectronics, MEMS/NEMS, power electronics, nanophotonics, integrated devices and systems at nanoscale),
2. **Circuits and computer engineering** (analog, RF, mm-wave, and digital circuits, emerging circuit design, computer engineering, IoT, embedded and cyber-physical systems), and
3. **Information and decision systems** (control, optimization, robotics, data science, network science, communications, information theory, signal processing, markets and social systems).

Prospective candidates in all areas are strongly encouraged to address large-scale societal problems in energy, transportation, health, food and water, economic and financial networks, critical infrastructure, and national security. We are especially interested in candidates whose interests are aligned with the school's strategic plan (www.seas.upenn.edu/PennEngineering2020).

Diversity candidates are strongly encouraged to apply. Interested persons should submit an online application at **http://www.ese.upenn.edu/faculty-positions** and include curriculum vitae, statement of research and teaching interests, and at least three references. Review of applications will begin on **December 1, 2016**.

The University of Pennsylvania is an Equal Opportunity Employer. Minorities/Women/Individuals with Disabilities/Veterans are encouraged to apply.

Duke University School of Medicine

FACULTY POSITION IN PHARMACOLOGY AND CANCER BIOLOGY

Applications are invited for a tenure track appointment as **Assistant Professor in the Department of Pharmacology and Cancer Biology** in the Duke University School of Medicine. We are seeking individuals with innovative research programs that will complement that of current faculty (http://pcb.duke.edu).

Applicants should submit a curriculum vitae, a brief statement of past research accomplishments and future research interests and arrange to have three letters of recommendation uploaded electronically to: **https://academicjobsonline.org/ajo/jobs/7731**.

Complete applications (including letters of reference) submitted by **November 30, 2016** will receive full consideration.

Duke University is an Affirmative Action/Equal Opportunity Employer.

NYIT College of Osteopathic Medicine

FACULTY POSITION: Neuroscience

The **Department of Biomedical Sciences of the New York Institute of Technology College of Osteopathic Medicine (NYITCOM)** seeks a faculty member at the **Assistant or Associate Professor** level with expertise in teaching and research in **Neuroscience**. Candidates who use model organisms and/or human subjects in **any area of neuroscience research** are encouraged to apply, especially those with expertise in sensori-motor control or touch/haptics. Current research in the Department includes microbial infection of the brain, nervous system development, and coordination of limb movements. Core facilities and institutional resources include confocal and scanning electron microscopy, animal facility, biomechanics lab, and an associated clinic for treating neuromotor disorders. Collaborative possibilities within the Department also include cardiovascular disease/heart failure, diabetes, microbiology, renal physiology and development, and thyroid hormone dysfunction. Successful candidates are expected to contribute to the medical school teaching effort and develop an active research program. The medical school provides strong institutional support for both teaching and research activities, including maintenance of core facilities, support for student workers, and access to IT resources.

The successful candidate will possess a Ph.D., D.O., M.D., or D.V.M., at least 2 years of post-doctoral research experience, and demonstrated successful medical education/teaching experience preferred. We offer institutionally-supported faculty salaries, a competitive benefits package, and a professional environment designed to enhance career development. To apply, please e-mail cover letter and resume to: **Dr. A. Martin Gerdes, Chair of Biomedical Sciences** at **agerdes@nyit.edu**.

EOE M/F/D/V.

UT Southwestern Medical Center

TENURE-TRACK POSITIONS

The Department of Physiology invites outstanding scientists with Ph.D., M.D., or equivalent degrees to apply for tenure-track faculty positions at the level of Assistant or Associate Professor. Candidates who use innovative optical, mechanical, electrical, molecular biological or computational methods with important applications to physiological systems, ranging from individual genes and proteins to cells and organs are encouraged to apply. The scientific excellence of the candidates is more important than the specific area of research.

These positions are part of the continuing growth of the Department at one of the country's leading academic medical centers. They will be supported by significant laboratory space, competitive salaries, state-of-the-art core facilities and exceptional start up packages. The University of Texas Southwestern Medical Center is the scientific home to six Nobel Prize laureates and many members of the National Academy of Sciences and Institute of Medicine. UT Southwestern conducts more than 3,500 research projects annually totaling more than $417 million. Additional information about the Department of Physiology can be found at **http://www.utsouthwestern.edu/education/medical-school/departments/physiology/index.html**.

Applicants should submit a CV, a brief statement of current and proposed research, and summary of your two most significant publications describing the importance of the work (100-150 words each). Please arrange to have three letters of recommendation sent on his/her behalf. All items should be submitted to **http://academicjobsonline.org/ajo/jobs/7612**. Completed applications will be reviewed starting **November 1, 2016**. You may email questions to **ron.doris@utsouthwestern.edu**.

UT Southwestern Medical Center is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans and individuals with disabilities are encouraged to apply.

Perelman School of Medicine University of Pennsylvania

Assistant/Associate Professor of Physiology

The Department of Physiology at the Perelman School of Medicine at the University of Pennsylvania seeks candidates for Assistant or Associate Professor positions in the tenure track. Rank will be commensurate with experience. Responsibilities include establishing and conducting an independent research program, and supervising, mentoring and teaching students. Applicants must have an M.D. or Ph.D. or equivalent degree and have demonstrated excellent qualifications in research.

The successful candidate will have experience in any aspect of physiology or a related field, including neuroscience and cardiovascular science. Candidates working at any or all ranges of physiology (molecular, cellular, and organismal) will be considered. Special attention will be given to investigators using state-of-the-art techniques with research programs that leverage molecular and cellular insights to inform physiological and pathophysiological functions. Research areas of particular interest include signaling, membrane transport physiology, organelle biology, metabolism and structural biology. Applicants at the Associate Professor level are expected to have funding and an internationally recognized track record of creative research productivity and excellence.

Highly competitive lab space and start-up funds are available. The Perelman School of Medicine, one of the top ranked medical schools in the nation for research and NIH funding, is a highly collaborative environment with strong core facilities. It is located on the campus of the University of Pennsylvania, a world-class institution located near the center of Philadelphia.

We seek candidates who embrace and reflect diversity in the broadest sense.

The University of Pennsylvania is an EOE. Minorities/Women/Individuals with disabilities/Protected Veterans are encouraged to apply.

Apply for this position online at:
https://www.med.upenn.edu/apps/faculty_ad/index.php/g311/d4042

Yale SCHOOL OF MEDICINE

Department of Neuroscience

New Haven, CT 06520-8001

http://medicine.yale.edu/neuroscience/index.aspx

CELLULAR NEUROSCIENCE FACULTY POSITIONS

The Department of Neuroscience at Yale University seeks to hire faculty who utilize **molecular, cellular and genetic approaches to study the development and function of the nervous system and its dysfunction in disease**. Candidates employing any model organism are welcome to apply. Emphasis will be placed on recruiting at the level of Assistant Professor, but outstanding applicants at Associate Professor level will also be considered. We seek candidates with an exceptional track record, potential for outstanding future achievements, and a wish to participate in a dynamic and growing neuroscience community at Yale that includes the Kavli Institute for Neuroscience, the Program in Cellular Neuroscience, Neurodegeneration and Repair (CNNR) and the Swartz Center at Yale. Candidates are expected to develop a productive and innovative research program and will have the opportunity to participate in graduate and medical education.

Candidates must hold a Ph.D., M.D., or equivalent degree. Please send a cover letter, curriculum vitae, up to 3 representative publications, a research plan (strictly limited to 2 pages), and arrange for submission of 3 letters of recommendation. All application materials should be submitted electronically to **apply.interfolio.com/37159**. Applications will be reviewed as they are received with a deadline of **November 18, 2016**.

SYSTEMS NEUROSCIENCE FACULTY POSITIONS

The Department of Neuroscience at Yale University seeks to hire faculty who use **neuronal or circuit level analysis to study the neural basis of behavior and cognition in health and disease**. Candidates employing any model organism, from invertebrates to non-human primates, are welcome to apply. Emphasis will be placed on recruiting at the level of Assistant Professor, but outstanding applicants at Associate Professor level will also be considered. We seek candidates with an exceptional track record, potential for outstanding future achievements, and a wish to participate in a dynamic and growing neuroscience community at Yale that includes the Kavli Institute for Neuroscience, the Program in Cellular Neuroscience, Neurodegeneration and Repair (CNNR) and the Swartz Center at Yale. Candidates are expected to develop a productive and innovative research program and will have the opportunity to participate in graduate and medical education.

Candidates must hold a Ph.D., M.D., or equivalent degree. Please send a cover letter, curriculum vitae, up to 3 representative publications, a research plan (strictly limited to 2 pages), and arrange for submission of 3 letters of recommendation. All application materials should be submitted electronically to **apply.interfolio.com/35942**. Applications will be reviewed as they are received with a deadline of **November 18, 2016**.

Yale is an Affirmative Action Equal Opportunity Employer. Yale values diversity among its students, faculty, and staff. Women, persons with disabilities, protected veterans, and underrepresented minorities are encouraged to apply.

PRINCETON UNIVERSITY

Assistant Professor Positions in Computational Biology Available

The Lewis-Sigler Institute for Integrative Genomics at Princeton University invites applications for tenure-track faculty positions at the Assistant Professor Level. We are seeking outstanding scientists in the areas of bioinformatics, computational biology or biostatistics. The successful candidate would have an established track record of collaborative research with experimental biologists. We are particularly keen to recruit faculty who will extend and complement our existing strengths in bioinformatics and biostatistics.

The Lewis-Sigler Institute for Integrative Genomics, housed in the Carl Icahn Laboratory at Princeton University, was established to innovate in research and teaching at the interface of modern biology and the more quantitative sciences. The Institute provides significant support to its faculty through heavy investment in state-of-the-art infrastructure, an NIH-funded graduate training program, and a high level of administrative support.

Faculty positions are jointly appointed in one of the following academic departments at Princeton: Computer Science, Ecology and Evolutionary Biology, Physics, Chemistry, Chemical and Biological Engineering, or Molecular Biology. These joint faculty positions offer significant benefits in terms of infrastructure, research funds, and collaborative opportunities, as well as an opportunity to interact with unusually able and interdisciplinary students. The successful candidate will have research laboratories at the Institute, and teaching responsibilities (both graduate and undergraduate) will be shared between the Institute and the home department.

Essential Qualifications: All applicants must have a Ph.D., M.D., or equivalent degree. In addition, applicants must have a very strong record of research productivity, demonstrate the ability to develop a rigorous research program, and be committed to teaching at both the undergraduate and graduate levels.

How to Apply: Applications must be submitted online at **http://jobs.princeton.edu**, requisition #1600658 (jobs.princeton.edu/applicants/Central?quickFind=68888) and should include a cover letter, curriculum vitae, a two-page research description, as well as contact information for at least three references. Applications will start being reviewed beginning on **December 1, 2016**.

Princeton University is an Equal Opportunity Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law. This position is subject to the University's background check policy.

PRINCETON UNIVERSITY

Assistant Professor Positions Available

The Lewis-Sigler Institute for Integrative Genomics at Princeton University invites applications for tenure-track faculty positions at the Assistant Professor level. We are seeking outstanding scientists with strong interest and experience in quantitative, systems-level approaches to understanding any area in modern experimental biology. Examples include, but are not restricted to, single-molecule live-cell imaging, whole-genome analysis of chromosome topology, high-throughput experimental analysis of aging, metabolomics, and animal development. We seek individuals who will extend and complement our existing strengths in experimental genomics and other –omics approaches, biological chemistry, and biophysics. A strong record of experimental work and quantitative analysis is essential.

The Lewis-Sigler Institute for Integrative Genomics, housed in the Carl Icahn Laboratory at Princeton University, was established to innovate in research and teaching at the interface of modern biology and the more quantitative sciences. The Institute provides significant support to its faculty through heavy investment in state-of-the-art research infrastructure, an NIH-funded graduate training program, and a high level of administrative support.

Faculty positions are jointly appointed in one of the following academic departments at Princeton: Physics, Chemistry, Molecular Biology, Ecology and Evolutionary Biology, Chemical and Biological Engineering, or Computer Science. These joint faculty positions offer significant benefits in terms of infrastructure, research funds, and collaborative opportunities, as well as an opportunity to interact with unusually able and interdisciplinary students. The successful candidate will have research laboratories at the Institute, and teaching responsibilities (both graduate and undergraduate) will be shared between the Institute and the home department.

Essential Qualifications: All applicants must have a Ph.D., M.D., or equivalent degree. In addition, applicants must have a very strong record of research productivity, demonstrate the ability to develop a rigorous research program, and be committed to teaching at both the undergraduate and graduate levels.

How to Apply: Applications must be submitted online at **http://jobs.princeton.edu**, requisition #1600659: (jobs.princeton.edu/applicants/Central?quickFind=68887) and should include a cover letter, curriculum vitae, **a concise 1-2 page research vision statement**, as well as contact information for three references. Applications will be reviewed beginning on **November 15, 2016**.

Princeton University is an Equal Opportunity Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law. This position is subject to the University's background check policy.

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Baylor College of Medicine

DEPARTMENT OF MOLECULAR & HUMAN GENETICS

FACULTY POSITION in GENETICS, GENOMICS, and BIOLOGY of CANCER

The Department of Molecular and Human Genetics and the Dan L. Duncan Comprehensive Cancer Center at Baylor College of Medicine invites applications for a tenure-track Assistant Professor, Associate Professor, or Professor position in cancer research. Applicants' research programs may focus on broad ranging topics in cancer genetic research including cancer genomics, mechanisms of genome instability, cancer model organisms, cancer therapeutics, epigenetics and gene expression, and others. Diverse areas of cancer genetics are welcome, and impact on basic and/or translational cancer research is strongly encouraged.

The Department of Molecular and Human Genetics (http://www.imgen.bcm.tmc.edu/molgen/) ranks #1 among all U.S. genetics departments in both number of grants and total funding from the National Institutes of Health. The Department provides a bridging environment for physicians and basic scientists, promoting a cross-species and multidisciplinary approach to cancer research. Faculty in The Department study disease mechanisms in many organismal contexts including bacteria, yeast, Drosophila, Dictyostelium, *C. elegans*, mouse, and humans.

The Department is committed to translation of basic science into clinical implementation and fosters technology transfer for faculty discoveries. Activities within the Department include basic and translational research, clinical genetics, a joint venture diagnostic laboratory, a long-standing NIH large-scale human genome sequencing center (https://www.hgsc.bcm.edu/), and an exceptional Ph.D. graduate program. More broadly, Baylor College of Medicine is the premier medical school of Texas, home to the NCI-designated Dan L. Duncan Comprehensive Cancer Center (https://www.bcm.edu/centers/cancer-center), and centered in Texas Medical Center, the world's largest biomedical research complex.

The Department has a total research funding of over $80 million, 62 primary tenured and tenure-track research faculty member and 180 total primary faculty members who are engaged in a variety of approaches to tackling cancer and other diseases. The Department and BCM offer premier recruitment packages and resources to enhance the candidate's research program. Qualified applicants should email a PDF of their curriculum vitae, 3 letters of reference, and a cover letter stating the position to which they are applying to:

Department of Molecular and Human Genetics
Baylor College of Medicine
One Baylor College of Medicine, ABBR Room R830
Houston, TX 77030
Phone: 713-798-5443
Email: mhgfacultyrecruits@bcm.edu

Review of qualified applicants will begin **November 1, 2016**.

Equal Opportunity, Affirmative Action and Equal Access Employer.

Baylor College of Medicine

DEPARTMENT OF MOLECULAR & HUMAN GENETICS

PHYSICIAN/SCIENTIST FACULTY POSITION

Among genetics departments at U.S. medical schools, the Department of Molecular and Human Genetics at Baylor College of Medicine (http://www.imgen.bcm.tmc.edu/molgen/) ranks number one in both number of grants and total funding from the National Institutes of Health. The Department of Molecular and Human Genetics provides a bridging environment for physicians and basic scientists, promoting a cross-species approach to functional genetics and a commitment to technology transfer. Activities within the Department include clinical genetics, basic and clinical research, a new joint venture diagnostic laboratory, long-standing association with an NIH large-scale human genome sequencing center, medical student teaching, a PhD graduate program, and residency/fellowship training in medical genetics.

The Department has total research funding of over $80 million, 62 primary tenured and tenure-track research faculty member and 180 total primary faculty members who are engaged in a variety of missions including basic and translational research, clinical diagnostic services, and pediatric and adult clinical care. To expand our translational impact, we developed a uniquely structured and governed joint venture with Miraca Holdings to form the Baylor Genetics Laboratories that will help translate genetic discoveries to cutting edge genetic testing for a world-wide audience. Model organisms studied by our faculty include bacteria, yeast, *Drosophila*, *Dictyostelium*, *C. elegans*, mouse, and humans.

Faculty also have an opportunity to interact with a vigorous Medical Genetics program with over 25 clinical MD and MD/PhD faculty, 35 genetic counselors, and 25 diagnostic laboratory PhD faculty. In addition, the Medical Genetics training program attracts 3-4 clinical fellows per year for ABMG-approved residency training. ABMG-approved training for DNA, biochemical, and cytogenetics diagnostic laboratories is also offered.

The Department staffs clinical genetics programs at Texas Children's Hospital, Baylor CHI St. Luke's Medical Center, Harris Health System, and the Michael E. DeBakey Veteran Affairs Medical Center.

The Department is currently seeking a physician scientist who has clearly demonstrated ability to conduct high-impact basic, translational and/or clinical research and to compete for scientific funding in the area of medical genetics and/or model organism genetics.

Appointment will be at the Assistant, Associate, or Full Professor level depending on experience.

Qualified applicants should email a .pdf version of their curriculum vitae and a cover letter stating the position to which they are applying to:

Department of Molecular and Human Genetics
Baylor College of Medicine
One Baylor College of Medicine, ABBR Room R830
Houston, TX 77030
Phone: 713-798-5443
Fax: 713-798-8515
Email: mhgfacultyrecruits@bcm.edu

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UPSTATE
MEDICAL UNIVERSITY
State University of New York

TENURE TRACK FACULTY POSITIONS
Department of Neuroscience and Physiology

The Department of Neuroscience and Physiology is seeking applications for two tenure-track faculty positions at the Assistant, Associate or Full Professor level in any area of Neuroscience. The rank and tenure status will be dependent on qualifications. The successful applicant will have demonstrated outstanding achievement and should have a strong publication record. Sustained grant funding is required for appointment to the rank of Associate Professor or Full Professor. The Department is collaborative with strengths in neural development, retinal degeneration, the neural extracellular matrix, and neural circuitry of feeding behaviors. The research laboratories are located in the brand new Neuroscience Research Building equipped with state-of-the-art facilities. Applicants will be expected to maintain a well-funded research program and to contribute to graduate and medical teaching. We offer a competitive startup package and salary compensation. Syracuse is a diverse and affordable metropolitan area located in central New York, with easy access to the Adirondacks, Catskills and Finger Lakes. Proximity to Syracuse University and SUNY ESF further enhance scientific collaboration and funding opportunities. Additional information at: http://www.upstate.edu/neuroscience-department/.

All applicants should e-mail a single PDF file containing a cover letter, curriculum vitae, teaching statement, summary of research accomplishments and plans to: neurosci@upstate.edu. Applicants for the Assistant Professor position should also make arrangements for 3 letters of reference. Address letters and inquiries to: **Chair, Faculty Search Committee, at neurosci@upstate.edu or Department of Neuroscience & Physiology, NRB 3706, 750 East Adams Street, Syracuse, New York 13210**. Review of applications will begin **November 1st, 2016** and continue until the positions are filled.

At SUNY Upstate Medical University we strive to promote a professional environment that encourages varied perspectives from faculty members with diverse life experiences. SUNY Upstate Medical University is an affirmative action, equal opportunity employer, including Veterans and Disabled, committed to inclusive excellence through diversity. A respect for diversity is one of our core values. We are committed to recruiting and supporting a rich community of outstanding faculty, staff and students. We actively seek applications from women and members of underrepresented groups to contribute to the diversity of our university community in support of our teaching, research and clinical missions.

Tenure Track Faculty
Biology of Aging Research

The University of Wisconsin-Madison is seeking applications for a tenure track Biology of Aging faculty position in the internationally renowned Division of Geriatrics and Gerontology of the Department of Medicine within the UW School of Medicine and Public Health. The position will include a joint appointment in the Geriatric Research, Education, and Clinical Center (GRECC) of the William S. Middleton Memorial VA Medical Center in Madison, WI.

The successful candidate will be expected to develop an independent research program in the Biology of Aging /Geroscience and to contribute to the growth and integration of extensive NIH-funded aging research programs on the UW Campus. Applications from individuals with a background in biochemistry, molecular and cell biology, or molecular studies will be prioritized. Candidates engaging in mechanistic research and those with existing extramural support will receive special attention. The academic rank, salary and benefits will be commensurate with prior qualifications and experience.

To apply, please send a letter of interest and a copy of your most recent curriculum vitae to: **Sanjay Asthana, MD, Associate Dean for Gerontology and Professor and Head, UW Division of Geriatrics, c/o Eva Gray, 2870 UNIVERSITY AVE, Room 106, Madison, WI 53705, or e-mail: sa@medicine.wisc.edu.**

School of Medicine and Public Health
UNIVERSITY OF WISCONSIN-MADISON

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Tenure-track faculty positions

The Department of Otolaryngology – Head and Neck Surgery at The Ohio State University Comprehensive Cancer Center – James Cancer Hospital and Solove Research Institute is seeking new tenure-track faculty at the assistant, associate or full professor level to join a basic and translational research program that focuses on head and neck cancer.

The program seeks several accomplished scientists within the fields of cancer cell biology, cancer genomics and immunology. Scientists with experience in tumor immunology, cancer stem cells, tumor lineage or single-cell analysis, and translational therapeutics will be strongly considered. Applicants with funded programs or strong evidence of funding potential will be given the highest priority and offered a highly competitive start-up package. Applicants should have a PhD and/or MD degree and will be expected to participate in the research and training mission of the department.

Applicants should send a cover letter describing their interest in the department, along with their CV and brief description of their research interests, as a single PDF document addressed to **James W. Rocco, MD, PhD, Professor, Mary E. and John W. Alford Research Chair in Head and Neck Cancer** at **James.Rocco@osumc.edu**. Review of applications will continue until the positions are filled.

The Ohio State University is an equal opportunity employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status or protected veteran status.

The James

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

cancer.osu.edu

Keck School of Medicine of USC

Department of Stem Cell Biology and Regenerative Medicine
Assistant Professorship in Stem Cells and Regenerative Medicine

The Department of Stem Cell Biology and Regenerative Medicine (http://keck.usc.edu/broadcenter/about-the-center/) is recruiting candidates exploring developmental and regenerative processes in mammalian organ systems. The Department is particularly interested in researchers applying tissue engineering, computational modeling, and genetics and genomic approaches. The Department is housed in the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research within the Keck School of Medicine of USC. Significant resources are available to support all aspects of stem cell research within the building and in adjacent centers. Excellent collaborative opportunities exist across the USC campuses. In addition to its research mission, all members will play an important role in the educational mission of this Department. Generous start-up packages will be awarded to the successful candidates.

Online applications will be accepted, please apply to **http://jobs.usc.edu/postings/74098**. Applications should include a letter of interest, curriculum vitae, brief 2-3 page outline of research past, present and future, and **four** letters of reference. The applicant is responsible for ensuring that the completed application is received before **October 31st, 2016**.

The University of Southern California strongly values diversity and is committed to equal opportunity in employment. Women and men, and members of all racial and ethnic groups, people with disabilities, and veterans are encouraged to apply.

Baylor College of Medicine

DEPARTMENT OF MOLECULAR & HUMAN GENETICS

MOUSE GENETICIST FACULTY POSITION

Among genetics departments at U.S. medical schools, the Department of Molecular and Human Genetics at Baylor College of Medicine (http://www.imgen.bcm.tmc.edu/molgen/) ranks number one in both number of grants and total funding from the National Institutes of Health. The Department of Molecular and Human Genetics provides a bridging environment for physicians and basic scientists, promoting a cross-species approach to functional genetics and a commitment to technology transfer. Activities within the Department include clinical genetics, basic and clinical research, a new joint venture diagnostic laboratory, long-standing association with an NIH large scale human genome sequencing center (https://www.hgsc.bcm.edu/), medical student teaching, a Ph.D. graduate program, and residency/fellowship training in medical genetics.

The Department has a total research funding of over $80 million, 62 primary tenured and tenure-track research faculty member and 180 total primary faculty members who are engaged in a variety of missions including basic and translational research, clinical diagnostic services, and pediatric and adult clinical care. Model organisms studied by our faculty include bacteria, yeast, drosophila, dictyostelium, C. elegans, mouse, and humans.

Faculty also have an opportunity to interact with a vigorous Medical Genetics program with over 25 clinical MD and MD/PhD faculty as well as a large research and diagnostic laboratory Ph.D. faculty. In addition, the Medical Genetics training program attracts 3-4 clinical fellows per year for ABMGG approved residency training. ABMGG-approved training for DNA, biochemical, and cytogenetics diagnostic laboratories is also offered.

The Department staffs clinical genetics programs at Texas Children's Hospital, Baylor CHI St. Luke's Medical Center, Harris Health System, and the Michael E. DeBakey Veteran Affairs Medical Center.

- The Department is seeking an individual for faculty appointment at rank appropriate for achievement and experience in mouse genetics.The Department has a distinguished history in mouse genetic study of development and disease. It is a center for the Knock Out Mouse Phenotyping (KOMP) project and has extensive core facility services. Candidates would be expected to make extensive use of mice in their research but might work in human genetics or with other model organisms; areas include models of human disease, developmental biology, neuroscience, or cancer genetics.

Appointment will be at the Assistant, Associate, or Full Professor level depending on experience.

Qualified applicants should email a .pdf version of their curriculum vitae and cover letter stating position to which they are applying to:

Department of Molecular and Human Genetics
Baylor College of Medicine
One Baylor College of Medicine, ABBR Room R830
Houston, TX 77030
Phone: 713-798-5443
Fax: 713-798-8515
Email: mhgfacultyrecruits@bcm.edu

Equal Opportunity, Affirmative Action and Equal Access Employer.

SANFORD RESEARCH

ASSOCIATE SCIENTIST/ASSISTANT PROFESSOR CANCER BIOLOGY AND CHILDREN'S HEALTH RESEARCH CENTERS

The Cancer Biology (CBRC) and Children's Health Research (CHRC) Centers (http://www.sanfordresearch.org/researchcenters/) at Sanford Research in Sioux Falls SD, invite applications from researchers for full time faculty at the rank of Associate Scientist within Sanford Research with commensurate rank of Assistant Professor in the Sanford School of Medicine at The University of South Dakota. An historic $400 million gift by philanthropist Denny Sanford has allowed for expansion of biomedical sciences at Sanford Research, an energetic and collegial research community focused on cancer and pediatric research.

For the CBRC we seek outstanding scientists with research programs that span all areas of cancer research, especially those relevant to cancer immunology or immunotherapy. For the CHRC we seek outstanding scientists with research programs on the underlying mechanisms and/or treatment of congenital defects, developmental disorders, pediatric diseases and regenerative cellular therapies. Applicants should hold a PhD, MD or MD/PhD degree and complement the existing strengths and the interdisciplinary and collaborative nature of Sanford Research. Physician Scientists are encouraged to apply. Candidates will be expected to develop independent research programs and secure extramural funding.

Significant institutional support, including modern laboratory space and state-of-the-art facilities, will be provided. In addition, a comprehensive compensation package will be tailored to the individual's qualifications.

Sanford Health is an Equal Opportunity/Affirmative Action Employer.

Candidates should submit a single PDF including a detailed *curriculum vitae*, description of research experience and future research plans with specific details on the relevance of their research to cancer biology, cancer immunology, or pediatric research. Candidates should also submit at least three letters of recommendation. Incomplete candidate packages will not be accepted. All application materials should be sent by email to:

Faculty Recruitment Committee
Sanford Research
2301 E. 60th Street North, Sioux Falls, SD 57104
Telephone: 605-312-6004
Email: researchrecruitment@sanfordhealth.org

NYU School of Medicine
NYU LANGONE MEDICAL CENTER

Director of the Stewart and Judith Colton Center for Autoimmunity

The NYU School of Medicine is accepting applications for the position of Professor and Director of the Judith and Stewart Colton Center for Autoimmunity. The mission of the Colton Center is to elucidate the initiating immunological events and to develop new diagnostics and treatments for autoimmune diseases (http://www.med.nyu.edu/colton-center-for-autoimmunity). The director will lead an extensive research effort among leading physicians and scientists with diverse expertise across the Medical Center's scientific community in order to to advance translational science in the field of autoimmune diseases.

To qualify, the director must have scientific expertise in the area of immunology, significant publications in high impact journals and a record of success in obtaining NIH funding. Candidates must be an associate or full professor, be able to provide leadership and a strategic vision for the Colton Center.

Applications are electronic only at **https://apply.interfolio.com/36764**, where you will be asked to upload the following documents:

1. Cover Letter
2. Curriculum Vitae

Equal Opportunity Employer: disability/veteran. VEVRAA Federal Contractor

UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

ASSISTANT PROFESSOR Faculty Positions in Biochemistry and Molecular Biology

The College of Medicine at the University of Arkansas for Medical Sciences is seeking applications for two tenure track faculty members at the level of Assistant Professor in the Department of Biochemistry and Molecular Biology (**http://www.uams.edu/biochem/**). The department is seeking highly qualified biochemists/molecular biologists who will establish research programs that seek to establish underlying mechanisms of biomedically important processes in order to enable development of therapies or diagnostics. Particular areas of interest include cancer biology, signal transduction, membrane traffic, DNA damage response, epigenetic mechanisms and systems biology. The positions offer competitive salary, benefits, start-up packages and research space. The positions are associated with the Winthrop P. Rockefeller Cancer Institute (**http://www.cancer.uams.edu**).

Candidates must possess a PhD and/or MD degree and postdoctoral experience. Successful candidates will be expected to have or develop an internationally leading research program and contribute to teaching medical and graduate students. Little Rock has an area population of 600,000, with many cultural amenities, an affordable cost of living, and beautiful natural surroundings.

Consideration of candidates will begin immediately and will continue until the positions are filled. Applicants should submit curriculum vitae, a brief statement of proposed research, and arrange for three reference letters to be sent to (electronic submission preferred): **Biochemistry Search Committee, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205. E-mail: Biochemsearch@uams.edu**.

University of Arkansas for Medical Sciences is an Equal Opportunity/Affirmative Action Employer.

regenerationNEXT

Duke University School of Medicine

FACULTY POSITION IN REGENERATIVE BIOLOGY AND MEDICINE

Regeneration Next Initiative (RNI) is a new venture to advance discovery research and education in the broad field of tissue regeneration, and to enable translational applications for regenerative medicine. RNI is partnering with basic and clinical departments throughout Duke University School of Medicine to hire a tenure-track faculty member at the Assistant Professor level. An appointment at the Associate or Full Professor level is possible for exceptional senior applicants. We invite applications from accomplished scientists with expertise and innovative approaches to developmental and cell biology, quantitative biology, imaging, stem cell biology, mechanisms of tissue regeneration, or tissue engineering.

A primary appointment will be made in a department most relevant to the candidate's research, with possibilities for joint appointments. Candidates must have a Ph.D., M.D., or equivalent degree and will have a strong record of creativity and productivity in a field broadly related to tissue regeneration. Qualified minority candidates are especially encouraged to apply.

Applicants should submit a cover letter, curriculum vitae, a 3-page total summary of accomplishments and research plans, a teaching statement, and at least 3 letters of recommendation by **November 15, 2016** to **https://academicjobsonline.org/ajo/jobs/7911**

Questions may be directed to: **Ken Poss, Director, RNI (regeneration@duke.edu)**

Duke University is an Affirmative Action/Equal Opportunity Employer committed to providing employment opportunity without regard to an individual's age, color, disability, genetic information, gender, gender identity, national origin, race, religion, sexual orientation, or veteran status.

McGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

Call for Nominations: Scolnick Prize in Neuroscience

The McGovern Institute for Brain Research is accepting nominations for the 14th annual Edward M. Scolnick Prize in Neuroscience. The Prize recognizes an outstanding discovery or significant advance in the field of neuroscience. The prize is $150,000. The recipient presents a public lecture at MIT, hosted by the McGovern Institute and followed by a dinner in Spring 2017.

Nomination Deadline: December 15, 2016

Nomination procedures:
Candidates for the award must be nominated by individuals affiliated with universities, hospitals, medicals schools, or research institutes, with a background in neuroscience. Self-nomination is not permitted. Each nomination should include:

- A biosketch or CV of the nominee;
- A letter of nomination with a summary and analysis of the major contributions of the nominee to the field of neuroscience.
- Up to two representative reprints will be accepted.

Selection Procedure:

- Members of the selection committee and faculty affiliated with MIT are not eligible.
- Announcement of the award recipient will be made in January 2017
- Recipient must attend all events to be awarded the prize.

Past Scolnick Prize Recipients: 2016: Cornelia Bargmann, HHMI, The Rockefeller University; 2015: Charles Gilbert, The Rockefeller University; 2014: Huda Zoghbi, HHMI, Baylor University; 2013: Thomas Jessell, HHMI, Columbia University; 2012: Roger Nicoll, UCSF; 2011: Bruce McEwen, The Rockefeller University; 2010: Lily and Yuh-Nung Jan, UCSF; 2009: Jeremy Nathans, Johns Hopkins University; 2008: Michael Davis, Emory University; 2007: David Julius, UCSF; 2006: Michael Greenberg, Children's Hospital/HMS; 2005: Judith Rapoport, NIH; 2004: Masakazu Konishi, CalTech

Send nomination packet to: **gwolf@mit.edu** or **Attn: Scolnick Prize Nomination, McGovern Institute for Brain Research, Massachusetts Institute of Technology, 77 Massachusetts Avenue 46-3160, Cambridge, MA 02139.**

For more information: http://mcgovern.mit.edu

RUTGERS
THE STATE UNIVERSITY OF NEW JERSEY

Open Rank Faculty Search
Department of Statistics & Biostatistics
Center for Integrative Proteomics Research

The Department of Statistics and Biostatistics and the Center for Integrative Proteomics Research at Rutgers, The State University of New Jersey seek to hire an outstanding faculty member with strong research and teaching interests in statistical analyses of biological and biomedical information. The search is open to all faculty ranks, including tenure-track Assistant Professor, tenure-track Associate Professor, tenured Associate Professor, and tenured Full Professor. Applicants must have Ph.D. in Statistics or related fields, and also a proven track record in research at the interface between advanced analytics and the biological/biomedical sciences. Responsibilities of the position include: teaching and academic advising of both undergraduate and graduate students in statistics, biostatistics or proteomics, and conducting original research. Pursuit of external research funding is expected.

The Department of Statistics & Biostatistics and the Proteomics Center have strong ties. Together they will provide access to a broad array of excellent research facilities and a competitive start-up package. The tenure line falls within the Department of Statistics and Biostatistics in the School of Arts and Sciences-New Brunswick Campus.

The Center is a newly built 75,000-square-foot facility dedicated to fostering interdisciplinary research in the biological and biomedical sciences using complementary quantitative tools of measurement and analysis. The Center is the home of the Institute for Quantitative Biomedicine at Rutgers and the RCSB Protein Data Bank. More information about our programs can be found in the websites: www.stat.rutgers.edu; www.proteomics.rutgers.edu; iqb.rutgers.edu; rcsb.org.

Interested individuals should apply online through the Rutgers Interfolio website link (**http://apply.interfolio.com/36128**) by providing a curriculum vitae, research summary, current research support, and teaching statement, and arranging for submission of at least three confidential letters of reference. Review of applications is expected to conclude in December 2016.

Rutgers, the State University of New Jersey, is an Equal Opportunity/Affirmative Action Employer. Qualified applicants will be considered for employment without regard to race, creed, color, religion, sex, sexual orientation, gender identity or expression, national origin, disability status, genetic information, protected veteran status, military service or any other category protected by law. As an institution, we value diversity of background and opinion, and prohibit discrimination or harassment on the basis of any legally protected class in the areas of hiring, recruitment, promotion, transfer, demotion, training, compensation, pay, fringe benefits, layoff, termination or any other terms and conditions of employment.

Caltech

CALIFORNIA INSTITUTE OF TECHNOLOGY invites applications for a tenure-track faculty position at the assistant professor level in the Division of Chemistry and Chemical Engineering. Candidates with strong commitments to research and teaching excellence are encouraged to apply.

BIOCHEMISTRY

Assistant professor level in biochemistry and related areas. The term of the initial appointment is four years and is contingent upon completion of all requirements for a Ph.D. in biochemistry or closely related fields. Interested candidates should apply electronically at **https://applications.caltech.edu/job/bmb**. Candidates unable to apply electronically may submit curriculum vitae, publication list, teaching statement, description of proposed research, and three letters of recommendation to: **Chair of the Biochemistry Search Committee, Division of Chemistry and Chemical Engineering, M/C 164-30, California Institute of Technology, Pasadena, CA 91125**. Applications should be received by **November 1, 2016**.

CHEMICAL ENGINEERING

Exceptionally well-qualified applicants at the associate or full professor level may also be considered. The term of the initial untenured appointment is four years and is contingent upon completion of all requirements for a Ph.D. in chemical engineering or in a related field. Interested candidates should apply electronically **https://applications.caltech.edu/job/chemeng**. Candidates unable to apply electronically may submit curriculum vitae, publication list, teaching statement, a description of proposed research, and three letters of recommendation to: **Chair of the Chemical Engineering Search Committee, M/C 210-41, California Institute of Technology, Pasadena, CA 91125**. Applications should be received by **December 15, 2016**.

CHEMISTRY

Exceptionally well-qualified applicants at the tenured level may also be considered. Areas of particular interest includes experimental physical chemistry, inorganic chemistry, and biochemistry, although applications in any area of chemistry broadly defined are welcome. The term of the initial appointment is four years, and the appointment is contingent upon completion of all requirements for a Ph.D. in chemistry or in a related field. Interested candidates should apply electronically at **https://applications.caltech.edu/job/chemistry**. Candidates unable to apply electronically may submit a curriculum vitae, publication list, teaching statement, description of proposed research, and three letters of recommendation to: **Chair of the Chemistry Search Committee, M/C 164-30, California Institute of Technology, Pasadena, CA 91125**. Applications should be received by **October 15, 2016**.

EOE of Minorities/Females/Protected Vets/Disability.

Assistant/Associate Professor of Cell Biology and Neuroscience

The Department of Cell Biology and Neuroscience at Rutgers, The State University of New Jersey, Piscataway, invites applications for a tenure-track position at the Assistant or Associate Professor level. We seek individuals with research programs that will advance our understanding of neurological disorders through the investigation of mechanisms affecting RNA metabolism or protein translation.

The Department is located on the Rutgers Busch Campus, and is part of the Division of Life Sciences, a group of Departments and Institutes that provides ample opportunities for interdisciplinary research. Current collaborations within the Division range from biomaterials and nanotechnology, RNA biology and neurosciences, to human genetics and stem cells. The campus is located near the Rutgers Robert Wood Johnson Medical School and is less than one hour away from New York City and Philadelphia. Rutgers offers excellent facilities and competitive start-up packages.

Applicants must have a Ph.D. in cell biology, biochemistry, molecular biology, neuroscience, or a related field and/or an M.D. with a minimum of four years postdoctoral experience. The successful candidate will be expected to teach courses at the undergraduate and graduate levels in the field of cell biology or neuroscience and to establish a productive, independent research program supported by external funding. Interested individuals are encouraged to apply online through the departmental website (**http://cbn.rutgers.edu**) with a curriculum vitae, a brief statement of research plans, and the names, addresses, and contact information of three individuals who will provide a letter of reference. Applications should be submitted as soon as possible but not later than **December 15, 2016**. Late applications will be considered only if positions remain available.

Rutgers University is an Equal Opportunity/Affirmative Action Employer.

Tenure-Track Faculty position Microbial Pathogenesis Yale School of Medicine

The Department of Microbial Pathogenesis of the Yale School of Medicine is seeking applicants for a tenure-track faculty position at the Assistant Professor level. Applications at other ranks from more established investigators with a strong record of accomplishments will also be considered. We are seeking applicants using multidisciplinary approaches to investigate host pathogen interactions. Individuals studying viral, bacterial, or protozoan organisms who are interested in the molecular mechanisms of microbial infection, the immunological response to infection, or the host microbiome are encouraged to apply. The position offers an attractive start-up package, excellent laboratory space and a stimulating scientific research environment. Candidates should have a Ph.D. and/or M. D. degrees, suitable postdoctoral research experience, a strong record of research accomplishments, a commitment to develop independent, innovative research programs, and an interest in graduate and medical education.

Review of applications will begin immediately and will continue until the position is filled. Applicants should electronically submit (to **bonnie.lemelin@yale.edu**) a curriculum vitae and a statement of current and future research interests. In addition, applicants should arrange for three letters of reference (addressed to: Chair, Search Committee, Department of Microbial Pathogenesis, Yale School of Medicine) to be sent to **bonnie.lemelin@yale.edu**.

Yale University is an Affirmative Action/Equal Opportunity Employer and welcomes applications from women, persons with disabilities, protected veterans, and members of minority groups.

TENURE TRACK FACULTY POSITION DEVELOPMENTAL/REGENERATIVE BIOLOGY

The Department of Biology invites applications for a **tenure-track Assistant or Associate Professor in Developmental or Regenerative Biology** with membership in the Allen Discovery Center at Tufts. We seek a creative scholar with primary expertise in large-scale pattern regulation in embryogenesis or regeneration. We expect this individual to use state-of-the-art technology and quantitative approaches toward an integrative, multi-scale understanding and control of anatomical patterning. Ideal candidates will address molecular and computational controls of large-scale morphology, synthesizing data and models across genetics, physiology, and anatomy. Integrating across levels of organization, from molecules to organs, is essential. Aquatic model species are especially welcome, as are approaches that involve biophysical forces, high-performance imaging, optogenetics, *in vivo* electrophysiology, and computational analysis and modeling beyond the cell level (see http://ase.tufts.edu/biology/ and http://allencenter.tufts.edu/).

The successful candidate is expected to develop an active externally-funded research program, and contribute to graduate and undergraduate courses. A doctoral degree and a record of research productivity are required.

Applicants should use the link **https://apply.interfolio.com/36975** to submit a cover letter, a curriculum vitae, and separate statements of (1) research interests and plans and (2) teaching experience and philosophy. Submission of one to three selected reprints in PDF format is encouraged. Applicants should also ensure that three confidential letters of reference are submitted to this link.

Review of applications begins **November 1, 2016**, and continues until the position is filled.

Tufts University is an Affirmative Action/Equal Opportunity Employer. We are committed to increasing the diversity of our faculty. Members of underrepresented groups are strongly encouraged to apply.

Tenure Track Faculty Position in Regenerative Medicine and Cell Biology

Medical University of South Carolina, Department of Regenerative Medicine and Cell Biology

A tenure track faculty position at the Assistant or Associate Professor level is available for a researcher who uses cellular and/or molecular-genetic approaches to address fundamental aspects of human disease. Candidates interested in the study of cardiovascular or digestive disease using stem cells are particularly encouraged to apply. Competitive salary, laboratory space and start-up funds are available. Candidates at the Associate Professor level are expected to bring a vigorous research program with significant extramural funding. Current research strengths in the department include tissue engineering, pluripotent stem cell differentiation, cardiovascular development, digestive disease, and molecular biology of cell function. Research is supported by several excellent core facilities specializing in imaging, genetically modified mice and rats, drug discovery, proteomics, genomics, and flow cytometry. A Ph.D. or M.D./Ph.D. degree (or equivalent), plus additional postdoctoral experience are required. Information about the Department can be found at http://cba.musc.edu.

Applicants should provide curriculum vitae, research plan, and the names of three references through the MUSC employment portal: **http://careers.pageuppeople.com/756/cw/en-us/job/493664/univ-assistant-or-associate-professor**. Review of applications will begin on **October 1, 2016** and continue until the position is filled.

The Medical University of South Carolina in Charleston was established in 1824 and is one of the oldest continuously operating medical schools in the United States. It is ranked in the upper quartile of all freestanding academic medical schools. The university has approximately 2,200 graduate and professional students that are supported by 2,000 faculty members. In 2014 total financial support topped $217 million with $100 million coming from the NIH. This includes funding that supports the NCI–designated Hollings Cancer Center as well as a Clinical and Translational Science Award.

MUSC is an Affirmative Action/Equal Opportunity Employer.

UC San Diego

The Division of Biological Sciences announces a *Division-wide Faculty Excellence Search*

The Division of Biological Sciences at the University of California, San Diego (www.biology.ucsd.edu) hereby announces a Division-wide Excellence Search for a tenure-track or tenured faculty position at assistant, associate or full professor level. We are seeking exceptional candidates in any Section within the Division of Biological Sciences: Cell & Developmental Biology; Ecology, Behavior & Evolution; Molecular Biology; or Neurobiology. Candidates working in any research area within these four Sections are welcome to apply. All candidates must have earned a Ph.D. or equivalent degree, and be committed to teaching at the undergraduate and graduate levels. In addition to excellence and creativity in research and scholarship, successful candidates must also demonstrate a commitment to equity and inclusion in higher education. A successful candidate must have a well-articulated plan of contributing to programs that increase access and success of underrepresented students, as well as faculty and local communities in the sciences. We are especially interested in candidates who have already created or contributed to such programs. A successful candidate will also have served as a role model in mentoring others, with a commitment to helping shape and expand the University's diversity initiatives (http://diversity.ucsd.edu/).

Review of applications will commence by **October 21, 2016** and will continue until the position is filled. Interested applicants must submit a cover letter, curriculum vitae, statement of research, statement of teaching, a statement describing their past experience and leadership in fostering equity and diversity and/or their potential to make future contributions, and 3-5 publications. Applicants at the Assistant Professor level need to submit 3-5 references, and applicants at the Associate or Full Professor level need to provide contact information for 3-5 references. Applications must be submitted through the University of California San Diego's Academic Personnel RECRUIT System at **https://apol- recruit.ucsd.edu/apply**.

The Division of Biological Sciences at UCSD is a vibrant center of scientific discovery, innovation, and collaboration. Our large research base spans many areas of biology and has one of the most celebrated graduate programs in the country. We are committed to academic excellence and diversity within the faculty, staff, and student body. This is where discovery comes to life.

UC San Diego is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to excellence through diversity (http://diversity.ucsd.edu/). All qualified applicants will receive consideration for employment without regard to gender, race, color, religion, sex, sexual orientation, national origin, disability, age or protected veteran status.

Faculty Positions in the Department of Biochemistry and Molecular Biology The University of Texas Medical Branch

The University of Texas Medical Branch (UTMB Health), Galveston, Texas, seeks outstanding faculty candidates at all ranks for tenure-track/tenured positions in the broad fields of Biochemistry and Molecular Biology (http://www.bmb.utmb.edu).

The ideal candidate will have extensive experience in elucidating the mechanisms of important biomedical problems at a molecular/atomic level. Applicants would be complementing growing strengths within the Department that include structural biology, RNA and DNA biology, molecular virology, and computational biology. The candidates should be energized by opportunities to interact with established biomedical researchers in a highly collaborative biomedical research community and to become outstanding mentors to students and postdoctoral fellows. Rich opportunities exist at UTMB for interactions and affiliations with centers of scientific excellence in structural biology and molecular biophysics, biodefense, molecular medicine, cancer cell biology, infectious diseases, environmental health, aging, and translational sciences (http://www.utmb.edu/centers). In addition to a highly collaborative environment, UTMB offers outstanding core services, including: next-generation sequencing, mass spectrometry, transgenic mouse facility, optical microscopy, flow cytometry, solution biophysics, NMR, X-ray crystallography, cryo-electron microscopy, and bioinformatics (see http://www.utmb.edu/core). Excellent opportunities for scientific interactions also exist through UTMB Health's participation in the Gulf Coast Consortia and the Keck Center for Interdisciplinary Bioscience (http://www.gulfcoastconsortia.org). UTMB is also a member of the Texas Medical Center.

A very attractive recruitment package of salary, start up funds and newly renovated space will be offered. Interested applicants should submit a curriculum vitae, a summary of research accomplishments, and future goals (max 3 pages), and contact information for three references to: **Mariano A. Garcia-Blanco M.D., Ph.D.** at **BMB.Recruiting@utmb.edu**. Applications must be received by **December 15, 2016** to be considered.

UTMB Health strives to provide equal opportunity employment without regard to race, color, national origin, sex, age, religion, disability, sexual orientation, gender identity or expression, genetic information or veteran status. As a VEVRAA Federal Contractor, UTMB Health takes affirmative action to hire and advance women, minorities, protected veterans, and individuals with disabilities.

utmb Health

UCLA

Tenure-Track Faculty Position Microbiology, Immunology and Molecular Genetics

The Department of Microbiology, Immunology and Molecular Genetics at the David Geffen School of Medicine at University of California Los Angeles invites applications for a TENURE-TRACK faculty position at the Assistant or Associate Professor level. Outstanding candidates working in the areas of immunology or virology are invited to apply. Successful candidates are expected to develop an innovative research program and join a highly interactive group of scientists within the Department of Microbiology, Immunology and Molecular Genetics (http://www.mimg.ucla.edu). The candidate will benefit from a collaborative research community spanning the basic and clinical sciences from the Schools of Medicine and Engineering, College of Letters and Science, California NanoSystems Institute, Jonsson Comprehensive Cancer Center, the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and the UCLA AIDS Institute. This tenure-track position includes a guaranteed salary, a highly competitive start-up package and laboratory space in a state-of-the-art research facility. Eligible individuals will also receive the Shaper Family Career Development Chair.

Applications will be screened beginning **November 1, 2016**. For full consideration applicants are encouraged to apply before the closing date of the search, **December 15, 2016**. Applicants must have a PhD, MD or equivalent terminal degree by the date of hire. Additionally, postdoctoral (or equivalent post terminal degree) experience, evidence of potential for establishing extramurally funded research program, a strong record of scientific publications, a demonstrated track record in teaching and mentoring students/trainees, and a commitment to mentoring underrepresented undergraduate/graduate students are preferred. Applicants should submit full curriculum vitae, a 2-page summary describing their current research program and future directions, a brief statement on teaching experience, and three letters of reference. Applicants with a history of commitment to mentoring underrepresented undergraduates are encouraged to apply and should indicate their experience in a cover letter. All applications are to be submitted through UC Recruit (**https://recruit.apo.ucla.edu/apply/JPF02546**) Job number **JPF02546**.

Inquiries, but not application material should be directed to:

Glen Fukui
Glenf@microbio.ucla.edu
Assistant to Chair, Faculty Search Committee
Department of Microbiology, Immunology and Molecular Genetics
University of California, Los Angeles
1602 Molecular Science Building
Box 951489
Los Angeles, CA 90095-1489

The University of California is an Equal Opportunity/Affirmative Action Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability, age or protected veteran status. For the complete University of California nondiscrimination and affirmative action policy, see: UC Nondiscrimination & Affirmative Action Policy.

POSITIONS OPEN

JUNIOR FACULTY POSITION in PHYSICAL CHEMISTRY
University of California Santa Barbara

The Department of Chemistry and Biochemistry at the University of California Santa Barbara announces a search for a tenure-track faculty member in physical chemistry for a position beginning Fall 2017 at the ASSISTANT PROFESSOR level. Outstanding candidates with research and teaching interests in all sub-areas of physical chemistry are invited to apply, including (but not limited to) biophysical chemistry, energy and catalysis, molecular spectroscopy and dynamics, and surface and interface science. In addition to contributing to the research, teaching and service missions of the Department of Chemistry and Biochemistry, we anticipate campus-wide interactions in interdisciplinary programs between departments in the Colleges of Letters and Sciences and Engineering, along with other University-based Centers and Institutes. Applicants should submit a curriculum vitae, description of their research plans, statement of graduate and undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to JPF00817 via website: **https://recruit.ap.ucsb.edu/.** Review of applications will begin **October 17, 2016** and will continue until the position is filled. A Ph.D. is required at the time of appointment. The Department is especially interested in candidates who can contribute to the diversity and excellence of the academic community through research, teaching and service.

The University of California is an Equal Opportunity/Affirmative Action Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law.

ASSISTANT PROFESSOR OF NEUROSCIENCE

The Department of Biology at the College of William and Mary invites applications for a tenure track Assistant Professor in Neuroscience beginning in Fall, 2017 with a preferred research focus using genetically tractable model systems, including, but not limited to, mice, zebrafish, and Drosophila. The successful candidate will employ a range of contemporary neuroscience techniques and is expected to maintain an externally funded research program involving both undergraduate and graduate students. We seek applicants whose teaching and research capabilities span from genes to behaviors. In addition to working with undergraduate and Master's students in Biology, there is the opportunity to mentor Ph.D. students whose focus is neuroscience as an affiliate of the Applied Science Department. Annual teaching expectations typically include an upper level course in molecular neuroscience along with a lab during one semester, and another smaller seminar-style course in the candidate's area of expertise for the following semester. A Ph.D. and postdoctoral research experience are required, and previous teaching experience will be viewed favorably. For full consideration, applicants should arrange to have all materials submitted before review begins on **October 28, 2016**. Applications received after this date will be reviewed if needed. Candidates must apply online at website: (**jobs.wm.edu/postings/25732**) and should include a curriculum vitae, a cover letter including statements of research and teaching interests, up to three representative publications and a list of courses taken/taught relevant to neuroscience. Candidates should also arrange to have three letters of reference submitted to the online jobs site. Additional information on the Biology Department and available research facilities may be obtained at *www.wm.edu/biology. The College of William & Mary values diversity and specifically invites applications from members of underrepresented groups who will enrich the research, teaching and service missions of the university. The College is an Equal Opportunity/Affirmative Action employer and strongly encourages applications from women, minorities, veterans, and individuals with disabilities.*

POSITIONS OPEN

DEPARTMENT HEAD OF ECOLOGY
Montana State University

The Ecology Department seeks an excellent scientist/experienced leader with strong academic credentials and a commitment to excellence in teaching and research to provide vision, leadership, direction, and expert administration for a dynamic, productive department. The department includes thirteen tenured or tenure-track faculty and two USGS Cooperative Fishery Research Unit faculty with research programs that are recognized regionally, nationally and internationally, a rapidly growing undergraduate program with more than 400 majors, and successful M.S. and Ph.D. programs that include more than 50 graduate students. The department offers B.S. degrees with emphases in Fish and Wildlife Ecology and Management, Conservation Biology and Ecology, Organismal Biology, and Biology Teaching and graduate degrees in Ecology and Environmental Science, Fish and Wildlife Biology and Management, and Biological Science. We seek a head to facilitate and promote the continued progress in this outstanding department. For more information and application instructions see website: **https://jobs.montana.edu/postings/5631**. Screening of applications will begin on or before **October 3, 2016**; however, applications will continue to be accepted until the position is filled. *Montana State Univeristy is an Equal Opportunity Employer, Veterans/Disabled*

ASSISTANT PROFESSOR, BIOLOGY

Muhlenberg College Biology Department invites applications for a tenure-track Assistant Professor position beginning August 2017. We are seeking candidates who are strongly committed to undergraduate teaching and research in a liberal arts college environment. Teaching responsibilities include an upper-level microbiology course and an upper-level course supporting the biology major or a related interdisciplinary major (e.g., biochemistry, environmental science, or public health). The successful candidate will also contribute to at least one of the following: our introductory biology sequence, a first-year seminar, a non-majors interdisciplinary cluster course, or a human difference and global engagement course.

Qualifications Include: a Ph.D., a record of excellent teaching, and an active research program that can involve undergraduate students. Post-doctoral research or similar experience expected. We are especially interested in applications from candidates with experience in emerging areas of study such as the microbiome, host-microbe interactions, pathogenesis mechanisms, or microbial evolution and ecology.

Muhlenberg is a highly selective liberal arts college located in southeastern PA within commuting distance of the New York City and Philadelphia areas.

Applicants should submit electronic copies of a letter of application that includes an explanation of how the candidate will contribute to Muhlenberg's efforts to become a more diverse and inclusive community, curriculum vitae, statement of teaching and research interests, evidence of teaching excellence, and sample publications. These and three confidential letters of reference must be sent to e-mail: **microbiologist@muhlenberg.edu. Chair of the search is Dr. Marten Edwards, Biology Department, Muhlenberg College, Allentown, PA 18104**. For detailed application information, visit website: **http://www.muhlenberg.edu/main/academics/biology/microbiologist**.

Review of applications will begin October 3, 2016 and continue until the position is filled.

Muhlenberg College is an Equal Opportunity Employer committed to recruiting and retaining outstanding faculty and staff from racial and ethnic groups that have been traditionally underrepresented in higher education. For additional information about Muhlenberg's commitment to diversity and inclusion, applicants can find the latest updates to the College's Diversity Strategic Plan at this link: http://www.muhlenberg.edu/main/aboutus/president/initiatives/diversityatmuhlenberg/.

POSITIONS OPEN

ASSISTANT OR ASSOCIATE PROFESSOR
Bioanalytical Mass Spectrometry; Metabolomics
Indiana University, Bloomington

The Department of Chemistry at Indiana University invites applications for a position in our chemical biology and analytical areas beginning August 2017 at the rank of **ASSISTANT OR ASSOCIATE PROFESSOR**. The search is open to candidates with expertise in bioanalytical mass spectrometry with a strong biological problem orientation in cancer biology, neurodegenerative disease, or childhood disease. The successful candidate will affiliate with Indiana University's emerging Center for Chemical Biology and Biotherapeutics (C2B2) organized under the Precision Health Initiative (PHI) in collaboration with the Indiana University School of Medicine and will be named a trainer in our Chemistry-Biology Interface (CBI) graduate training program in Quantitative and Chemical Biology (website: **http://www.chem.indiana.edu/qcb/**). A Ph.D. in chemistry, biochemistry or a related field and postdoctoral experience are required. Successful candidates will be expected to develop or already have a visible, externally funded research program. Primary teaching assignments will involve graduate level chemical biology, biochemistry, or bioanalytical chemistry and undergraduate chemistry or biochemistry.

Applications completed by **October 15, 2016** will receive full consideration, but the review will continue until the position is filled. Interested candidates should review the application requirements and submit a complete Curriculum vitae, a summary of future research plans, and arrange to have four letters of recommendation to be sent to (website: **https://indiana.peopleadmin.com**). Questions regarding the position or application process can be directed to: **Professor David Giedroc, Chair, Faculty Search Committee, Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405, E-mail: chemchair@indiana.edu.**

Indiana University is an Equal Employment and Affirmative Action Employer and a provider of ADA services. All qualified applicants will receive consideration for employment without regard to age, ethnicity, color, race, religion, sex, sexual orientation or identity, national origin, disability status or protected veteran status.

ASSISTANT PROFESSOR

The Department of Earth, Atmospheric, and Planetary Sciences at the Massachusetts Institute of Technology invites qualified candidates to apply for a tenure track position at the assistant professor level beginning July 2017 or thereafter. Applicants with research interests in Planetary Science are encouraged to apply. We seek an outstanding scientist with interest in and potential for innovation and leadership in teaching at the undergraduate and graduate levels and research. The search is in the broad area of Planetary Science encompassing our Solar System as well as exoplanets, including theory, observation, and instrumentation. However, we are especially interested in individuals whose research complements existing MIT expertise.

Applicants must hold a Ph.D. in Planetary Science or related field by the start of employment and must demonstrate ability to excel in teaching. A complete application must include curriculum vitae, two-page description of research and teaching plans and three letters of recommendations.

Applications are being accepted at Academic Jobs Online: **https://academicjobsonline.org/ajo/jobs/7724** To receive full consideration, complete applications must be received by: **December 1, 2016**.

Search Contact: **Ms. Karen Fosher, HR Administrator, EAPS, Massachusetts Institute of Technology, 54-924, 77 Massachusetts Avenue, Cambridge, MA 02139-4307; email: kfosher@mit.edu**

MIT is an Equal Employment Opportunity Employer. All qualified applicants will receive consideration for employment and will not be discriminated against on the basis of race, color, sex, sexual orientation, gender identity, religion, disability, age, genetic information, veteran status, ancestry, or national or ethnic origin.

University of Massachusetts Medical School Department of Biochemistry and Molecular Pharmacology

CHEMICAL BIOLOGY

The Department of Biochemistry and Molecular Pharmacology at the University of Massachusetts Medical School (http://www.umassmed.edu/bmp/) invites applications for a tenure-track faculty position in Chemical Biology at the rank of Assistant or Associate Professor level. We seek to recruit a dynamic individual who brings a strong chemical perspective to complex systems in biology and medicine. The recruit will join our robust Program in Chemical Biology (http://www.umassmed.edu/bmp/chemical-biology/), a leading center of research that includes more than twenty faculty spanning multiple departments. The position will be highly competitive with regard to start-up funds, laboratory space and salary. The applicant must have a PhD or equivalent and is expected to develop an internationally recognized research program.

Our department offers a vibrant and interactive research community with many opportunities for collaborations within the broader UMass Medical School research community. We are housed in a state-of-the-art building and facilities include x-ray, NMR, Cryo-EM, Mass Spectrometry/Proteomics, Small Molecule Screening, Single Molecule Fluorescence, DNA Sequencing, and High Performance Computing.

Applicants should upload a cover letter, CV, a three-page statement of research (consisting of a one-page summary of past research accomplishments and a two-page summary of future research plans) and the names and contact information of three references to **http://www.academicjobsonline.org**. After you have finalized and approved the submission of your application materials, references will be notified via email instructing them to upload their letters of support. Please ensure that requested referees upload their support letters by the deadline. **The deadline for completed applications and submission of all reference letters is Sunday, November 13, 2016**. Review of applications will begin on **November 14, 2016** and continue until the position is filled.

Inquiries, but not application materials, may be directed to the Search Committee Chair, **Dr. Paul Thompson** (**paul.thompson@umassmed.edu**).

As an Equal Opportunity and Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives and backgrounds.

Penn
University of Pennsylvania

Assistant Professor in Experimental Quantum Condensed Matter Physics

The Department of Physics and Astronomy at the University of Pennsylvania seeks applications from outstanding candidates for an appointment as Assistant Professor in experimental quantum condensed matter physics. Appointment at higher rank can be considered in truly exceptional cases. The successful candidate will develop an innovative research program on quantum phenomena in modern materials that attracts the participation of graduate and undergraduate students and creates collaborative links with other Penn scientists and engineers. We anticipate the candidate's program will contribute actively to the Laboratory for Research on the Structure of Matter and the newly established Singh Center for Nanotechnology. Applicants must apply online at **http://facultysearches.provost.upenn.edu/postings/952**. Required application materials include: curriculum vitae with a list of publications, a research statement and a teaching statement. Applicants should also submit the names and contact information for three individuals from whom we will request letters of recommendation.

Review of applications will begin no later than **November 1, 2016** and will continue until the position is filled. The Department of Physics and Astronomy is strongly committed to Penn's Action Plan for Faculty Diversity and Excellence and to creating a more diverse faculty (for more information see: http://www.upenn.edu/almanac/volumes/v58/n02/diversityplan.html).

The University of Pennsylvania is an EOE. Minorities/Women/Individuals with disabilities/Protected Veterans are encouraged to apply.

Penn
University of Pennsylvania

Assistant Professor in High Energy Theory

The Department of Physics and Astronomy at the University of Pennsylvania invites applications for an Assistant Professor in high energy theory, broadly defined. Applicants from all areas of high energy physics, including particle physics, string theory, quantum field theory, particle cosmology, and the interface with mathematics are encouraged to apply. Senior appointments may be considered in exceptional cases. Applicants should have a Ph.D., an outstanding research record demonstrating independence and innovation, and strong interest in teaching and mentoring students. Interested candidates should submit materials online at **http://facultysearches.provost.upenn.edu/postings/951** and include a curriculum vitae with publications, statements of research and teaching interests, and the name and contact information of at least three referees. Recommenders will be contacted by the University with instructions on how to submit a letter to the website.

Review of applications will begin **November 1, 2016** and continue until the position is filled. The Department of Physics and Astronomy is strongly committed to Penn's Action Plan for Faculty Diversity and Excellence and to creating a more diverse faculty (for more information see: http://www.upenn.edu/almanac/volumes/v58/n02/diversityplan.html).

The University of Pennsylvania is an EOE. Minorities/Women/Individuals with disabilities/Protected Veterans are encouraged to apply.

POSITIONS OPEN

CHEMICAL BIOLOGY FACULTY POSITION
Boston College Chemistry Department

The Chemistry Department of Boston College invites applications for a tenure track position to be effective in the fall of 2017. Applicants will be evaluated based on their potential to establish a prominent and well-funded research program and to excel in teaching at the graduate and undergraduate levels. Successful applicants will join a department of approximately 120 doctoral students, 30 postdoctoral fellows, 200 undergraduate majors, and an internationally recognized faculty.

Assistant Professor in the area of Chemical Biology requires a Ph.D. in Chemistry or related areas; postdoctoral experience is desirable but not required. The candidate is expected to have published in top refereed journals and demonstrated the ability to perform outstanding independent research.

Interested applicants must submit a cover letter (which includes the names of three references), a graphical executive summary of research plans (one page), curriculum vitae, a summary of research plans (eight pages maximum), a statement of teaching philosophy and arrange to have three letters of reference submitted via the online faculty application at website: **http://apply.interfolio.com/30499**.

All application materials must be submitted electronically on or prior to October 15, 2016.

Boston College, a university of eight schools and colleges, is an Equal Opportunity Employer and supports Affirmative Action.

CHEMISTRY DEPARTMENT FACULTY POSITION

The Department of Chemistry at the University of Michigan invites applications for a tenure-track position in any area of chemistry or biochemistry (including analytical, chemical biology, education, inorganic, materials, organic and physical) with an anticipated start date of September 1, 2017. The position is expected to be filled at the assistant professor level; but, applicants at all levels of professor rank will be considered. This position will be a University year appointment (9-months academic year salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels.

Detailed information regarding the electronic application process and required materials is available online web-site: **https://www.chem.lsa.umich.edu/chem/facultyrecruit/**.

Review of applications will begin on **October 1, 2016**. Information about the Chemistry Department is available on the web: **www.lsa.umich.edu/chem**.

Questions about the application process may be sent to e-mail: **chemfacrecruit@umich.edu**.

The University of Michigan is an Equal Opportunity/Affirmative Action Employer and is supportive of the needs of dual career couples, women and minorities are encouraged to apply.

FACULTY CHAIR POSITION in MOLECULAR, CELLULAR, and DEVELOPMENTAL BIOLOGY

We seek an internationally recognized scientist with a highly regarded research program and a commitment to graduate and undergraduate education. MCDB is a distinguished and diverse department with a current faculty of 30. Attractions of this position include a substantial endowment for the department, the ability to recruit new faculty and a vibrant environment for innovations in STEM education. The new chair will have an unprecedented opportunity to implement a vision of the future for MCD Biology in an outstanding academic environment.

Appointment could begin as early as spring 2017. Please submit curriculum vitae, description of research and teaching interests, and the names of three references electronically at website: **www.cu.edu/cu-careers**. Dossiers will be considered upon receipt until position is filled. More information is available at website: **http://mcdb.colorado.edu/**.

The University of Colorado Boulder is committed to diversity and equality in education and employment.

POSITIONS OPEN

FACULTY POSITION in MOLECULAR, CELLULAR and DEVELOPMENTAL BIOLOGY

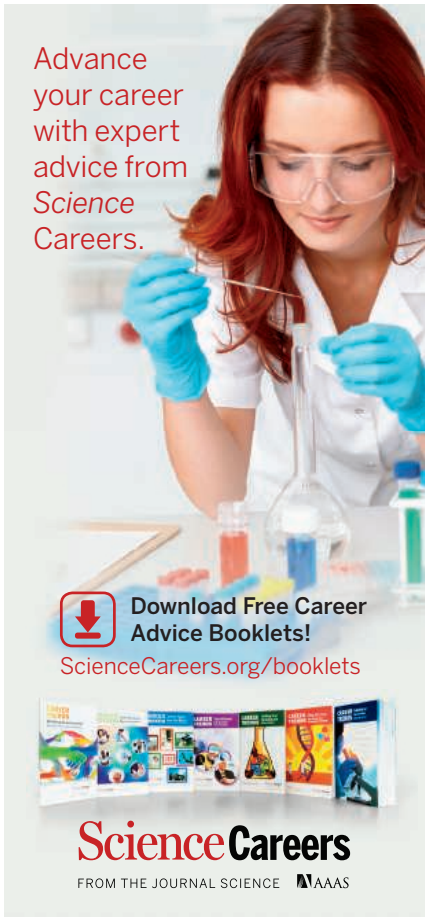
The University of Colorado Boulder invites applications for a **TENURE-TRACK ASSISTANT PROFERSSORSHIP** in the Department of Molecular, Cellular and Developmental Biology. We seek to hire an outstanding scientist in molecular or cellular biology with preference for candidates using electron cryomicroscopy to study important biological questions. The successful candidate will be expected to develop a creative and innovative research program, to provide research training for graduate and undergraduate students, and to teach biology courses at both the undergraduate and graduate level. A Ph.D., postdoctoral research experience and a strong publication record are required.

Submit a curriculum vitae, statement of research and teaching interests, and contact information for three references electronically at website: **www.cu.edu/cu-careers**, posting number #06672. Applications will be reviewed beginning October 15, 2016 until the position is filled.

The University of Colorado Boulder is committed to diversity and equality in education and employment.

TENURE-TRACK FACULTY and CURATOR POSITION
Department of Biological Sciences
Humboldt State University (HSU) - Job #7710

Inviting applications for an academic year tenure-track faculty position in Mammalogy starting August 2017. This individual will also serve as Curator of the HSU Vertebrate Museum. To view full vacancy announcement and to apply, please visit website: **http://apptrkr.com/869762**. Position open until filled. First review of applications is **November 1, 2016**. *HSU is a Title IX/AA/EOE.*



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WASHINGTON STATE UNIVERSITY

Faculty Position in Tissue Regeneration
Assistant/Associate Professor
School of Molecular Biosciences

The School of Molecular Biosciences in the College of Veterinary Medicine at Washington State University in Pullman, WA, seeks to hire an outstanding individual for a full-time tenure-track position at the rank of Assistant or Associate Professor of Molecular Biosciences, (http://www.smb.wsu.edu/), who will contribute to a recently funded campus-wide interdisciplinary initiative in Functional Genomics through their research involving progenitor cells and their role in tissue development and repair. Candidates should also use CRISPR-Cas technology in their research. The successful applicant will have the opportunity to establish their research programs in a state-of-the-art building specifically designed and equipped for biological research. **Duties will include** maintaining a strong, externally funded research program, teaching undergraduate and graduate courses on topics of cell biology and molecular genetics, and participating in service at the school, college, and/or university level. **Job requirements include** a PhD degree in biosciences or a closely related field with a minimum of two years of post-doctoral training in basic science, veterinary medicine, medicine, or a related discipline by the date of hire. Applicants for an Assistant Professor position must demonstrate the potential to establish and maintain an externally funded research program. Applicants for an Associate Professor position must have established an extramurally-funded research program with a record of peer-reviewed publications and national/international recognition. The successful candidate must demonstrate commitment and the ability to successfully advocate for diversity and values of diversity. Applications, including a cover letter, curriculum vitae, and statements of research interests and teaching philosophy should be submitted electronically via: https://www.wsujobs.com. The position number is **71788**. The candidate should also solicit three letters of recommendation to be **submitted electronically** to the same address. Screening of applications will begin **November 1, 2016**. Start date is open, and salary will be commensurate with experience. Direct questions to **pinter@vetmed.wsu.edu**.

Washington State University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.

Polymer Science/Engineering Faculty

The Department of Macromolecular Science & Engineering at Case Western Reserve University (CWRU) is soliciting applications for a full time, tenured or tenure-track faculty position. This position is open to candidates at all ranks, but preference will be given to experienced faculty who would start at the Associate Professor or Professor level. All areas of expertise in Polymer Science and Polymer Engineering will be considered, but it is anticipated that applications in the area of polymer chemistry/synthesis will be preferred. Candidates should possess a Ph.D. or equivalent degree in Chemistry, Chemical Engineering, Material Science or Polymer Science/Engineering. Applications should include a full CV, statement of research plans, statement of teaching, and a list of at least three individuals who can be contacted for letters of recommendation. Complete applications should be sent via email to **Charlotte.foster@case.edu**, with the subject line "**Faculty Application**". The Department of Macromolecular Science & Engineering (Polymers.case.edu), founded 53 years ago, is the home for 14 tenure track faculty, and over 200 B.S., M.S. and Ph.D. students, operating in a research intensive, but highly collegial team. CWRU is located in the beautiful University Circle neighborhood of Cleveland Ohio, home of internationally famous music, art and historical institutions, and a lively student/faculty-focused location.

Competitive candidates for associate professor should have a substantial publication record and a national reputation. Competitive candidates for professor should have achieved records of leadership in the profession and have a strong record of scholarly publications.

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. Women, veterans, members of underrepresented minority groups, and individuals with disabilities are encouraged to apply

Case Western Reserve University provides reasonable accommodations to applicants with disabilities. Applicants requiring a reasonable accommodation for any part of the application and hiring process should contact the Office of Inclusion, Diversity and Equal Opportunity at 216-368-8877 to request a reasonable accommodation. Determinations as to granting reasonable accommodations for any applicant will be made on a case-by-case basis

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WESTERN MICHIGAN UNIVERSITY

Tenure Track Faculty Position
Anatomy or Physiology

The Department of Biological Sciences at Western Michigan University seeks applications for a position at the Assistant/Associate Professor level beginning in fall 2017, pending budgetary approval. The successful candidate will pursue research that complements existing strengths within the department, establish an extramurally funded research program, teach graduate level courses in their area of expertise, participate in the training of Master and Ph.D. students and serve on departmental and University committees. The successful candidate will also be required to teach an undergraduate course in anatomy or physiology. A Ph.D. and relevant post-doctoral experience are required. Competitive salary and startup funding will be offered. Information concerning the Biological Sciences Department's programs and faculty can be obtained at www.wmich.edu/biology/.

Western Michigan University is a learner centered, discovery driven and globally engaged university with high research activity, provides Master's and Ph.D. degrees, and offers a unique opportunity for individuals seeking a balanced research and teaching career. Applicants must apply at **http://www.wmich.edu/hr/jobs**. Please have three letters of reference sent to **Cindy Linn, Ph.D. (cindy.linn@wmich.edu), Anatomy/Physiology Search Committee, Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008-5410**. Review of applications will begin on Nov 1, 2016, but will be considered until position is filled.

WMU is an Equal Opportunity/Affirmative Action Employer. Minorities, women, veterans, individuals with disabilities and all other qualified individuals are encouraged to apply.

ScienceCareers online @sciencecareers.org

香 港 大 學
THE UNIVERSITY OF HONG KONG

Founded in 1911, the University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. The University has a comprehensive range of study programmes and research disciplines spread across 10 faculties and over 140 academic departments and institutes/centres. There are 28,000 undergraduate and postgraduate students who are recruited globally, and more than 2,000 members of academic and academic-related staff coming from multi-cultural backgrounds, many of whom are internationally renowned.

Post-doctoral Fellowships and Research Assistant Professorships

Applications are invited for a number of positions as Post-doctoral Fellow (PDF) and Research Assistant Professor (RAP), at the University of Hong Kong. Appointments will be made for a period of 2 to 3 years and the appointees must be in post on or before July 31, 2017.

PDF and RAP posts are created specifically to bring new impetus and vigour to the University's research enterprise. Positions are available from time to time to meet the strategic research needs identified by the University. Positions are available in the following Faculties/Departments/Schools/Centres/Units:

- Real Estate and Construction
- School of Modern Languages and Cultures
- Faculty of Dentistry
- Centre for the Enhancement of Teaching and Learning
- Computer Science
- Electrical and Electronic Engineering
- Mechanical Engineering
- School of Biomedical Sciences
- School of Chinese Medicine
- Clinical Oncology
- Research Centre of Heart Brain Hormone and Healthy Aging
- Centre of Influenza Research
- Medicine
- Psychiatry
- Centre for Reproduction, Development and Growth
- School of Biological Sciences
- Chemistry
- Physics
- Geography
- Social Work and Social Administration

Post-doctoral Fellows

PDFs are expected to devote full-time to research. Applicants should be doctoral degree holders having undertaken original research that has contributed to the body of knowledge. A highly competitive salary commensurate with qualifications and experience will be offered. Annual leave and medical benefits will also be available.

Research Assistant Professors

The main focus of an RAP's duty is research. RAPs can however be assigned some teaching duties, up to 50% of the normal teaching load. Applicants should be research active and have a proven publication record. A highly competitive salary commensurate with qualifications and experience will be offered, with a contract-end gratuity and University contribution to a retirement benefits scheme (totalling up to 15% of basic salary). Annual leave and medical benefits will also be offered.

Procedures

Prospective applicants are invited to visit our web page at http://jobs.hku.hk/ to view the list of the Faculties/Departments/Schools/Centres/Units and their research areas for which PDF/RAP positions are currently available. Before preparing an application, they should contact the Head of the appropriate academic unit, or the contact person as specified, to ascertain that their research expertise matches the research area for which a vacant PDF/RAP post is available.

Applicants must submit a completed University application form, which should clearly state **which position** they are applying for; and in which academic discipline. They should also provide further information such as details of their research experience, publications, research proposals, etc.

Application forms (341/1111) can be downloaded at http://www.hku.hk/apptunit/form-ext.doc and further particulars can be obtained at http://jobs.hku.hk/. **Closes October 15, 2016**. The University thanks applicants for their interest, but advises that only candidates shortlisted for interviews will be notified of the application result.

The University is an equal opportunities employer and is committed to equality, ethics, inclusivity, diversity and transparency; and is committed to a Non-smoking Policy

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SCIENCE & DIPLOMACY

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Bayer is a global enterprise with core competencies in the Life Science fields of health care and agriculture. Its products and services are designed to benefit people and improve their quality of life. At Bayer you have the opportunity to be part of a culture where we value the passion of our employees to innovate and give them the power to change.

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- Ensure the group has adequate personnel, analytical technology and appliances for timely and cost-efficient analysis and characterization of biological final drug products, API's, intermediates and raw materials including method development and validation
- Provide effective leadership including developing and implementing objectives and business strategies, selecting key personnel and motivating members of the functional area
- Direct the establishment of and have approval authority for Quality Control functions, systems, procedures and specifications for raw materials, intermediates, API's and drug products
- Ensure compliance with all obligations under drug legislation and cGMP-compliant working practices in the laboratories
- Represent areas of responsibility in external regulatory inspections and serve as a technical resource expert (m/f) in these areas
- Provide expertise and support for training, product investigations, CAPA (Corrective Actions & Preventive Actions), quality and operational improvements to ensure compliance with regulations

WHO YOU ARE

- PhD in chemistry or biochemistry
- Long-term experience in a bio-pharmaceutical Quality Control function, including personnel responsibility
- Advanced knowledge and experience in multiple Quality Control subject matter areas
- Broad cGMP experience and knowledge of Quality Assurance/ Quality Control and regulatory compliance (US and EU)
- Strong leadership and interpersonal skills
- Being open-minded and challenging the status quo
- Excellent communication skills in English and German

YOUR APPLICATION

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The Jackson Laboratory for Genomic Medicine (JAX-GM), a nonprofit biomedical research institute in Farmington, CT. is inviting applications for Assistant, Associate, and full Professors. The campus is dedicated to advancing precision medicine using genomic strategies to understand underlying health and disease, and the development of novel diagnostics and therapeutics. Areas for recruitment include:

- Cell Biology
- Cancer Immunology
- Clinical Genetics
- Human Population and Evolutionary Genetics
- Microbial Genomics, Microbiome Research and Infectious Diseases
- Computational Biology and Bioinformatics (systems biology/interactome analysis)

JAX offers a uniquely collaborative scientific research environment, which is supported by outstanding scientific services, unparalleled mouse and genomic resources, pre- and post-doctoral training programs, and numerous courses and conferences.

Candidates must have a Ph.D., M.D. or D.V.M. degree, and 2-5 years of relevant postdoctoral training, with an exceptional record of research accomplishment, and the ability to develop a competitive, independently funded research program. Opportunities are available for shared mentorship of trainees, and integration with the Jackson Laboratory campuses in Maine and California.

Applicants must apply online. Please submit a curriculum vitae and a concise statement of research interests as one document to *www.jax.org/careers*, select Faculty Positions, position #5800. In addition, please have three letters of reference sent to: *facultyjobs@jax.org*.

The Jackson Laboratory is an Equal Opportunity/Affirmative Action Employer. We consider all qualified applicants and employees for hiring, placement and advancement, without regard to a person's race, color, religion, national origin, age, genetic information, military status, gender, sexual orientation, gender identity or expression, disability or protected veteran status.

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Smithsonian Tropical Research Institute

TWO RESEARCH POSITIONS IN TROPICAL BIOLOGY:
MICROBIAL ECOLOGY; and FOREST ECOLOGY AND EVOLUTION

The Smithsonian Tropical Research Institute (STRI; www.stri.si.edu) is pleased to invite applicants to fill two permanent research positions. The first position is part of a new initiative, made possible by generous support from the Simons Foundation, to conduct research on functional roles played by **microbes in tropical forests**. The second position is for a quantitative biologist conducting research on **the ecology and evolution of tropical forests**. Successful candidates will develop outstanding, independent research programs, supervise graduate student researchers and post-doctoral fellows, collaborate with Smithsonian staff, and provide service to STRI and the Smithsonian Institution.

STRI is headquartered in the Republic of Panama, with modern research facilities, a library with extensive holdings in the natural and anthropological sciences, and field stations throughout the Republic of Panamá. Staff scientists maintain research programs within a worldwide network of collaborators at diverse academic institutions. Opportunities for mentoring young scientists are available through an internal fellowship program. Formal teaching is possible through educational programs with affiliated universities, but not required.

Incumbents will complement existing strengths at STRI, and within STRI's Center for Tropical Forest Science / Smithsonian Forest Global Earth Observatory, including, but not limited to, community and population ecology, ecosystem biology, evolutionary ecology, soil-plant interactions, microbial ecology of forests, plant physiology, animal behavior and human impacts on tropical forests. Both positions are based in the Republic of Panama. Early- to mid-career candidates are especially encouraged, but applicants at all postdoctoral stages will be considered.

Minimum Qualifications: A Ph.D. and post-doctoral research experience in a relevant field; an outstanding publication record; demonstrated success in obtaining grants; a commitment to collaborating with a broad array of organismal biologists; and a commitment to communicating science to the public. Demonstrated experience with bioinformatics (microbial biologist) and database management (quantitative biologist) will be an advantage.

To Apply: Interested candidates should submit a cover letter, a summary of research accomplishments and interests, curriculum vitae, three to five significant publications as separate PDFs, and the names and contact information of three references. **Send applications electronically to strisearch @si.edu**. Address inquiries to Dr. Allen Herre, Chair, Microbial Ecology Search Committee at HerreA@si.edu or Dr. Helene Muller-Landau, Chair, Quantitative Forest Ecology Search Committee, at MullerH@si.edu.

Positions are open until filled; review of applications will begin on **2 December 2016**.

STRI is an Equal Opportunity Employer and is committed to diversity in its workforce. Appointments are made regardless of nationality.

CALL FOR PAPERS!
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Gopinath Sutendra and Evangelos D. Michelakis, "Pulmonary Arterial Hypertension: Challenges in Translational Research and a Vision for Change", *Sci. Transl. Med.* 5, 208sr5 (2013) Credit: *Science* Source

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Its History & Future

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Poster deadline: October 1

Topics

The Story of Animal Retroviruses
The Pandemic Begins: Early Discoveries
Human and Primate Retroviruses, HIV Origin
The Extraordinary Virus: Molecular Biology
Immunology and Prevention
Antiretroviral Therapy
Pathogenesis and Prospects

Organizers

Robert Gallo, Institute of Human Virology, University of Maryland School of Medicine, Baltimore
John Coffin, Tufts University
Mila Pollock, Cold Spring Harbor Laboratory
Bruce Walker, Ragon Institute of MGH, MIT and Harvard

Confirmed Speakers

Salim Abdool Karim, CAPRISA, Columbia University
David Baltimore, Caltech
Francoise Barrè-Sinoussi, Institut Pasteur, Paris, France
Edward Berger, National Institute of Allergy and Infectious Diseases (NIAID)
Samuel Broder, Intrexon Corporation
Dennis Burton, The Scripps Research Institute
John Coffin, Tufts University
Jon Cohen, Science Magazine
Larry Corey, Fred Hutchinson Cancer Research Center
James Curran, Emory University
Ronald Desrosiers, University of Miami, Miller School of Medicine
Michael Emerman, Fred Hutchinson Cancer Research Center
Emilio Emini, Bill & Melinda Gates Foundation
Myron Essex, Harvard University
Anthony Fauci, National Institute of Allergy and Infectious Diseases (NIAID)
Genoveffa Franchini, National Cancer Institute
Robert Gallo, Institute of Human Virology, University of Maryland School of Medicine, Baltimore
Glenda Gray, South African Medical Research Council, South Africa
Beatrice Hahn, University of Pennsylvania
Victoria Harden, NIH, Office of NIH History & Stetten Museum (Founding Director, Emerita)
Mark Harrington, Treatment Action Group
Barton Haynes, Duke University School of Medicine
Daria Hazuda, Merck Research Labs
Staffan Hildebrand, Face of AIDS Foundation
Sharon Hillier, Magee-Womens Hospital of UPMC
David Ho, The Rockefeller University
Sharon Lewin, University of Melbourne
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John Martin, Gilead Sciences, Inc.
John Mellors, University of Pittsburgh
Nelson Michael, US Military HIV Research Program
Peter Mugyenyi, Joint Clinical Research Centre, Uganda
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Susan Zolla-Pazner, Mount Sinai School of Medicine

Financial support provided by: Bill & Melinda Gates Foundation, Gilead Sciences Inc., Merck & Co., National Institute of Allergy & Infectious Diseases

CSHL Genentech Center Conferences on the History of Molecular Biology and Biotechnology

Photo: Early October at Cold Spring Harbor

Remaining 2016 Meetings

Axon Guidance, Synapse Formation and Regeneration
Sept 20 - 24
Greg Bashaw, Linda Richards, Peter Scheiffele

Mechanisms of Aging
Sept 26 - 30
Vera Gorbunova, Malene Hansen, Scott Pletcher

Germ Cells
Oct 4 - 8
Robert Braun, Geraldine Seydoux

Biological Data Science
Oct 26 - 29
Bonnie Berger, Jeff Leek, Michael Schatz

Transposable Elements
Nov 2 - 5
Rob Martienssen, Phoebe Rice, Donald Rio

Neurodegenerative Diseases: Biology & Therapeutics
Nov 30 - Dec 3
Karen Duff, Richard Ransohoff, John Trojanowski

Blood Brain Barrier
Dec 7 - 10
Robert Bell, Chenghua Gu, Stefan Liebner

www.cshl.edu/meetings

By **Marie Rippen**

Taking ownership of luck

When people ask me how I got my job as the facility director for a biotechnology incubator right after finishing my Ph.D., my answer used to be, "I was lucky." I felt lucky to land an intellectually engaging job that gives me the freedom to shape the future of a company in the city where I want to live. Actually, I felt lucky to get a job at all. But recently, I've realized that luck doesn't deserve all the credit—I do. I'm not saying that I made all the right choices, and serendipity played its role, but landing my job was first and foremost a result of my own hard work and determination. I'm now learning to take ownership of my success, which will help me continue to progress in my career.

As I was finishing graduate school, my plan was to write a paper while continuing my research as a postdoc before figuring out my next move. (I knew I wanted to pursue something other than a typical academic career.) But the postdoc position lost funding right before I defended my thesis. Suddenly, I had to choose between writing my paper and finding a job. One of my ambitions in grad school had been to publish in a respected journal, and shelving that goal made me feel like I was failing in a major part of my education. But papers don't pay rent, so I chose to leave it unwritten and find a job.

I figured that I was most likely to succeed if I cast a wide net, so I applied for many different types of positions—including grant writer, part-time lecturer, and laboratory manager—but nothing was panning out. With the possibility of living with my parents as a 28-year-old looming, I was getting a little desperate when I happened upon a job ad for a biotechnology incubator facility director, which an acquaintance of mine had posted on LinkedIn.

As I looked into it, I realized that I really wanted this job. The responsibilities included managing the daily operations of the facility, writing grants, promoting the space to potential clients, doing local outreach, and accounting. I didn't know how to do most of those things, but I was confident that I could learn and would enjoy the variety of tasks. I also loved the mission of the organization—to help early-stage biotech startups by providing quality labs. This was a chance to help nurture companies that would offer satisfying work to young scientists like myself.

When I got the job, which started the day after my grad school stipend ran out, I thought, "How lucky! What a serendipitous series of events that led me to a job I actually wanted."

"Each of my choices and accomplishments contributed to my success."

Now, I know better. On my 1-year anniversary at this job, I reflected on all the new experiences I've had and how surprisingly well prepared I was. But the more I thought about it, the less surprising it became. Luck wasn't the only factor in landing this job. My actions led me here.

When I decided in my third year of grad school that I didn't want a career in academia, I took the initiative to seek out experiences that would help me build skills for other jobs. I wrote for a hospital media relations department, planned events, tutored, and attended every networking session I could. These experiences helped me submit a strong application package for the incubator job, and they have served me well as I have written marketing materials, run outreach events, and secured new clients over this past year. Each of my choices and accomplishments contributed to my success, so it's time that I own it.

Adopting this mindset hasn't been easy, in part because I am a woman, brought up with certain social norms. I am used to waiting for compliments only to humbly deny them, to declining to offer ideas if I'm not 100% sure that I'm right, to not talking about my accomplishments unless I'm asked. In being more vocal about how my choices and talents got me the job I wanted, I know that I risk being seen as arrogant. But I remind myself that no one will know what I can do if I don't tell them. ■

Marie Rippen is the facility director for LabLaunch-Monrovia in California. Do you have an interesting career story? Send it to SciCareerEditor@aaas.org.

ILLUSTRATION: ROBERT NEUBECKER

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