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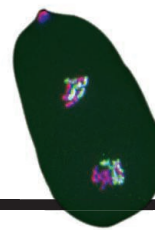
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ON THE COVER



Artist's impression of how a bacterial immune complex, called Cascade (foreground structure), from the CRISPR-Cas system recognizes invading DNA (red-blue-black strand) from a bacteriophage. Cascade,

a complex of 11 proteins and a guide RNA, uses the sequence of the guide RNA to detect invading DNA. See pages 1452, 1473, and 1479 for a close-up look at Cascade before and after it binds to its target DNA. *Illustration: Valerie Altounian/Science; protein coordinate data supplied by Scott Bailey*

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2014 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$153 (\$74 allocated to subscription (51 issues)); \$1282; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request. GST #R1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A. **Change of address:** Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. **Single-copy sales:** \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. **Authorization to photocopy** material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$30.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.

Be one of the first

On 3 July 1880, “a weekly journal of scientific progress” published its first research articles under the banner *Science*. From that original print medium, the inaugural set of papers has been reprinted, photocopied, posted online, converted to PDF, and even etched in glass blocks to serve as special gifts. Being part of the vanguard is indeed a privilege, and opportunities to be one of the first are rare. For that reason, I proudly announce that *Science Advances*, a new open-access journal for all of the sciences, officially joins the *Science* family of journals (*Science*, *Science Translational Medicine*, and *Science Signaling*) and is now accepting submissions (go to www.scienceadvances.org). In February 2015, the inaugural papers will be released, heralding the start of something big in scope, reach, and influence.

The publishing landscape has been changing rapidly, with many new options for disseminating research. In defining the scope of *Science Advances*, our vision is to provide a venue for publication that will allow authors across the entire range of scholarly pursuits—computer, engineering, environmental, life, mathematical, physical, and social sciences—to communicate to others beyond their disciplinary boundaries, and globally without limits, through open access. As the readers’ time is valuable, the editors and reviewers will apply policies for peer review that are similar to those of *Science* to select the most important, noteworthy manuscripts for publication.

The complaint I hear most often from authors is that “*Science* doesn’t publish enough” of their particular field of research. Indeed, *Science* is forced to turn away excellent research in all fields because of its limited space. At *Science Advances*, we can publish more of everything,

subject only to the quality of what is submitted. That makes *Science Advances* an excellent option for a manuscript for which you desire the selectivity and visibility of a top-tier journal and to reach the broadest possible audience, including readers outside of the academic community, through open access. Alternatively, *Science Advances* could be your choice if your paper involves a particularly broad multidisciplinary or interdisciplinary study, including, for example, engineering or social sciences, which puts it beyond the scope of some other research journals. Finally, you might select *Science Advances* because you seek the reputation of the *Science* family, but your research is best represented in a longer format, integrating material that would otherwise be in a separate supplement.

The launch of *Science Advances* will see every article prominently highlighted on the journal’s website and advanced to the public through press releases and social media. And we are pleased to offer a special publication rate to authors who submit their papers early.

The mission of the American Association for the Advancement of Science, the nonprofit publisher of *Science Advances*, is to promote science for the benefit of all humankind. We challenge the scientific community to submit research that addresses the most important problems facing society, reaches across diverse fields to create successful partnerships, and provides

exciting and significant science advancement in any relevant discipline. I cannot begin to speculate about the many ways, 140 years from now, in which those inaugural papers will have been reproduced and spurred discovery and progress. We all believe that this is the start of something big.

– Marcia McNutt



Marcia McNutt
Editor-in-Chief
Science journals



“...our vision is to provide a venue for publication that will allow authors across the entire range of scholarly pursuits...to communicate to others beyond their disciplinary boundaries, and globally without limits, through open access.”

The Pope tackles sustainability

The war on environmental degradation has a powerful new ally: Pope Francis. Prompted by a Joint Workshop of the Pontifical Academy of Sciences and the Pontifical Academy of Social Sciences on sustainability that was convened in May 2014, the Vatican has articulated some of its strongest environmental statements to date, calling for all of us to take personal responsibility and redirect our relationship with nature to ensure the future habitability and sustainability of this planet (see the Perspective on p. 1457). The problems that motivate the Vatican are no different from those that concern the scientific community: depletion of nonrenewable resources, loss of ecosystem services, and risks from changing climate. But what the Vatican contributes is the rationale for taking action: because it is our moral responsibility to bequeath a habitable planet to future generations.

When I received an invitation to present a paper on “The Risks of Rising Seas to Coastal Populations” to the group of distinguished international scholars assembled by the Pontifical Academy, my expectations were high that the threat of climate change could relate to broader concerns about poverty and equity. The latest report from the Intergovernmental Panel on Climate Change* places “death or harm from coastal flooding” as the number-one risk from climate warming, and estimates that the current investment in adaptation to sea-level rise is orders of magnitude less than what is needed to address the critical problem: a rapidly expanding population of poor people living within reach of ever-rising seas. This meeting promised to be interdisciplinary in more dimensions than others I had attended, with the addition of the Vatican’s influence. The proceedings were overseen by His Excellency Monseigneur Marcelo Sanchez Sorondo, a Catholic bishop with degrees in philosophy and theology and Chancellor of the Pontifical Academy of Sciences and Pontifical Academy of Social Sciences. The addition of leaders

from the Vatican enriched discussions of ethics, values, morality, and social justice with regard to climate change and sustainability.

An elite minority of the world’s population consumes the majority of resources, especially energy, with climate change being one of the most universal impacts, affecting everyone, even those who may have contributed the least to greenhouse gas emissions. The diverse group convened by the Vatican called for a universal change in convictions and behaviors that currently condone an attitude of indifference with respect to what we leave for future generations. We advocated for new

measures of wealth that replace income or gross domestic product with more constructive metrics such as natural capital, environmental and human health, social equality, and educational attainment. Science and engineering can deliver the technological capability to be good stewards of the planet, but we must counteract the forces that resist change. As a society, we urgently need to make the choice to redirect our relationship with nature by adopting, for example, the United Nations Sustainable Development Goals to promote a sustainable pattern of economic development and social inclusion.

Pope Francis took the time to personally meet with all of the members of the workshop and even suggested some of the session themes. He struck me immediately as a very humble and kind person—and yet someone with the power to change the world. Although the meeting

was convened by the Catholic Church, the attendees included Hindus, Muslims, Protestants, Jews, atheists, and agnostics, all willing to follow this leader, not because of his religious significance, but because of his moral high ground. We need more leaders to step forward, claiming this moral high ground. Although the current trajectory we are on is dangerous, the message is also one of hope. A safer, more just, more prosperous, and sustainable world is within reach.

– Marcia McNutt



Marcia McNutt
Editor-in-Chief
Science journals



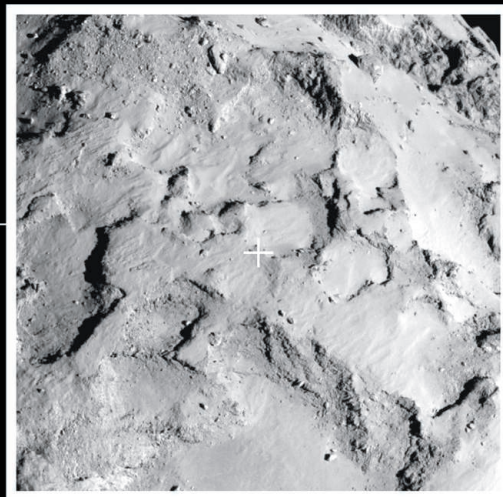
***“...the Vatican contributes...
the rationale for taking
action: because it is our
moral responsibility to
bequeath a habitable planet
to future generations.”***

*Intergovernmental Panel on Climate Change, Working Group 2.
Climate Change 2014: Impacts, Adaptation, and Vulnerability, www.ipcc.ch/report/ar5/wg2/.

“I’ve always been interested in light, since very early in my youth. My first word after ‘mama’ and ‘papa’ was ‘light,’ believe it or not.”

Physicist Ed Moses, former associate director of the National Ignition Facility, to *Physics*
Today on why he’s a good fit for his new job: head of the Giant Magellan Telescope.

IN BRIEF



Rosetta lander aims for site ‘J’

The European Space Agency’s (ESA’s) Rosetta mission has chosen its landing site on comet 67P/Churyumov-Gerasimenko, ESA mission managers announced this week. Rosetta arrived at the comet on 6 August; it’s the first spacecraft to accompany a comet as the sun heats it up and turns on its jets of gas and dust. On 11 November, Rosetta’s Philae lander will set down at a site near the top of the comet’s head known as site J. It will scoop up a sample of dust and ice

from the site, which has an area of about a square kilometer, and analyze its composition. Scientists and engineers picked the site from five candidates, based on surface terrain, dynamical considerations of the descent, and, of course, prospects for exciting science. The primary goal: a scoop of the comet’s black, organic-rich dust, which may be material unaltered since the earliest days of the solar system, more than 4.5 billion years ago.

<http://scim.ag/Rosettaland>

AROUND THE WORLD

Horizon 2020 reopened to Swiss

BRUSSELS | Starting 15 September, scientists in Switzerland will again be able to apply for some research funds from the European Union’s Horizon 2020 program. Switzerland lost its “associated country” status after a referendum to curb immigration in February. As a result, Switzerland said it couldn’t include Croatia, which entered the union last year, in its agreement on the free movement of

labor. The European Commission and Switzerland have now agreed to a short-term deal to restore Switzerland’s associated country status for the “first pillar” of Horizon 2020, worth €24.4 billion for 7 years, but Swiss researchers will be considered third-country applicants for most of Horizon 2020, including its €29.7 billion third pillar. If Switzerland doesn’t extend free movement of persons to Croatia before 9 February 2017 it will again lose its associated status. <http://scim.ag/Swiss2020>

Ebola response ramps up

GENEVA, SWITZERLAND | After weeks of increasingly desperate calls from the World Health Organization (WHO) and medical charities for help fighting West Africa’s Ebola epidemic, several countries have pledged substantial new assistance. On 12 September, WHO and the Cuban government announced the country would send 165 doctors and nurses to Sierra Leone in October. On 16 September, WHO announced China would send 59 more

BY THE NUMBERS

28%

Fraction of its players that the NFL expects will have long-term cognitive problems, according to a report provided for a settlement between the league and 5000 former players.

68%

Five-year U.S. survival rate for all cancers from 2003 to 2009, compared with 49% from 1975 to 1977, according to the American Association for Cancer Research's *Cancer Progress Report 2014*.

29%

Jump in the rate of deforestation of the Amazon rainforest over the past year, according to the Brazilian government, which documented the destruction through satellite data.

experts to join 115 already at the China-Sierra Leone Friendship Hospital. The same day, U.S. President Barack Obama said the U.S. military would dedicate 3000 U.S. forces and \$500 million to an outbreak containment effort headquartered in Monrovia that will build treatment centers, recruit and train health care workers, and distribute more than 50,000 home health care kits to help people care for patients outside treatment centers. <http://scim.ag/CubaEbola>; <http://scim.ag/USaidEbola>

Woman gets iPS cell treatment

TOKYO | In the first human use of tissue derived from induced pluripotent stem (iPS) cells, on 12 September surgeons implanted a 1.3-millimeter-by-3-millimeter sheet of retinal pigment epithelium cells into the eye of a Japanese woman suffering from macular degeneration. The patient is the first of six to undergo the procedure to determine its safety. "This is the first step toward using iPS cells in regenerative medicine," said Masayo Takahashi, an ophthalmologist heading the research effort at the RIKEN Center for Developmental Biology in Kobe, Japan, in a statement. Routine use of the procedure is years away, but may still represent remarkably quick progression from basic finding to clinical use: Kyoto University researcher Shinya Yamanaka announced the discovery of mouse iPS cells in 2006 and human iPS cells in 2007, earning him a Nobel Prize in 2012.

Stem cell papers didn't convince

WASHINGTON, D.C. | The fallout from two now-retracted stem cell papers includes a national hero accused of scientific fraud, the revamping of one of Japan's major research institutes, and the suicide of a respected cell biologist. Last week, *Science* obtained e-mail correspondence between a *Nature* editor and Haruko Obokata, lead author of the papers, that revealed the work initially received a rocky reception.

Dated 4 April 2013, the e-mail includes criticisms of the two papers and suggestions for new data to support claims of a simple, novel way to make stem cells that could form the myriad cell types within a body (dubbed stimulus-triggered acquisition of pluripotency, or STAP). The *Nature* editor initially rejected the papers, but they were accepted 10 months later. A representative for *Nature* declined to comment. <http://scim.ag/NatureSTAP>

Plan to save bluefin tuna

TOKYO | The multinational Western and Central Pacific Fisheries Commission (WCPFC), which coordinates fishing activities in the region, is throwing a lifeline to heavily overfished Pacific bluefin tuna. During a 10 September press briefing, Japanese officials provided details on a new plan agreed to by a WCPFC subcommittee this month: The proposal



Alpha male Pimu was killed by males in his own community in the Mahale Mountains of Tanzania in 2011.

Chimp violence is adaptive behavior

Pity poor Pimu, an alpha male chimp at Mahale national park in Tanzania, pictured here being murdered in 2011 by members of his own troop. But such warfare actually serves an evolutionary purpose, concludes a new study of 18 chimpanzee and four bonobo communities across Africa published this week in *Nature*. Warfare makes the winners more fit by giving them greater access to food resources and females to mate with, the study finds. Those findings contradict claims by some anthropologists that chimp warfare is a response to the stress of human impacts on their habitats, such as deforestation and hunting, although it confirms previous assumptions that bonobos are relatively more peaceful than chimpanzees. Thus, of 153 total killings, only one involved a bonobo. But researchers are still arguing about whether chimp warfare has anything to tell us about the origins of human conflict. <http://scim.ag/chimpwar>



Lonesome George's shell on a lightweight sculpture, part of the taxidermy process (left); the giant tortoise in 2005 (right).



Remembering Lonesome George, an icon of the Galápagos

Lonesome George, a giant long-necked, saddle-backed tortoise who came to embody biological conservation in the Galápagos Islands, died of old age in June 2012 at about 102 years old. First spotted on Pinta Island in 1971, the tortoise was the last known member of his subspecies, *Chelonoidis nigra abingdonii*. Multiple attempts to mate him with closely related subspecies failed, and his death, conservationists fear, marked the end of the subspecies (though researchers have now discovered hybrid

tortoises descended from Pinta Island tortoises on nearby Isabela Island). Even after death, Lonesome George remains an important conservation icon: The body was placed in deep freeze and sent to taxidermists at the American Museum of Natural History (AMNH) in New York City, who began preserving it in 2013, placing him in a classic Lonesome George pose—neck extended, head raised. From 19 September through 4 January 2015, he'll be on display at AMNH, before being sent back to the Galápagos for permanent display.

calls for keeping total Pacific bluefin catches below 2002 to 2004 annual average levels and for reducing catches of juveniles too young to spawn to 50% of those levels. The full commission will almost certainly adopt the proposal at a meeting to be held in Samoa starting



Frozen bluefin tuna at Tsukiji fish market in Tokyo.

1 December, said Masanori Miyahara, an adviser to Japan's ministry of agriculture who chaired this month's meeting. <http://scim.ag/bluefincut>

Academics support student strike

HONG KONG | Hong Kong's academics are being drawn into a debate over election procedures as student activists organize a boycott of classes to protest what they argue are undemocratic restrictions proposed by Beijing. More than 500 professors and staff members at 20 of the city's colleges and universities have signed a statement supporting the students. Universal suffrage by 2017 in elections for Hong Kong's chief executive was a key principle underlying agreements to transfer Hong Kong from British to Chinese sovereignty in 1997. But on 31 August, a committee of China's National People's Congress announced that only two or three people should be eligible to run and that candidates should be selected by a nominating committee widely seen as favoring Beijing. <http://scim.ag/HKstrike>

NEWSMAKERS

New head of European research



Carlos Moedas, secretary of state to Portugal's center-right prime minister, last week was appointed European commissioner in charge of research, science, and innovation. If approved by the

European Parliament, he will take over from Máire Geoghegan-Quinn for a 5-year term at the European Commission, the European Union's executive branch. Moedas will oversee the use of funds from Horizon 2020, the European Union's €80 billion research program. Moedas, 44, earned his civil engineering degree at the Higher Technical Institute of the University of Lisbon, and after earning an MBA from Harvard Business School in 2000, he worked as a banker for Goldman Sachs and Aguirre Newman and founded his own investment company in 2008. <http://scim.ag/CarlosMoedas>



A meteorite brightens the dawn over northeastern Spain on 7 September. The hunt is on for fragments.

PLANETARY SCIENCE

All eyes on shooting stars

By tracking meteorite falls, camera and radar networks help searchers find precious fragments

By **Eric Hand**, in Casablanca, Morocco

Some meteorites announce themselves to the world with a fireball, a sonic boom, and havoc on the ground. Nakhla, for instance, a meteorite from Mars, struck and killed a dog in Egypt in 1911. Other famous falls have hit mailboxes, cars, horses, and, in rare cases, people. But countless others plummet to Earth unseen. A few may be picked up eventually by meteorite hunters—finds outnumber witnessed falls by 50 to 1—but most are lost to time.

Last week, at the annual meeting of the Meteoritical Society here, scientists explained how they are bettering the odds of catching falling space rocks in the act—even the ones that aren't so showy. They are expanding automated camera networks that monitor meteorites' initial fiery descent. Weather radar is also playing an increasingly important role in charting a fall's final stages, and its use has begun to allow hunters to narrow their search to patches as small as a square kilometer.

Scientists who study meteorites are always eager for more samples. But the expanded tracking efforts are paying off in other ways, too. By following a meteorite as it enters the atmosphere, scientists can learn how big the original rock was and what orbit it followed. And the smaller search areas can help hunters snap up falls while the meteorites, and the mineralogical clues they carry about the history of the solar system, are uncontaminated, says Michael Zolensky, curator of the cosmic dust program at

NASA's Johnson Space Center (JSC) in Houston, Texas. "A lot of information is lost the first time it rains on them," he says.

At the conference, Zolensky and Marc Fries, a curation scientist at JSC, offered a workshop on spotting falling meteorites with radar. When a meteorite enters the atmosphere, its outer edges heat up in a glowing plasma. This fireball stage typically occurs at altitudes between 20 and 30 kilometers. That's when cameras—or human eyes—can trace its path. Soon atmospheric friction slows the meteorite and it enters a "dark flight" stage. At this point, it can't be seen by fireball surveys, but it can be spotted by the weather stations that use radar to scan for rain, snow, and hail. They can track the fall-

ing object at altitudes of between about 2 and 10 kilometers. After accounting for winds, Fries says he can produce a more accurate model of a meteorite's trajectory from radar data than from images of the fireball alone.

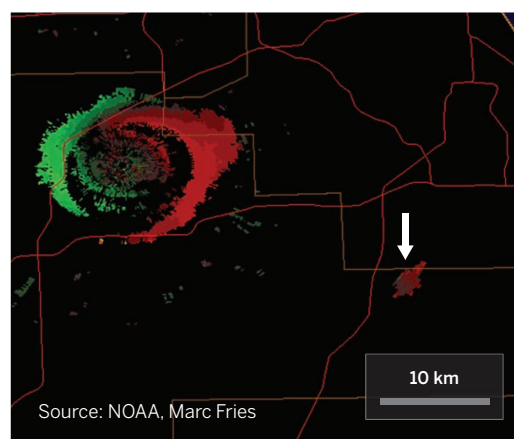
By starting with known fireballs and combing through radar data, Fries and his colleagues have spotted 18 falling meteorites. An important demonstration of the technique came in April 2012, when the Sutter's Mill meteorite broke up and fell in a hailstorm of fragments over several small towns in California (*Science*, 21 December 2012, p. 1521). "We pulled up the radar data and there it was," Fries recalls. He directed a search party to a parking lot, where three pieces were quickly found. Dozens more were found later—after it had rained. That experience underlined the value of speed. The first finds contained oldhamite, a fragile, reactive mineral preserved since the early days of the solar system. It had vanished from the other samples. "As soon as it gets wet, [oldhamite] turns into calcium sulfate with reckless abandon," Fries says.

He is now going through archived radar data to try to reconstruct the trail of older falls. For instance, meteorite hunters have found a few bits and pieces of the January 1998 Elbert, Colorado, fall, but nothing commensurate with the size of the fireball. From radar data, Fries calculated the trajectory of the missing main mass and its size: bigger than a loaf of bread, he says. Finding it will be difficult. Fries's model has the main mass hitting "the only forest area for miles around."

He also wants to expand the technique geographically. According to the World Meteorological Organization, nearly 80% of the world's radar stations lie outside the United States—and that's discounting China, India, and Russia. Fries says the main obstacle to searching for meteorites in the data amassed by those stations is wresting it from local weather agencies, and he urged his colleagues in

A hard rain

Weather radar captured a shower of fragments (arrow) from a 2007 Illinois meteorite fall.



other countries to push for access.

Meanwhile, camera networks continuously monitor the night skies with fisheye lenses. Some, such as the European Fireball Network, are well established, but many rely on enthusiastic amateurs and outdated film cameras. Philip Bland, a researcher at Curtin University, Bentley, in Australia, has larger ambitions. By the end of this month, he and his colleagues will have finished installing some 30 camera stations in the Australian outback, covering an area the size of Alaska.

Each AU\$7000 station in the Desert Fireball Network includes a high-resolution camera that takes 30-second time-lapse stills for calculating trajectories and brightnesses. It also has a video camera to determine the exact duration of the fireball, which can be translated into its speed. The solar-powered stations can collect data, unattended, for months on end.

Bland expects to catch between five and 10 fireballs a year. He also believes that recovering the meteorites will be easier than it is in places such as Europe—even though he often won't be able to enlist radar to refine his trajectories. This part of Australia largely lacks people and precipitation, and hence has few weather radars. But the dark meteorites should be easy to spot against the light-colored, largely barren desert. A camera-equipped drone will aid the search.

With such a wide net for trapping space rocks, Bland believes that he can start to determine the rate at which material from space hits the Earth—and not just extra-terrestrial material. Humanmade space junk regularly falls to Earth and can be distinguished by its slower speed: Anything that strikes the atmosphere at less than 11 kilometers per second can be assumed to have been lofted by humans.

All this technology could eventually make retrieving meteorite fragments routine and efficient. The old way—rare, momentous fireball sightings followed by frenzied yet agonizingly slow searches on foot—would be history. But for now, researchers still thrill to the chase. During the radar technique workshop, Josep Trigo-Rodríguez, a researcher at the Institute of Space Sciences in Barcelona, Spain, learned that a massive fireball, brighter than the full moon, had been seen over Catalonia early on 7 September. Trigo-Rodríguez worked through the week to refine the meteorite's trajectory, based on data from five fireball network cameras and two pictures from eyewitnesses.

He would not reveal its exact location; he sent out a search party last week and did not want to be scooped by local meteorite hunters. But he suspects that several kilograms of fresh meteorite are waiting for him somewhere in the northeast of Spain. ■

CLINICAL TESTING

Harmful mutations can fly under the radar

With more sensitive genetic tests, researchers are hunting the roots of disease in the human “mosaic”

By Kelly Servick

Last month, researchers at Boston Children's Hospital published a study that closely examined the DNA of 158 people with brain malformations who had been unable to identify the cause of their disorders through clinical genetic testing. With highly sensitive sequencing, the group found eight disease-causing mutations that occurred in only a small fraction of each person's cells, making them too subtle for conventional genetic diagnostic tests.

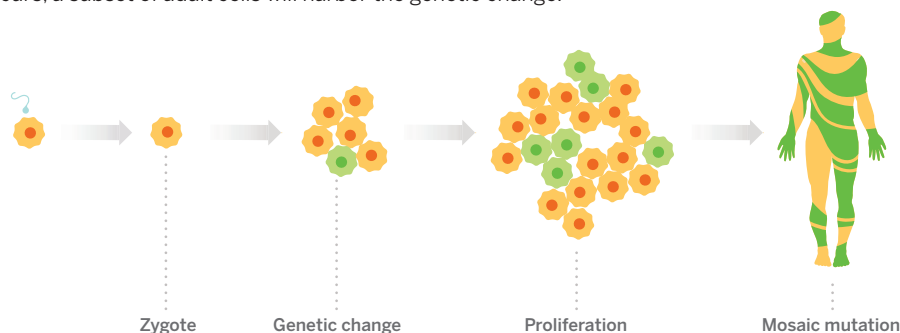
Such genetic variation among cells, known as mosaicism, is present in all of us and has long been common knowledge. The phenomenon was a feature of “college dorm

disease remain invisible to even the best clinical tests.

Traditionally, genetic disease research has focused on mutations inherited through the germ line—the legacy of parents who are carriers, or of genetic mistakes during the formation of their eggs and sperm. These DNA mishaps end up in every cell of a child. But once an embryo starts developing, genetic errors can arise every time a cell divides. And because cells can then proliferate and spread their mutations, each body is “a kind of microevolutionary experiment,” says Leslie Biesecker, a geneticist at NIH's National Human Genome Research Institute in Bethesda, Maryland. The number of cells with a given mutation depends on when and where the mutation

Building a human mosaic

Depending on when and where in embryonic development a mutation occurs, a subset of adult cells will harbor the genetic change.



room conversations in the decades past,” says Chad Shaw, a statistician at Baylor College of Medicine in Houston, Texas. “It was like, ‘Hey, this will blow your mind.’”

But now that genetic technologies make it possible to hunt down specific mutations in small numbers of cells, researchers, clinicians, genetic counselors, and funders are paying closer attention to mosaicism. Next month, the U.S. National Institutes of Health (NIH) will begin reviewing applications for grants to find genetic variation in brain tissue that may help explain neuropsychiatric disorders. And as researchers find more cases of mosaicism, they're wondering how many genetic causes of

arose and how it changes a cell's function. Many such mutations are benign, but some cause disease.

The systematic hunt for disease-causing mosaic mutations is only beginning. Some are obvious—cases where “nature is sticking its finger in our eye,” Biesecker says. In conditions such as McCune-Albright syndrome, the abnormal cells can be seen as patches or patterns on a person's skin. But any genetic disease could be mosaic. Cancers, which consist of genetically distinct cells, are a familiar example, and are a common focus of sequencing efforts. But other studies have shown that mosaic mutations can underlie dozens of conditions,

from Duchenne muscular dystrophy to hemophilia to cardiac arrhythmia.

In some, such as Down syndrome, a mosaic form can produce less severe symptoms than a form that affects all of the body's cells does. In others, such as the brain overgrowth syndrome megalencephaly, a germline mutation is presumably always fatal, and even a low concentration of mutated cells—less than 20% of neurons, according to recent studies—is enough to cause debilitating symptoms.

Finding these scarce mutations isn't easy. To examine a specific gene or a full genome, researchers normally pool DNA from a large number of cells—typically from blood—and sequence it many times. From these repeated reads, a sequencing machine essentially reaches a consensus about the likely identity of each base along the DNA strand. "We can sequence the genome," Shaw says, "but what exactly does that mean? It means we can sequence the average genome."

To detect deviations from the average, researchers have to increase the numbers of reads. But DNA sequencing, although speedy and ever-cheaper, presents a trade-off between breadth (how much of a genome is read) and depth (how many times each point in the genome is read). In whole-genome sequencing, a depth of 50 to 60 is typical, says Christopher Walsh, a neurologist and geneticist at Boston Children's Hospital. Mutations that pop up in only a handful of reads will be interpreted as part of the machine's inherent error rate, rather than as actual differences in DNA sequence between the cells sampled—i.e., a case of mosaicism. "You can only find them if you deliberately go looking for them," he says.

That's what Walsh and colleagues recently did in the study of people with brain malformations. None of the known genetic causes for these disorders had turned up in standard clinical tests. But "deep sequencing"—sequencing this handful of candidate gene regions to an average depth of 300—allowed the group to identify mutations that would otherwise be dismissed as read errors. Of the eight mutations, five were present in so few cells that they were undetectable using traditional Sanger DNA sequencing, still the gold standard for validating mutations, the group reported in the 21 August issue of *The New England Journal of Medicine*. Walsh says deep sequencing might eventually allow scientists to find genetic mosaicism underlying

milder neurological conditions, including intellectual disability and autism spectrum disorders.

Others looking for mosaicism are pursuing an even more sensitive and targeted approach: polymerase chain reaction (PCR)-based techniques, which can detect mutations that exist in less than 1% of cells. Such tests won't reveal all the variations of a given gene—just the presence of a single mutation for which the test was designed. But one such PCR approach proved revealing in a recent study of healthy parents whose children had genetic diseases.

The parents had originally tested negative for the mutations, which were detected in all the cells of their children. In 4% of cases, however, a small number of parental blood cells had the mutation, which was

likely also present in their eggs or sperm, says Pawel Stankiewicz, a geneticist at Baylor who led the research, reported online in July in *The American Journal of Human Genet-*

"Many, many families every day are misinformed."

Pawel Stankiewicz,
Baylor College of Medicine

ics. But when genetic labs test parents to see if they are genetic carriers of disease, they don't typically sample germline tissue. Testing of sperm is impractical and would require multiple ejaculates for an accurate test, Stankiewicz says, while obtaining eggs involves an invasive biopsy. Given current methods, Stankiewicz contends, "many, many families every day are misinformed" about their risk of passing on a disease to their next child. To counsel families accurately, labs will need more sophisticated tests.

But the highly sensitive approaches like those used by Walsh and the Baylor group aren't common outside of university labs and research hospitals. That's partly because it can be complicated and time-consuming to confirm that a sequence appearing very rarely in a DNA sample is a real, disease-causing mutation, not a sequencing error, Walsh says. And because looking deeply into the genome now requires narrowing your focus to a set of known candidate genes or even specific mutations, researchers are limited to diseases where some of the genetic causes are already understood. Walsh predicts that improvements in DNA testing will eventually allow broader searches for mosaicism. But until then, he says, studies revealing overlooked cases of mosaicism serve as a warning for many clinical diagnostic labs: "They're not realizing they might be missing important stuff." ■

SCIENTIFIC COMMUNITY

Who are the science stars of Twitter?

Science communicators and some highly cited researchers, but few women, make top 50 list

By Jia You

Genomicist Neil Hall sparked an online tempest this summer by proposing a “Kardashian Index,” or K-index—a comparison of a scientist’s number of Twitter followers with their citations. Scientists with a high score on the index, named after the reality TV star Kim Kardashian, one of the most popular celebrities on the social media platform, should “get off Twitter” and write more papers, suggested Hall, who works at the University of Liverpool in the United Kingdom.

Though Hall says he meant his K-index lightheartedly, his article in *Genome Biology* sparked a Twitter storm of criticism. So just who are the Kardashians of science, and is Hall’s criticism justified? Hall tactfully declined to provide a K-index for anyone specific, but *Science* was curious about the names and the numbers. We have compiled a list of the 50 most followed scientists on the social media platform and their academic citation counts—and calculated their K-index.

Rather than identifying “Science Kardashians”—those who are, as Hall put it, “famous for being famous”—the list reveals that a majority of science Twitter stars spend much, if not all, of their time on science communication. For them, Twitter popularity can amplify their efforts in public outreach. A case in point is Neil deGrasse Tyson, director of the Hayden Planetarium in New York City and host of the science TV show *Cosmos: A Spacetime Odyssey*. With more than 2.4 million followers and fewer than 200 citations, the astrophysicist is the top-ranking celebrity scientist on Twitter—and has the highest K-index of anyone on the list. Yet few would consider his Twitter fame unwarranted.

Although the index is named for a woman, *Science*’s survey highlights the poor representation of female scientists on Twitter, which Hall hinted at in his commentary. Of the 50 most followed scientists, only four are women. Astronomer Pamela Gay of Southern Illinois University, Edwardsville, whose more than 17,000 Twitter followers put her 33rd on the list, says that doesn’t surprise her because society still struggles to recognize women as leaders in science. Female scientists are also more likely to face sexist attacks on-

line, she adds. “At some point, you just get fed up with all the ‘why you are ugly’ or ‘why you are hot’ comments.”

Twitter stardom need not exclude research achievements, as our top 50 Twitter list shows. Many have thousands of citations and seven of the people listed also appear on two recent citation-based rankings of influential scientists. (Find an expanded story, the full top 50 list, and details on how we compiled the story’s data at <http://scim.ag/Twitterstars>.) Even so, most high-performing scientists have not embraced Twitter. *Science* sampled Twitter usage among 50 randomly chosen living scientists from Scholarometer’s top 100 authors ranking. Only a fifth of the scientists have an identifiable Twitter profile.

Even some who do dislike the medium.

Chad Mirkin of Northwestern University in Evanston, Illinois, the highest ranking chemist on Scholarometer’s list, considers Twitter a waste of precious time that he’d much prefer spending on reading and writing scientific papers. “A lot of social media is ... time spent aggrandizing one’s accomplishment,” says Mirkin, who registered on Twitter just to keep up with his son’s tennis scores.

So why do the highly cited researchers who are also Twitter science stars make the time to engage in social media? Geneticist Eric Topol of the Scripps Research Institute in San Diego, California (17th place; 44,800 followers), who boasts more than 150,000 citations, says he once thought the social media platform was only for “silly stuff” like celebrity news. Then he tried

Twitter’s scientific celebrities

The top 20 of the 50 most followed scientists on Twitter

SCIENTIST	FOLLOWERS	CITATIONS	K-INDEX	EXPERTISE
Neil deGrasse Tyson	2,400,000	151	11129	Astrophysicist
Brian Cox	1,440,000	33,301	1188	Physicist
Richard Dawkins	1,020,000	49,631	740	Biologist
Ben Goldacre	341,000	1,086	841	Physician
Phil Plait	320,000	254	1256	Astronomer
Michio Kaku	310,000	5,281	461	Theoretical physicist
Sam Harris	224,000	2,416	428	Neuroscientist
Hans Rosling	180,000	1,703	384	Global health scientist
Tim Berners-Lee	179,000	51,204	129	Computer scientist
P. Z. Myers	155,000	1,364	355	Biologist
Steven Pinker	142,000	49,933	103	Cognitive scientist
Richard Wiseman	134,000	4,687	207	Psychologist
Lawrence M. Krauss	99,700	10,155	120	Theoretical physicist
Atul Gawande	96,800	13,763	106	Surgeon/public health scientist
Oliver Sacks	76,300	13,883	83	Neurologist
Dan Ariely	73,000	16,307	76	Psychologist/behavioral economist
Eric Topol	44,800	151,281	23	Geneticist
Brian Greene	38,700	11,133	45	Theoretical physicist
Marcus du Sautoy	34,200	1,461	77	Mathematician
Sean M. Carroll	33,200	14,208	36	Theoretical physicist

Twitter during a TEDMED conference in 2009, as a tool to gauge reactions to his talk. Now, he starts his workday browsing through his Twitter feed for news and noteworthy research in his field. During the day, he checks Twitter several times and spends another 10 to 20 minutes on an evening roundup. “It actually may be the most valuable time [I spend] in terms of learning things that are going on in the world of science and medicine,” says Topol, who reciprocates by daily tweeting papers, presentations, and more to his followers.

Psychologist Daniel Gilbert of Harvard University (36th; 15,500 followers) views Twitter as a natural extension of his public outreach efforts, which include hosting the PBS science documentary, *This Emotional Life*. “It’s another teaching tool,” he says.

Jonathan Eisen of the University of California, Davis (25th; 24,900 followers), says that consistently tweeting ongoing research at his lab has helped attract graduate students as well as two grants for science communication. He suggests an active social media presence might even aid applications for research funding, as it demonstrates a commitment to public outreach. But the spontaneity of Twitter can backfire, too. Eisen, for one, has live-tweeted brusque criticism at academic conferences that came back to bite him. “You can seem like a jerk, an idiot, or both,” he says.

The K-index gets it wrong by suggesting that science communication and research productivity are incompatible, says Albert-László Barabási, a network theorist at Northeastern University in Boston who studies social media. Research on altmetrics—alternative metrics for measuring scientific impact—has found no link between social media metrics such as number of tweets and traditional impact metrics such as citations, he says. “We should really not mix the two ... because they really probe different aspects of a scientist’s personality.”

For his part, Hall says others have read too much into his satire, which originated after seeing conference organizers factor Twitter follower numbers into speaker considerations. “I don’t mean to criticize anyone for having a lot of Twitter followers,” he says. “My criticism is only of using it as a metric on research scientists.”

It might be premature, in any case, for the scientific community to worry about “Science Kardashians” when it faces a more pressing challenge of staying relevant in public discussions. Even Tyson’s Twitter popularity is dwarfed by that of the real Kim Kardashian, who boasts 10 times as many followers. ■



Okairos uses a “wave bag” to shake up cells and produce small lots of vaccine.

INFECTIOUS DISEASE

Ebola vaccine: Little and late

Scaling up production of Ebola vaccines and treatments will take many months

By Jon Cohen

As the Ebola outbreak in West Africa accelerates, the containment measures that worked in the past, such as isolating those who are infected and tracing their contacts, clearly have failed. This has spurred hopes that biomedical countermeasures, such as monoclonal antibodies and vaccines, can help save lives and slow spread. But as President Barack Obama calls for an aggressive ramp up of the U.S. government’s response (see p. 1434), resolve is colliding with a grim reality: The epidemic is outpacing the speed with which drugs and vaccines can be produced.

Administration officials have begun working with industry to speed manufacturing of experimental drugs and vaccines. “We’re trying to do everything we can to scale up product,” says Nicole Lurie, assistant secretary for preparedness and response at the U.S. Department of Health and Human Services (HHS). But the logistical obstacles are huge, and makers are getting a late start.

An Ebola vaccine made by GlaxoSmith-Kline (GSK) in Rixensart, Belgium, is the furthest along, having entered phase I human trials on 2 September. GSK has committed to manufacturing up to 10,000 doses of the vaccine, which consists of an Ebola surface protein stitched into a weakened chimpanzee adenovirus, by the end of the year. If it passes muster in the early studies, it could be given to health workers as soon as November. But hundreds of thousands of doses

would be needed to slow the outbreak. That “would take one-and-a-half years at the scale we’re working at,” says Ripley Ballou, who heads the Ebola vaccine program for GSK.

The scientific hurdles are not particularly high. Companies have made similar vaccines at high volume, and animal studies have shown that Ebola virus is fairly easy to defeat with the proper immune response. “Although Ebola is a very scary, hemorrhagic virus, all you need is fairly modest neutralizing antibody response and you’re protected,” says John Eldridge, chief scientific officer at Profectus BioSciences, a Maryland and New York-based company making an Ebola vaccine that has struggled to attract funding.

Ballou says GSK is considering several options for speeding production. But first the company wants to be sure that there’s a market for the vaccine. He says when the company contacted the World Health Organization at the start of this outbreak in March, no one showed much interest. “The answer was, ‘Thanks, we’ll get back to you.’”

NewLink Genetics of Ames, Iowa, has a second vaccine in a phase I trial that consists of a crippled vesicular stomatitis virus (VSV), which infects livestock, with the gene for the Ebola virus surface protein. Only 1500 doses exist. Profectus makes a similar vaccine that should be ready for human testing next June. Like GSK, Profectus needs a commitment from a funder before it can scale up production from the planned 5000 to 20,000 doses, Eldridge says.

In principle, vaccine production is

straightforward: Grow a “master seed” strain of virus on a large scale, then harvest and process it. Okairos, a small company that produces the GSK vaccine at a facility near Rome, grows its virus in a human cell line in up to 200-liter wave bags, so-called because they rock back and forth on a platform. It will take about 2 months to grow, harvest, and prepare the promised doses.

Company founder Riccardo Cortese says with an investment of about \$10 million, they could convert their facility to process several 400-liter bags simultaneously in as little as 3 months. At that scale, he says, “I calculated we could make 100,000 doses per month.”

GSK’s Ballou is more circumspect. First, they need more master seed. “There were only so many vials made, and we’re depleting them,” he says. And mass production, he says, would work best in huge bioreactors, stainless steel tanks that hold 1000 liters or more. But the cell lines that produce the vaccine virus are finicky. “The driver is really the productivity of the cell line,” Ballou says. He puts the most optimistic timeline for 100,000 to 500,000 doses at 9 months—and the cost at \$25 million.

Treatment is stuck in a similar rut. A cocktail of Ebola antibodies called ZMapp has shown remarkable efficacy in monkey experiments and has been given to seven people. But there’s less than one dose left. Made by Mapp Biopharmaceutical of San Diego, California, ZMapp contains three monoclonal antibodies produced in tobacco plants. The process from plants to product takes a few months and now yields just tens of doses.

HHS’s Biomedical Advanced Research and Development Authority (BARDA), which was established to speed development of treatments and vaccines for emergencies, has contacted two other outfits that can make the antibodies in tobacco plants. But this will boost production to only hundreds of doses per month. BARDA is now exploring the possibility of producing the antibodies in Chinese hamster ovary (CHO) cells—the standard system for making monoclonals. But a 2012 study suggested that the antibodies would have reduced potency if made in CHO cells. “You’re not going to make 100,000 doses anytime in the near future,” says someone familiar with the discussions who did not want to be named.

A bill now moving through the U.S. Congress would give BARDA \$58 million for Ebola vaccines and treatments. That’s sure to help, but not soon enough. “I don’t know that we’re going to have these countermeasures in time to make a big dent now,” says HHS’s Lurie. “But hopefully we’ll have the countermeasures by the next time there’s an outbreak so this never, ever happens again.” ■

SCIENCE POLICY

Play it again, Uncle Sam

Prominent academics recommend growth in federal basic research budget that matches previous golden era

By Jeffrey Mervis

A report out this week urges the U.S. government to lift spending on basic research to historically high levels as a way to ensure prosperity and preserve American preeminence in science and technology.

The recommendations include boosting real spending on basic research by more than 4% annually, making multiyear funding commitments, streamlining regulations on academic research, and creating an optimal biomedical research workforce. The proposals echo those in a half-dozen other reports in the past decade from similarly high-level panels. Coming from the American Academy of Arts & Sciences in Cambridge, Massachusetts, the advice may also seem self-serving. But panel co-chair Neal Lane, former director of the National Science Foundation and presidential science adviser, says the message bears repeating as tight overall federal bud-

ing for basic research that occurred between 1975 and 1992 (see graph). Funding for the biomedical sciences has stalled since the end of a 5-year doubling of the National Institutes of Health’s budget in 2003 except for a spike in 2009 to 2010, for example, and Lane says that boom-and-bust cycle has been devastating to the community. At the same time, a budget doubling for agencies funding basic research in the physical sciences, proposed in the 2005 National Academies’ report and embraced by Congress and the White House, never materialized.

The new report calls for federal spending on basic research, now \$32.3 billion, to reach 0.3% of the nation’s gross domestic product by 2032. Such a leap “is rather ambitious,” Lane admits. That ratio has hovered around its current level of 0.19% for the past 2 decades. But he says it’s needed at a time when “science and engineering have become even more important as drivers of economic growth.”

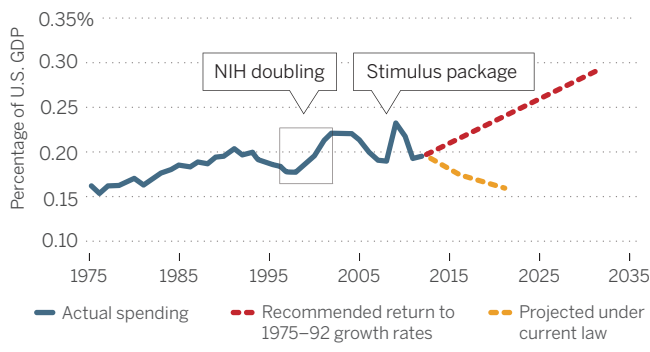
The report also proposes that the White House issue a biennial report on the “state of American science and engineering,” that research universities strengthen ties with industry, and that professional and scientific organizations speak with one voice about how research has created jobs and improved public health and welfare. Even then it’s going to be an uphill battle, says panel member James Duderstadt, an

engineer and president emeritus of the University of Michigan. “Sometimes I feel that we’re plowing the ocean,” says Duderstadt, who also served on a 2012 National Academies’ committee that suggested ways to improve the health of U.S. research universities.

Duderstadt cites last week’s triple announcement by Apple of a new smart watch, iPhone, and banking app as evidence for the need for a continued federal investment in research. “They are doing wonderful things with basic research from 30 years ago,” he remarks. ■

A boost for basic research?

U.S. government spending has been inconsistent and is headed down.



gets have put the squeeze on research.

“The response from policymakers to date has been disappointing,” admits Lane, a physics professor at Rice University in Houston, Texas. (The panel’s co-chair is Norman Augustine, who also led the influential 2005 National Academies’ report, *Rising Above the Gathering Storm*.) “But you can’t give up on the political process. We hope that this report will help start a conversation about the things that really matter.”

The panel’s first suggestion is to return to the annual rate of growth in federal fund-

FEATURES



Evolutionary theory helps Russell Gray explain why crows can make tools like this hooked lever.

No miracles

Biologist Russell Gray uses evolutionary ideas to probe the origin of languages and complex thinking

By Virginia Morell, in Auckland, New Zealand

“I’m a man of enthusiasms,” said Russell Gray, binoculars and fishing pole in hand as he bounded up a trail on Tiritiri Matangi Island, a wildlife sanctuary off the coast of the city, “and birding and fishing are just two of them.” Fair and almost boyishly animated, Gray,

54, set off with long, energetic strides into the forest, where he eagerly pointed out the island’s rarest birds, expounded on its ecological history, and happily described a giant fish he’d once caught, as well as the sauce with which he’d served it. “I’m enthusiastic

about food and wine, too,” he said, beaming.

Then he paused for a moment to point out a pair of pied cormorants, large, long-necked waterbirds at the water’s edge. “The subject of my first evolutionary study,” Gray said, “so I have a fondness for them.”

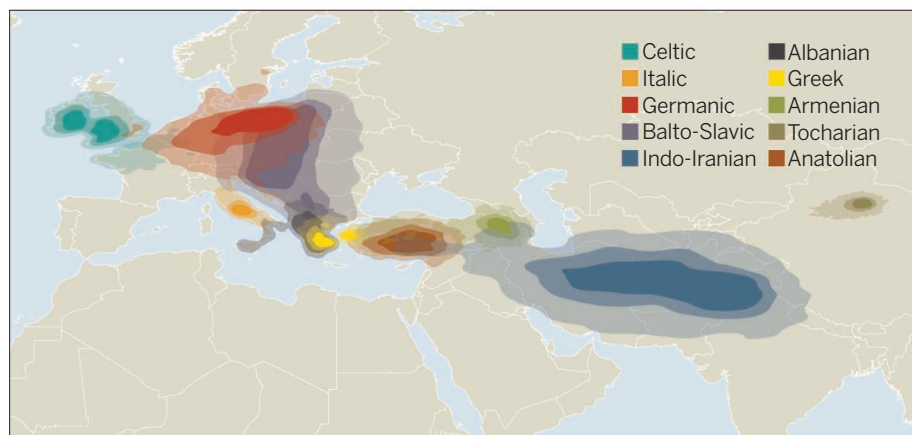
As unlikely as it might seem, the cormorants and other seabirds set Gray on a career path into the evolution of human linguistics and culture. Although the full list of Gray’s enthusiasms would fill this page, suffice it to say that evolutionary biology is at the top. Using its principles, Gray, an evolutionary biologist and comparative psychologist at the University of Auckland, has helped

crack open two areas—animal cognition and historical linguistics—long regarded by many as black boxes, impenetrable to the scientific method.

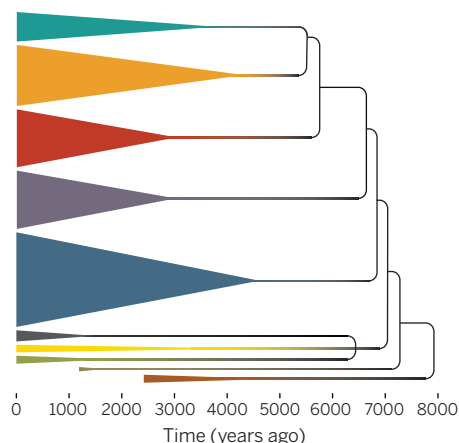
Because languages change at unpredictable rates, analyzing their relationships was for many linguists more of an art than a science. But by applying evolutionary methods borrowed from genetics, Gray and his colleagues are transforming the discipline, shaping it into a science of prehistory. His group has unraveled the histories of the Austronesian and Proto-Indo-European (PIE) languages and peoples, and traced their migrations over vast distances.

The roots of Indo-European languages

Indo-European languages today extend from the British Isles to India, as seen in a map of some of the major subfamilies, but Gray's work roots them all in Anatolia (brown). He dates the origin of Proto-Indo-European to about 7600 years ago (right).



Each triangle represents a group of related languages (see key, left) that blossomed over time from a single tongue.



Although controversial, his papers have so stirred the field that several researchers credit him with being “in the vanguard of a revolution,” as evolutionary biologist Mark Pagel of the University of Reading in the United Kingdom puts it. Gray’s fresh approach prompted Germany’s Max Planck Society to tap him as a co-director of its new Institute for History and the Sciences in Jena.

The Max Planck officials who interviewed Gray weren’t even aware of his other research focus, animal cognition. The question of how complex thinking evolved, like the origin of languages, was thought to be beyond the ken of science.

But this black box, too, yields to evolutionary thinking, insists Gray, who leads the University of Auckland’s highly regarded project on New Caledonian crows, which have astonished biologists and the public with their skill at fashioning simple tools. “There’s a reason these crows make tools and other birds don’t,” he says. “It’s not a miracle.”

THAT SENTENCE, along with the even shorter phrase “no miracles,” is a favorite of Gray’s.

The power of evolution “to explain many things,” as he puts it, struck him when he was still in high school in Tauranga, south of Auckland, after reading Richard Dawkins’s *The Selfish Gene*. Hooked, Gray pursued this enthusiasm, earning his doctorate and landing a position at the University of Otago, Dunedin. “It’s on South Island, where there are fantastic seabirds, and I developed a new interest—seabird behavior.” Using existing descriptions of the social displays of Pelecaniformes (an order

of waterbirds that includes cormorants and pelicans), in 1996 Gray and his colleagues created 12 behavioral family trees and compared them with published morphological and genetic trees. The trees largely matched, showing that birds with similar behaviors, such as open-billed courtship displays, shared a common ancestry.

For Gray, “it didn’t seem a huge leap to think of human languages in the same way. ... Words are inscribed, in their shape and form, with a powerful record of the past.” Growing up in New Zealand, he had heard Polynesian languages, including Maori, Ton-

lished a *Nature* article using language trees to test competing hypotheses about the settlement of the Pacific by people speaking ancestral Austronesian: a rapid “express train” of peoples who spread from Taiwan across the widely scattered islands in a few thousand years, or an “entangled bank” of Austronesian and other speakers who mixed more slowly over a longer period. Using 77 Austronesian languages and 5185 words and phrases, they found that Taiwanese languages were the oldest, and that their spread matched that of express train settlement, with Indonesian and coastal New

Guinean languages hiving off before those in New Zealand and Hawaii.

“I was just delighted when that paper came out,” says Pagel, who also studies the evolution of language. “He showed that you can test questions of human history with linguistic data.”

The team extended their Austronesian analysis with more sophisticated statistics in 2009, finding that these languages diverged and changed in fits and starts that could be timed and tied to specific inventions, such as the outrigger canoe and sail (*Science*, 23 January 2009, p. 479). “It’s kind of wild to think you can get these types of results from comparing language lists,” says Andrew Garrett, a historical linguist at the University of California, Berkeley.

But when Gray and his colleagues applied their techniques to an even bigger question—the origin of the Indo-European language family, which includes English, Spanish, Russian, Hindi, and Urdu among many others—Garrett and other linguists recoiled at the results.

“It’s hard to overstate just how contentious my linguistic work is. People ... have left the room when we present our results.”

Russell Gray, University of Auckland

gan, and Samoan, and just by listening he could tell that they must be closely related. As with the seabirds’ behaviors, he suspected that the Pacific Island languages had come about through “some kind of descent with modification.” His key insight: “Words are just like genes,” in that they resemble each other because of shared ancestry.

For example, “mother” in English and *mutter* in German sound alike because they derive from the same PIE word, *mehter*. Linguists call such words cognates, and they’ve long used them to construct trees showing the relationships among languages.

Gray took the analysis further: He realized that the sophisticated software designed to trace genetic lineages could be applied to languages. In 2000, he and a colleague pub-

As with the settlement of the Pacific, there were two competing hypotheses: that PIE originated among Anatolian farmers about 8000 to 9000 years ago and expanded with their settlements, or that it arose only about 6000 years ago on the steppes north of the Black and Caspian seas, and spread via the horses and wheeled carts of semi-nomadic herders and warriors. Most linguists favored the latter hypothesis.

In successive papers, Gray's group used evolutionary software to analyze the vocabularies of 103 ancient and modern European and Asian languages. They produced phylogenetic trees and ended up with a date for the origin of PIE at 7600 years ago. Then they applied a method from another branch of biology, using a software program developed to determine the geographic origin of viral pathogens (*Science*, 24 August 2012, p. 957). The resulting trees and maps showed Anatolia as the homeland and supported the earlier date.

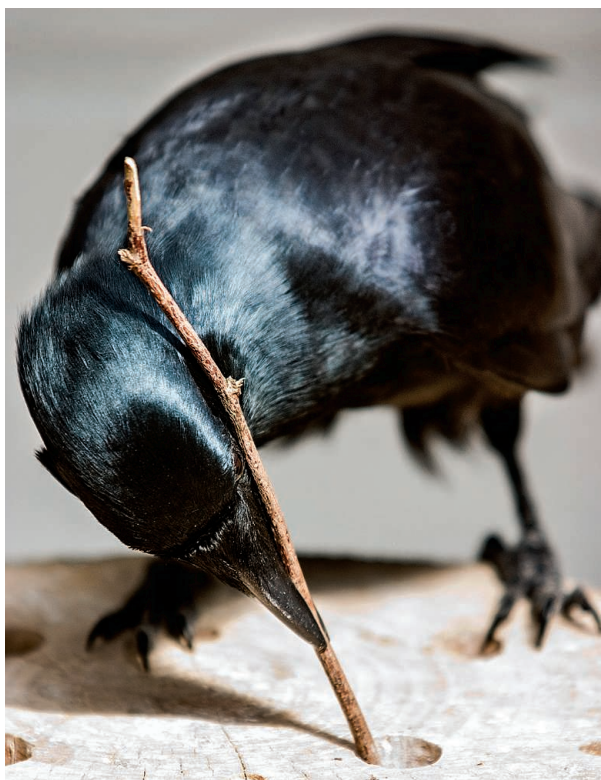
Many linguists disliked both the results and the methods. "It's hard to overstate just how contentious my linguistic work is," Gray says. "People at linguistic conferences have left the room when we present our results."

Linguists don't object to Gray's language family tree, which basically matches the one devised using traditional methods. But they object to his place and date of origin for PIE. His conclusions are at odds with "the best archaeological scenario for the origin and diversification of the Indo-European language family," says Don Ringe, a historical linguist at the University of Pennsylvania. That scenario is based in part on cognates for the word "wheel," which dot numerous branches of the Indo-European language tree, from Sanskrit to Greek to Germanic. Linguists say these cognates all stem from a PIE word reconstructed as *k'ek'los*, which must have first been spoken about 5500 years ago—the archaeological date of the first wheels. And those early wheels and axles are associated with steppe peoples, not Anatolian farmers.

Critics like Ringe also cringed at Gray's quantitative method, because it uses the varying rates at which words change. Such rates are a staple of glottochronology, a now-discredited technique once used to date the timing and spread of languages. "The problem with glottochronology was that it assumed words change at a steady rate," Gray says. Biologists made the same error when constructing their first molecu-

lar clocks, he notes. But they found a way around the problem by creating a "relaxed" clock—software programs that allow the rates of change to vary and statistically compare numerous trees.

Despite the criticisms, Garrett says that he and many other historical linguists now recognize the power of Gray's biological approach and are employing it themselves. He and his colleagues will soon publish their own phylogenetic analysis of PIE, which incorporates what he considers more accurate cognates and models the relationships among languages differently. Their findings support the steppe hypothesis.



A New Caledonian crow pries out dinner with a tool of his own manufacture.

Still, Garrett adds, "the fact that we're doing these phylogenetic analyses of language at all is entirely due to [Gray]. In 10 to 20 years, every historical linguist will be doing research this way because the tools are so powerful—and Gray has been so creative in their use."

AT THE MAX PLANCK, Gray won't be exploring only linguistics. His mandate extends to culture, and he's already enthusing over the prospect of applying phylogenetic methods to questions such as what societal factors lead to belief in an omnipresent deity or how large states develop from small tribal bands. "Cultural anthropologists think there aren't any rules for how political structures or religions come about,"

Gray says. "But to me, these are empirical questions. We don't have to just wave our arms. There are no miracles."

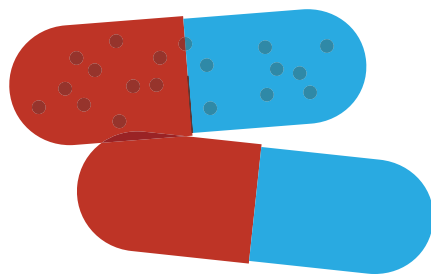
Gray intends to keep his New Caledonian crow project going, too. In his view, the field of animal cognition suffers from the same deficiency as linguistics once did: "Its theories haven't been sufficiently shaped by evolutionary thinking." Researchers often discover one or two species doing something clever and immediately "compare them to humans. That's not thinking like an evolutionist," he says. Instead, Gray and his team seek intermediate steps, devising tests for the crows that reveal mental limitations as well as talents.

In a hotel lobby, he produced a palmlike pandanus leaf that a crow had carefully crafted into a stepped rake; the birds use these to forage for grubs and insects. "When you see a bird make a tool like this," he explained, "you immediately think they have some understanding of the physical properties of things and cause and effect." But that's an assumption that may not be warranted.

For instance, Gray's group has probed the crows' understanding of a string-pulling test that's been cited as evidence that the birds think about a problem the way a human might. In the test, a crow sees a piece of meat dangling from the end of a string that's attached to a branch. She flies to the perch, reels in some of the string with her beak, and steps on it to keep it anchored while she pulls in more, until she gets the treat. She gives the impression that she understands that stepping on the string helps keep it short. Yet when Gray's group challenged her with variations of the string-pulling

test (none of which would have stumped a human), she failed them all. "So, no, she isn't thinking about the problem exactly like we would," Gray says. "And we shouldn't expect that. That's not the way evolution works."

Back on Tiritiri Matangi Island, Gray's passion for birding beat out that for fishing, as rarely seen species flew into view. As he boarded the ferry back to Auckland, talk turned to the sea north of New Zealand. There lie the islands of Vanuatu, site of another enthusiasm Gray plans to explore at the Max Planck. "It has the greatest density of languages; it's like the Galápagos of language evolution, and we're going to find out why," he says. "I can guarantee you, it's not a miracle." ■



Outsmarting the placebo effect

Can a genetic test to predict a person's level of placebo response help new drugs win approval?

By Kelly Servick

Kathryn Hall's early career was built on pharmaceuticals, not placebos. A molecular biologist, she had spent 2 years identifying drug targets at Millennium Pharmaceuticals, when in 2005, she found herself in an acupuncturist's office seeking any possible relief from persistent carpal tunnel syndrome in her wrists and hands. "I remember sitting there thinking, I can't believe I'm doing this. This is so ridiculous," she says.

As the first needle slid in, Hall felt a "god-awful" shooting sensation, and she hasn't had carpal tunnel problems since. The needles might have had a physiological effect, Hall acknowledges. Yet she also became fascinated by the possibility that her relief was partially due to the placebo effect: genuine benefit that patients derive from the mere expectation of treatment.

Years later, Hall is a researcher in the Program in Placebo Studies and the Therapeutic Encounter at Harvard Medical School's Beth Israel Deaconess Medical Center (BIDMC) in Boston. And her first published work on the subject, describing a gene that in a small study correlated with a person's degree of placebo response, has become the basis of an unusual company called Bio-

metheus. The firm's CEO, Gunther Winkler, hopes to offer drug developers a tool that predicts and controls for the placebo effect in clinical trials, where a strong response to a sugar pill can muddy the data and derail the approval process.

Drug developers have longed for a way to control for the placebo effect. So the prospect of a predictive test is "certainly intriguing and worth investigating," says Amir Kalali, a neuroscientist and drug development expert at the large contract research organization Quintiles in San Diego, California. Kalali was among those attending a session at the Biotechnology Industry Organization's annual meeting in June in San Diego, where Winkler and Hall presented the new company's premise. Yet some veterans of research on the mysterious, complex placebo effect are cautious. "It feels a little premature at this point in time to think that one single gene will give us predictive value in clinical trials," says Jon-Kar Zubieta, a psychiatrist and neuroscientist at the University of Michigan, Ann Arbor.

THE PLACEBO EFFECT can be a gift to people suffering from a variety of conditions, but it's a bedeviling problem for drug companies. "Drug discovery is not about whether a

drug works," says Ted Kaptchuk, who directs the Harvard placebo lab. "It's about whether it works better than a placebo control." For conditions where placebo groups tend to show huge improvements in clinical trials—especially pain, depression, and other psychiatric disorders—outperforming a fake treatment is a real hurdle. One study found that only 53% of acute depression trials submitted to the U.S. Food and Drug Administration (FDA) between 1983 and 2008 showed the antidepressant under review beating the placebo.

Winkler wrestled with unpredictable placebo responses during his 23 years developing drugs and running clinical trials for Biogen Idec. While preparing to test a treatment for psoriasis, a poorly understood condition that often causes skin irritation and joint pain, he researched previous trials of potential treatments and found placebo response rates from 5% to 30%. "Where do you start?" he wondered. "Assume 5%, run a small trial, and fail? Or start with the assumption of 30%, and have a very big trial which costs a lot of money and may preclude the drug developer from running other studies?"

Some people may show a placebo response simply because they signed up for a trial at a low point in their illness and then

naturally get better. But some fraction may experience a genuine placebo effect—which appears to be stronger for some individuals than others. Personality tests have linked a strong response to traits such as “agreeableness,” “ego-resilience” and “novelty-seeking,” but these associations have been hard to generalize across disease groups or studies. So have reported links between responsiveness to placebo and distinctive features seen in brain imaging.

Hall joined the Harvard lab hoping to identify genetic factors in the placebo response. She was quickly enticed by a gene that encodes the enzyme catechol-O-methyltransferase (COMT), which breaks down catecholamines—a family of compounds that includes the neurotransmitters dopamine and epinephrine. In pain studies done by other labs, people who inherited two copies of the “val” form of the gene reported feeling less pain than those with two of the “met” form. If the met-met types sense pain more acutely, Hall thought they might also be more sensitive to the pain relief of the placebo effect.

Hall’s logic was straightforward: Placebo response has been linked to release of dopamine, a major player in the brain’s reward system, in the prefrontal cortex, and the met form of *COMT* is three to four times slower to break down dopamine than the val form of the enzyme is. As a result, Hall suspected, the met form allows dopamine to linger, creating a more intense feeling of pain relief after treatment—whether it’s drug or placebo.

In the first group of patients she observed, Hall found a tidy correlation: People with irritable bowel syndrome (IBS) seemed to fall along genetic lines in their response to a sham acupuncture treatment (where needles only appeared to penetrate the skin). Overall, the sham acupuncture recipients reported significant improvements compared with those who got no treatment. But met-met types had especially large gains—on average, 50 points higher than val-vals on a 500-point scale of severity. Patients with one of each allele fell in between, Hall reported in *PLOS ONE* in 2012.

Hall is quick to point out that *COMT* influences many neural pathways, so its relationship to placebo is probably much more complicated than dopamine breakdown. “What it’s actually doing to which molecules when, that’s the mystery,” she says.

Because Hall’s IBS study relied on self-reported pain, other researchers caution that it can’t distinguish between a real improvement in symptoms and so-called response bias—a patient’s tendency to report improvements on a questionnaire. In other words, met-met subjects might just be more likely to say they feel better.

Nor is Hall’s gene likely to be the full story. In 2008, psychologist Tomas Furmark of Uppsala University in Sweden and colleagues reported that a variant of a gene involved in regulating serotonin production predicted which people with social anxiety disorder symptoms responded most strongly to a sugar pill. Yet for this disorder, Furmark has found no link between *COMT* and placebo response. In 2013, a group led by Zubieta found a gene associated with placebo-induced relief from a painful stimulus among healthy controls, but he doesn’t expect the same mechanisms to be at play in lots of other settings.

Helen Mayberg, a neurologist who studies depression at Emory University in Atlanta, sees Hall’s *COMT* research as a small piece of what should be a much larger search for biomarkers to gauge both placebo and drug responses. “We are working a la carte,” she says, “and this is going to need to be a buffet.”

Yet Hall’s finding caught the eye of Winkler, a member of BIDMC’s board of trustees. If companies could identify and exclude strong placebo responders (met-met types make up roughly 25% of the population, he says), they could create smaller, more statistically powerful trials. Winkler and BIDMC filed for a patent on the concept of screening and excluding potential trial participants based on the *COMT* gene, and another on using the gene to predict placebo responders in the clinic. Hall now serves as an unpaid scientific adviser to Biometheus and would get some modest royalties if it successfully markets a test.

Winkler says several companies researching pain, central nervous system diseases, and autoimmune conditions have expressed interest in the test. He hopes to use large genetic data sets from their completed trials to look for links between *COMT* variants and placebo response in other diseases.

There’s reason to think that FDA would support companies using *COMT* to screen trial participants, Winkler says. The agency already endorses a variety of so-called enrichment strategies, which aim to reduce response in the placebo group through crafty

trial design. The most common strategy is to give all potential participants a placebo, and then “drop them out if they get too much better,” says Robert Temple, deputy center director for clinical science at FDA’s Center for Drug Evaluation and Research in Silver Spring, Maryland. But better ways to minimize response in placebo groups “are on everybody’s mind.”

Yet using the *COMT* gene to select trial participants could raise new complications. For one, it relies on the assumption that strong placebo responders will not also be exceptional drug responders, says Luana Colloca, a clinical neuroscientist with the National Institutes of Health’s National Center for Complementary and Alternative Medicine in Bethesda, Maryland. If placebo response and drug response share common mechanisms, eliminating people most susceptible to placebos may also obscure the actual effects of the drug.

Kevin Weinfurt, a psychologist at the Duke University Clinical Research Institute’s program for empirical bioethics in Durham, North Carolina, raises another problem: “You’ve created a population that doesn’t look like the population that’s going to receive the drug,” he says, “so you’re getting the wrong picture of how it will actually work out in practice.”

The question is at the front of Winkler’s mind, he says, and it came up in a recent meeting with FDA. He says regulators were enthusiastic about using such an approach in early-phase clinical testing, to cut down the cost of finding out if a drug is worth pursuing further. Kalali of Quintiles says that makes the most sense to him, too. “What you really want is the least amount of people possible at the early stages to get the scientific answer that you need.”

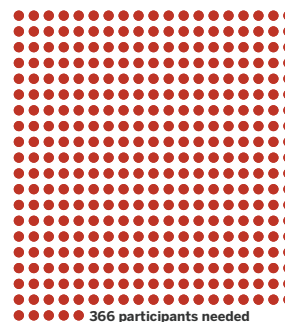
Meanwhile, Hall has turned to research on how variants of *COMT* influence cardiovascular health and

disease. She still “enjoys” acupuncture, but although she’s curious which version of the *COMT* gene she has, she decided not to find out. In future studies of the placebo response, Hall says, not knowing her own type may help her stay objective. ■

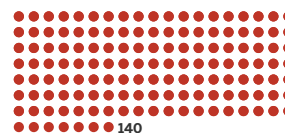
How to shrink a drug trial

Fewer subjects (●) are needed to see a drug effect when fewer people improve on placebo. Assuming **59%** improve on actual drug:

Placebo response rate: **44%**

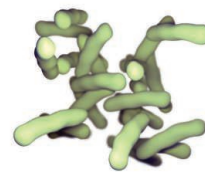


Placebo response rate: **34%**



Placebo response rate: **24%**





PERSPECTIVES



A Tokay gecko
(*Gekko gecko*).

Adhesive strength. The remarkably adhesive feet of geckos are paved with microscopic setae, which enable them to run upside down on surfaces including glass.

MATERIALS SCIENCE

Biomimetics and evolution

Linking biomechanical and evolutionary analysis helps to create viable biomimetic products

By S. N. Patek

Biomimetics is based on the idea that living systems offer novel solutions to engineering challenges, as exemplified by the legendary story of how a stubbornly attached burr led to the development of Velcro. Yet, despite dramatic increases in biomimetic research, marketable products remain rare. Evolutionary analysis can be essential for pinpointing biological features that can be emulated in engineered structures. A recently published approach that links biomechanics and evolutionary analysis (1) points to a powerful

strategy for biomimetics, as illustrated by the development of gecko-inspired adhesives (2, 3).

Geckos are renowned for their ability to run upside down on ceilings and stick to glass. Biomimetic studies have mostly focused on the fine hairs (setae) covering gecko feet. The setae enable close surface contact, thus allowing adhesion to a wide range of surfaces without sticky secretions (4). However, a problem emerged for large-scale fabrication: Biomimetic setae do not scale up to allow large forces (5, 6). Evolutionary analyses of setal scaling presaged these fabrication challenges by revealing

that setal adhesion does not fully explain the relationships between setal pad area, setal contact splitting, and animal body mass; the latter should correlate with adhesive properties, given that the feet must support body weight (7, 8).

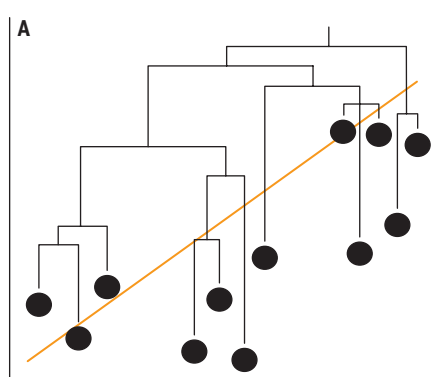
A team of researchers realized the import of these evolutionary studies, turned away from the prevailing focus on setae, and connected key insights from evolutionary analyses of adhesive scaling to the physics of adhesion (2, 3). They proposed and tested a scaling equation that relates the maximum adhesive force to the strength of the intermolecular bonds of the interface, the surface

area of adhesion, and the compliance, or inverse of stiffness, of the system (3, 9). The work led to the invention of Geckskin (developed at the University of Massachusetts Amherst), which combines fabric and soft materials, such as polydimethylsiloxane (PDMS), that can make contact at micrometer and submicrometer scales without the need for extremely fine fibers.

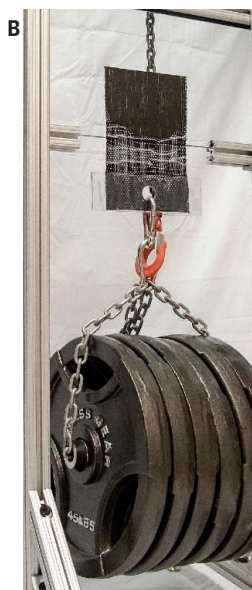
Geckskin's properties are ideal for effective, scalable adhesion, providing a balance of draping and stiffness, abrupt detachment at a critical force (unstable failure), and minimal use of energy through optimal elastic energy storage. These features differ from those of traditional fiber-reinforced adhesives, which typically use the viscoelasticity of the material for elastic energy transitions and require continuing force to achieve detachment (stable failure) (2, 3). Geckskin materials can generate substantial adhesive forces, be scaled to large areas (see the figure), and be applied over a wide range of surface types (2, 3, 10). In addition to circumventing the setal-scaling issues, Geckskin products are dry (not sticky), cheap, reusable, scalable, and cleanable, fulfilling many of the goals of setal biomimetics.

The invention of Geckskin relied on key insights from previous evolutionary analyses of adhesive scaling combined with the physics of adhesion. Comparative studies are increasingly common in biomimetics (11, 12). However, statistical analyses that do not include evolutionary analysis can result in spurious correlations (13). For example, a comparative study of biological adhesion incorrectly reported a correlation between setal pad area and body mass (14). Statistical and methodological incorporation of evolutionary relationships and multispecies data sets is essential for correctly estimating correlations among traits, identifying the origins of traits, and revealing the pathways and timing of these relationships (13). Indeed, Geckskin scaling analyses also need to statistically incorporate phylogenetic relationships (10).

Taylor and Thomas (1) chart a path for a robust evolutionary approach to biomechanics that can also be applied to the growing need for quantitative evolutionary analysis in biomimetics. The first, and perhaps most difficult, step is to define equation-based relationships among the variables of interest,



Learning from evolution. Many biomimetic studies have aimed to mimic setal adhesion. The developers of Geckskin instead used evidence from evolutionary analyses [see schematic in panel (A)] together with a guiding equation of adhesive scaling to create an effective, dry adhesive. The material adheres so strongly that a 100 cm² pad can hold 300 kg (B).



focusing on the desired engineering output (such as increasing adhesive force with size of adhesive). The explanatory power of this relationship is then tested by incorporating data from multiple species. If the focal biological variables do not fully explain the relationship (for example, setal contact splitting does not adequately explain adhesive force), the deviation from the guiding equation (outside of measurement error) is considered equation error. Equation error typically indicates an incorrect or incomplete equation.

Rather than viewing equation error as problematic, Taylor and Thomas use it to refine the hypothesized variables. For example, in gecko setal scaling studies, equation error indicated that compliance and compliant structures needed to be incorporated into the scaling equation. Equation error points to alternative mechanical hypotheses by indicating the presence of important but unmeasured variables. It also reveals the presence of shared variation in these variables among closely related species. These evolutionary relationships among species can be used to correct for the nonindependence of the data. By pinpointing shared variation in unmeasured variables that may also play distinct roles in a subset of species, evolutionary analysis is an important step toward finding good model species for biomimetics.

Taylor and Thomas' framework includes another key insight into the information provided by evolutionary analysis of mechanical systems. Biological systems typically navigate a range of objectives depending on their environment. When there are trade offs among traits in different environments, there can be multiple equally effective solutions to these trade offs. Cross-

species studies provide a database of these equally effective solutions (called Pareto sets) that can be used for biomimetics. Variation in biological trait combinations across a range of conditions can inform biomimetic systems for use in specific environments or for particular functions.

This approach to biomimetics demands a physical understanding of a system through equation-based analysis and a direct connection to biological features through both mechanical and evolutionary analysis. It can even incorporate environmental variation that maps to equally viable combinations of traits. For setal biomimetics, this approach could have more

quickly focused efforts on solving the adhesive scaling conundrum. Further insight may come from studies showing that across gecko evolutionary history, setae originated and were lost repeatedly (15), suggesting varying utility of setal attachments under particular conditions. These data provide a rich evolutionary data set for defining suites of traits that are equally effective across a range of environmental settings or conditions. In this and other areas of biomimetics, recognizing the limitations of single-species studies and embracing the data set of evolutionary history will be key for achieving cost-effective and efficient translation from biology to marketable products. ■

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ACKNOWLEDGMENTS

I thank A. J. Crosby and D. Irschick and their research groups at the University of Massachusetts Amherst for providing images and members of the Patek Laboratory for their constructive feedback.

APPLIED PHYSICS

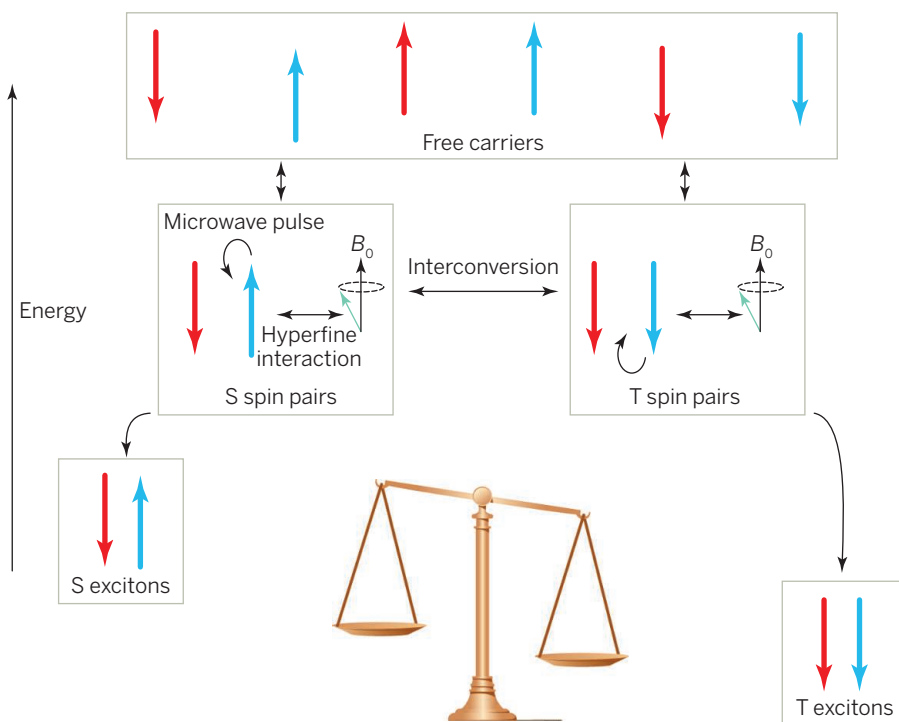
Manipulating spin in organic spintronics

Probing the interplay between the electronic and nuclear spins in organic semiconductors

By Peter A. Bobbert

The growing interest in spin manipulation in the field of spin electronics, or “spintronics,” is due to the wealth of exciting possibilities that it offers in areas of magnetic sensing, new types of information storage, low-power electronics, and quantum information processing. Nuclear spin manipulation is especially attractive, as nuclear spin lifetimes can reach minutes or more. Addressing nuclear spins in a direct way is, however, difficult. Fortunately, indirect addressing is possible via their hyperfine interaction with electronic spins, which can be conveniently probed electronically or optically. To date, research efforts have focused mainly on conventional semiconductors such as silicon, germanium, diamond, and III-V compound semiconductors. On page 1487 of this issue, Malissa *et al.* (1) demonstrate the combined manipulation of the electronic and nuclear spins in an organic semiconductor.

The use of organic materials for organic spintronics opens many new roads, mainly because of the sheer number of varieties in which they can be synthesized. It even appears that nature utilizes organic spintronics in a quantum-biological compass that allows organisms to probe Earth’s magnetic field (2). A clear indication of a spin-related compass is the disruption of the orientation capability of migratory birds by electromagnetic noise (3). The proposed functioning mechanism of this biological compass and the detection mechanism in the spin manipulation of Malissa *et al.* are closely related. The common key ingredient is pairs of electronic spins undergoing spin-selective reactions to a spin-0 (singlet, S) or a spin-1 (triplet, T) state (see the figure). In the compass of the bird, these spin pairs are believed to be created by optical excitation of a donor-acceptor protein complex in the retina, where a negatively charged electron is transferred from the donor to the acceptor, leaving a positively charged “hole” on the donor. Malissa *et al.* conduct their experiments on organic light-emitting diodes (OLEDs) with a conjugated polymer as the active layer, where spin pairs are created by



Balance between singlet and triplet spin pairs. A key ingredient in the work of Malissa *et al.* is the presence of singlet (S) and triplet (T) spin pairs of an electron and a hole (blue and red arrows) that have almost no mutual spin interaction but can form S and T excitons (electron-hole bound states) with different rates. The balance between the amounts of S and T spin pairs is then a measure of their rate of interconversion, which can occur by rotation of one of the spins driven by a microwave pulse or by hyperfine interaction with a proton spin (green arrow). Precession of the proton spin modulates the interconversion and therefore the balance, which is observed in a modulation of the current.

encounters of electrons and holes injected into the polymer by opposite electrodes.

A key aspect of the process is a decoupling of the evolution of the spins in the pair. Small external magnetic couplings of the spins to a static or time-varying magnetic field and to surrounding nuclear magnetic moments via the hyperfine coupling are then able to change the interconversion rate between the S and T spin-pair states. Because of the different reaction rates to form an S or T state with strongly coupled spins (excitons, or strongly bound electron-hole pairs, in the OLEDs of Malissa *et al.*), the balance between the amounts of S and T spin pairs is a measure for the $S \leftrightarrow T$ interconversion rate in the spin pair and therefore of external magnetic couplings of the spins. In the bird’s compass, the $S \leftrightarrow T$ spin-pair balance is assumed to be “measured” by chemical detection of a signaling reaction product (2). In the OLED of Malissa *et al.*, a change in the $S \leftrightarrow T$ balance changes the current, allowing the convenient electrical detection of

external magnetic couplings. It has become clear that the initially very puzzling effect of organic magnetoresistance in OLEDs and other organic devices (4) is based on a very similar mechanism (5).

By a clever combination of microwave pulses in the presence of a static magnetic field B_0 (creating an easily detectable feature called a “Hahn echo” in the current, in an electron-spin resonance setup), Malissa *et al.* deliberately manipulate the electron spin by rotating it over prescribed angles and detect the resulting disturbed $S \leftrightarrow T$ spin-pair balance by monitoring the current as a function of a delay time between two of the pulses. The fascinating aspect of this experiment is that a modulation in the current is observed that corresponds precisely with the nuclear spin precession frequency of a proton in the field B_0 : It appears that the rotation of electron spins has brought proton spins into precessional motion around B_0 via the hyperfine interaction, “kicking back” with their precession

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frequency at the electron spins via the same interaction. Performing the experiment with a deuterated instead of hydrogenated polymer changes the modulation frequency to the deuteron precession frequency (a factor of 6.6 times smaller), which is a nice confirmation of this picture.

In a second experiment, Malissa *et al.* manipulate both the electron and nuclear spins (in an electron-nuclear double resonance setup) by using, in addition to the microwave pulses, a radiofrequency pulse to rotate the proton spins. Because the hyperfine couplings with surrounding proton spins create a small random magnetic field, the field B_0 at which resonance with the microwave field occurs is slightly different for each electron spin. A specific field B_0 can therefore select a subensemble of electron spins coupling predominantly to proton spins that are directed, say, upward. After a radiofrequency pulse that swaps the proton spins by 180° downward, the subensemble of these electron spins is no longer in resonance with the microwave field, and therefore these spins become insensitive to subsequent microwave pulses. The swapping of the proton spins can then be observed via the current. This provides an intriguing new approach to detect nuclear spin manipulation electrically.

The study of Malissa *et al.* is an important step toward the use of organic materials in quantum-coherent spin manipulation. A great advantage as compared to other spin manipulation approaches is the operation at room temperature. Nature may have paved the way here for us in the development of a quantum-biological compass, obviously operating at ambient temperature. However, the disadvantages at present are the lack of control over the involved electronic processes, which are mostly due to the morphological disorder of the used conjugated polymer. Coherence times are presently limited by the uncontrolled lifetime of the spin pairs. Increasing and controlling morphological order by embedding a semiconducting organic molecule in the pores of a zeolite crystal have led to a spectacular increase in organic magnetoresistance and the option to address only a few molecules (6). This may also be the way to bring spin manipulation in organic materials to a next level of control. ■

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The modern Periodic Table. Similar chemical behavior might be expected for the homologs (Mo, W, Sg), (Hg, Cn) and (Pb, Fl), but relativistic effects can cause deviations from the expected behavior.

CHEMISTRY

Superheavy carbonyls

The radioactive superheavy element seaborgium can form a carbonyl compound during its short lifetime

By **Walter Loveland**

Elements with atomic numbers $Z > 94$ are radioactive and cannot be found naturally in Earth. Efforts to create these elements, particularly the superheavy elements ($Z \geq 104$), in the laboratory typically use “hot fusion” reactions involving extremely high temperatures. On page 1491 of this issue, Even *et al.* (1) show how these atoms can be cooled down and used to synthesize a new class of chemical compounds, the superheavy metal carbonyls. The results confirm a 15-year-old prediction of relativistic quantum chemistry.

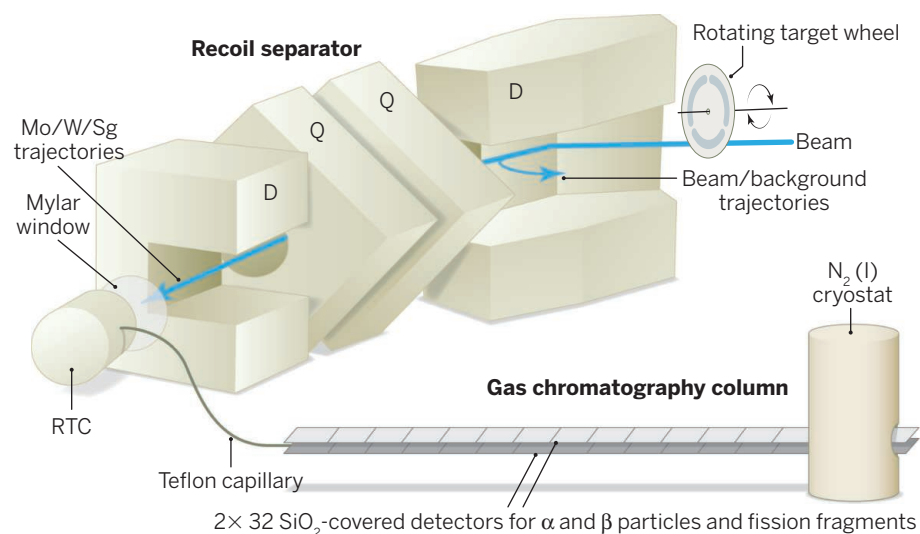
The known superheavy elements, referred to as the transactinides, range in atomic number from 104 (Rf) to the as yet unnamed element 118 (see the first figure). The lifetimes of many of these elements range from milliseconds to minutes. In the case of $Z = 112$ (Cn) to $Z = 118$, current methods only allow the production of a few atoms per week. Chemistry must be done on a “one atom at a time” basis, making these studies very difficult.

Relativistic effects shape the chemistry of these elements. The speeds of the innermost electrons in these atoms approach the speed of light, requiring the use of relativistic quantum mechanics to describe the atoms and molecules. The relativistic effects consist of a contraction and stabilization of the innermost s and p orbitals, the splitting

of electron energy levels due to spin-orbit coupling, and an expansion (and destabilization) of the outer d orbital and all f orbitals. In the transactinides, stabilization of the 7s orbital of Cn doubles its binding energy. In Sg, the 7s and 6d orbital ordering is inverted, possibly changing its oxidation properties. The spin-orbit splitting, like the other relativistic properties, increases roughly as Z^2 , approaching the energies of chemical bonds in magnitude.

The periodic table of the elements is not a list of the elements ordered by increasing atomic number, but rather a grouping of the elements by chemical properties. It is thus a living document. The placement of new elements such as the transactinides in the table depends on measurements of their chemical properties. Experimental studies of the chemistry of the transactinides (2) support the placement of the elements Rf (104), Db (105), Sg (106), Bh (107), Hs (108), and Cn (112) into groups 4, 5, 6, 7, 8, and 12 of the periodic table, respectively, forming the fourth row of transition metals; however, the real possibility of deviations exists. For example, element 112 (Cn) is nominally a homolog of Hg; element 114 (Fl) is a homolog of Pb; thermochromatography experiments have shown that Cn behaves like Hg (3); but it has been difficult to determine whether Fl is a noble gas or a noble

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Making Sg carbonyl. In Even *et al.*'s experiment, hot Sg atoms are produced in a nuclear reaction, transported through the GARIS magnetic separator, and slowed down in the recoil transfer chamber (RTC), where they react with CO. The resulting Sg(CO)₆ products are transported to the thermochromatography apparatus for analysis.

metal (4, 5). Gas-phase chemical studies have been important tools for studying the chemical behavior of short-lived elements. The use of physical recoil separators that isolate the nuclear reaction products has allowed the synthesis of compounds, such as the metal carbonyls. The metal carbonyls of the group 6 elements—Cr(CO)₆, Mo(CO)₆, and W(CO)₆—are well known. U(CO)₆ is unstable above 30 K, in accord with predictions of relativistic quantum chemistry (6). However, Sg(CO)₆ is predicted (6) to be stable because of relativistic effects that lead to stronger molecular bonding.

Actinide carbonyls have been difficult both to synthesize and characterize. Laser ablation experiments produce U(CO)₆ and Th(CO)₆ only as transient species. The synthesis of transactinide carbonyls looked to be forbidding because of the harsh synthesis conditions required to create these elements. Even *et al.* use a novel separation of the recoiling reaction products to overcome these problems. Having synthesized a Sg carbonyl complex, they used thermochromatography to characterize it.

The authors carried out the experiment at the GARIS recoil separator at the RIKEN accelerator facility in Japan. They first generated Mo, W, and Sg nuclei in nuclear reactions at the target portion of the separator (see the second figure). The recoiling nuclei were then separated from the incident projectile beam by the magnetic elements of the GARIS separator and entered a recoil transfer chamber. In that chamber, the Mo, W, and Sg nuclei were thermalized in a He/CO mixture and reacted with the CO molecules to form carbonyls. These compounds were transported to the gas chromatography

column, where a temperature gradient from 25°C to −120°C was maintained. The column consisted of 32 pairs of Si semiconductor detectors covered with SiO₂ that recorded the decay of the radioactive nuclei.

Mo and W formed volatile carbonyls that adsorbed on the column in temperature regions characteristic of their known enthalpies of adsorption. A volatile Sg species adsorbed on the column at a similar position. Monte Carlo simulations of the adsorption process allowed the scientists to deduce an adsorption enthalpy of 50 ± 4 kJ/mol for the Sg species, in good agreement with the theoretical predictions (7) and similar to the measured value for W(CO)₆.

Even *et al.* conclude that they have formed a volatile Sg carbonyl complex, probably Sg(CO)₆. This represents the first synthesis of a new class of superheavy compounds—a finding that could lead to the synthesis of carbonyl complexes of elements 104 to 109. The implications of this development are widespread. For example, the chemistry of element 109, Mt, has not been studied. Using carbonyl complexes, it should now be possible to study Mt chemistry. Thermal dissociation experiments with the carbonyls should enable studies of the strengths of metal-carbon bonds in all the superheavy elements. ■

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10.1126/science.1259349

STRUCTURAL BIOLOGY

Cascading into focus

Structures of a multisubunit protein-RNA complex reveal how the CRISPR system recognizes DNA targets

By Yan Zhang and Erik J. Sontheimer

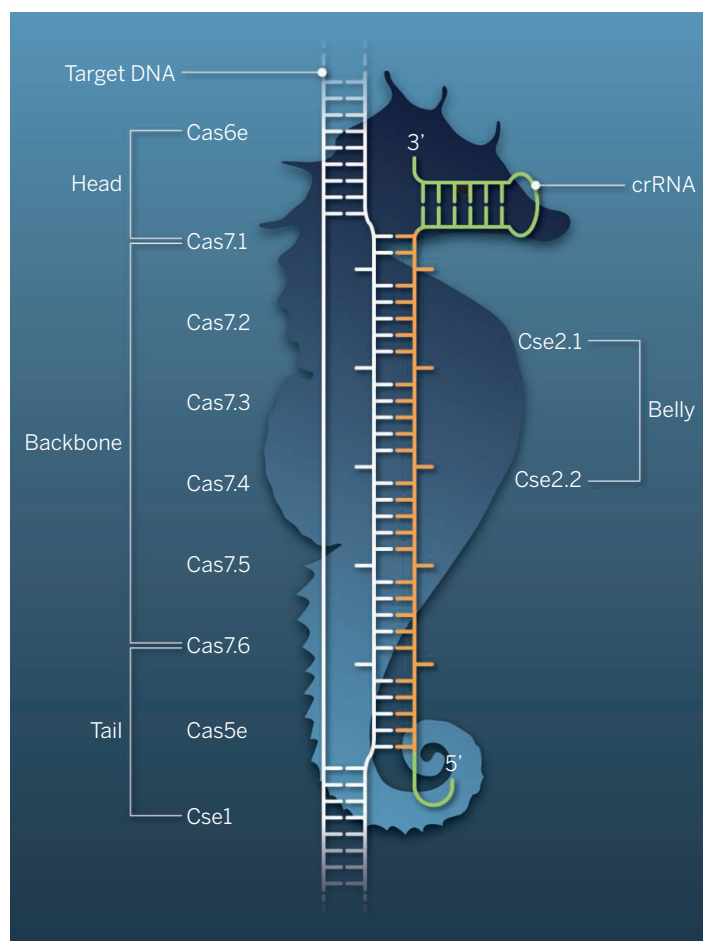
An adaptive immune pathway in bacteria and archaea is specified by clustered, regularly interspaced, short palindromic repeat (CRISPR) sequences (1). Research into CRISPR RNAs (crRNAs) and the CRISPR-associated (Cas) proteins have revealed the ability of most of these systems to target foreign DNA molecules for destruction. On pages 1473 and 1479 of this issue, Jackson *et al.* (2) and Mulepati *et al.* (3), respectively, and Zhao *et al.* (4) describe high-resolution structures of the multiprotein assembly called CRISPR-associated complex for antiviral defense (“Cascade”), which drives CRISPR interference in many strains of *Escherichia coli*. The structures show how Cascade presents crRNA to its DNA target, and demonstrate that DNA recognition occurs through a configuration that, surprisingly, is not double-helical.

Diverse flavors of CRISPR systems have been organized into three families (types I, II, and III) with additional subdivisions (5). All use crRNA precursors (pre-crRNAs) with multiple repeat sequences that are separated by “spacers.” These spacers match “protospacer” sequences that are usually present within phage genomes or plasmids. Pre-crRNAs are processed into individual crRNAs, each with a single spacer that guides the effector machinery to its DNA target. Type II systems require only a single protein, Cas9, for crRNA-guided DNA targeting. By contrast, crRNAs in type I and type III systems assemble into large multiprotein complexes. Cascade was first defined for the type I-E system from *E. coli* strain K12 (6) and includes one to six copies each of five distinct Cas proteins. Many of *E. coli* Cascade's important interactions, mechanistic features (6–12), and structural properties (7–9, 12) have been described, including its seahorse-like shape. Double-stranded DNA (dsDNA) recognition by Cascade requires not only crRNA-DNA complementarity, but also a 3-base pair

protospacer adjacent motif (PAM) (6–11). Target recognition by Cascade activates a separate protein, Cas3, which uses its nuclease domain to destroy the target dsDNA (11).

Jackson *et al.* and Zhao *et al.* report the structure of the 405-kilodalton *E. coli* Cascade in its pre-target-bound state. The complex contains 12 components (11 proteins, one RNA) and exposes most of the 32-nucleotide crRNA spacer (see the figure). The head of the seahorse-like complex includes the Cas6e pre-crRNA-processing enzyme, tightly bound to the 3'-terminal stem-loop of the crRNA. The “head” and “tail” regions are connected along the complex’s “backbone” by a helical arrangement of six Cas7 proteins (Cas7.1 to 7.6), as well as a “belly” that includes two Cse2 proteins. In the tail, the 5'-terminal crRNA “handle” anchors the assembly of the Cas5e, Cse1, and Cas7.6 proteins. The Cas5e and Cas7 subunits each resemble the shape of a right hand with finger, palm, and thumb-like domains. The thumb of one Cas7 extends toward the fingers of the preceding Cas7, stabilizing the Cas7 filament. Cas7.1 (at the head) and Cas7.6 (at the tail) cap the filament via distinct interactions with Cas6e and Cas5e, respectively.

One of the most intriguing aspects of these structures is the apparent manner of crRNA presentation to target DNA. The thumbs of five Cas7 proteins (Cas7.2 to 7.6) project out and fold over every sixth nucleotide of the crRNA, with concomitant introduction of a kink at each of those sites. Consequently, the crRNA is displayed as five discrete pentanucleotide segments, each poised for base pairing with the target DNA. The bases of the kinked nucleotides are flipped outward and apparently unavailable to participate in DNA recognition. Functional tests demonstrate that DNA recognition is insensitive to mismatches at kinked nucleotides, whereas mismatches elsewhere have much more dramatic effects on binding. The half-helical-turn segments in the crRNA suggest that the DNA protospacer could be recognized in five-nucleotide blocks.



Views of engagement. The Cascade multiprotein complex has a seahorse-like shape. Its 11 protein subunits associate with crRNA. The crRNA then forms a ribbonlike duplex with target DNA. Six “kinked” nucleotides in the crRNA stick outward, allowing five regions of contact with DNA.

Mulepati *et al.* report the structure of *E. coli* Cascade bound to a single-stranded DNA target. The overall architecture of the DNA-bound complex is in superb agreement with the two apo structures of Jackson *et al.* and Zhao *et al.*, and further reveals the configuration of the crRNA-DNA duplex. Sure enough, the DNA is engaged in 5-base pair blocks, with every sixth base unpaired.

This underwound, ribbonlike arrangement allows the target DNA recognition pathway to circumvent a potential topological challenge. If the target DNA strand were to be bound as a fully paired double helix, it would either have to thread the gap (twice!) between the crRNA and Cascade, or the complex would have to risk releasing a crRNA end to allow it to wrap around the DNA strand. Instead, each unpaired position enables the DNA to reset its trajectory for the next 5-base pair block without continuing around the other side of the crRNA.

DNA binding energetics is dominated by a subset of crRNA positions known as the

“seed,” which in *E. coli* lies within the 5'-terminal eight spacer nucleotides (10). Mismatches in the seed are far more deleterious to CRISPR interference than are mismatches elsewhere (1). One unexpected feature of the *E. coli* seed’s stringent complementarity requirement is that a single position within it—the sixth nucleotide—is excluded (10). The Cascade-DNA structure, with its unpaired configuration at precisely that position, explains why. In addition, the apo structures suggest that all five of the displayed pentanucleotide blocks (not just those that include seed residues) are preordered for DNA pairing, which suggests that the seed’s disproportionate importance is not due to a unique entropic advantage. Instead, Jackson *et al.* suggest that the seed bases are crucial to nucleate crRNA-DNA duplex formation because of their proximity to the PAM, the recognition of which could initiate local unwinding. Zhao *et al.* also point out that the seed region is relatively free of obstruction by the Cse2 dimer along the belly. Further work will be required to understand PAM recognition, as the complementary-strand

PAM nucleotides are disordered in Mulepati *et al.*’s structure and the other DNA strand is absent.

Nonetheless, the three structures provide the first high-resolution views of Cascade, before and after engagement with its target DNA. Additional frames of this “movie” will hopefully reveal how subsequent Cas3-catalyzed DNA degradation occurs. ■

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MICROBIOLOGY

Tracking antibiotic resistance

Sequencing plasmids can reveal the transmission of resistance among bacteria from patients in a clinical setting

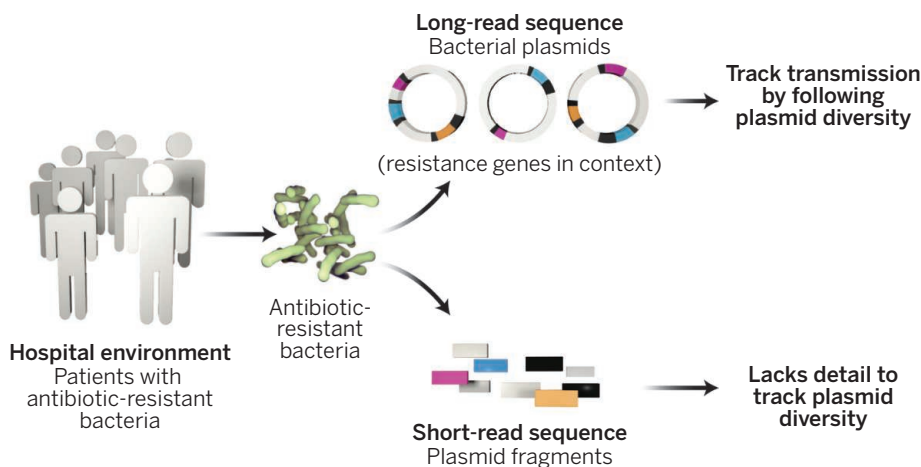
By Scott A. Beatson^{1,2} and Mark J. Walker^{1,2}

According to the World Health Organization (1), “Antimicrobial resistance...threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi....A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st Century.” Often, antibiotic-resistance genes acquired by bacteria are associated with mobile genetic elements that mediate their exchange between pathogens, or between commensal and pathogenic bacterial populations. Thus, the genetic context in which an antibiotic-resistance gene is placed can reflect its mobility within the bacterial population. Although the presence of resistance genes in clinical isolates can be detected with either polymerase chain reaction technology or high-throughput short-read length DNA sequencing, their precise genetic context may be overlooked because of the limitations of these methodologies. As Conlan *et al.* (2) demonstrate, the use of single-molecule long-read DNA sequencing can address this limitation and opens the way to track the transmission of antibiotic-resistant bacteria in a health care environment.

The transfer of genetic elements between bacteria, known as horizontal transfer (3), can occur through plasmids, small DNA molecules that are separate from bacterial chromosomal DNA. Conlan *et al.* applied long-read genome sequencing to identify plasmids harboring the gene *bla*_{KPC}, which encodes a carbapenemase that hydrolyzes carbapenem antibiotics. This analysis was carried out after an outbreak in 2011 at the U.S. National Institutes of Health Clinical Center in which six infected patients died from carbapenem-resistant *Klebsiella pneumoniae* (4). Conlan *et al.* surveyed more than 1000 patients for carbapenem-resistant bacteria. The bacterial genomes from such infected patients were analyzed

with long-read sequencing (single-molecule real-time sequencing). Sixty-three plasmid sequences were completed from patient or hospital environmental isolates, providing a unique snapshot of plasmid complexity from a single geographical site. The sequences allowed elucidation of the fine detail of plasmid diversity that would not have been obvious otherwise. Two patients infected with near-identical

compelling evidence to exclude nosocomial transmission may have been gathered from short-read sequencing, but long-read sequencing not only produced the required diagnostic result, but additionally provided reference sequences for all chromosomes and plasmids to aid future surveillance. Plasmid comparisons also disproved the hypothesis that transfer of a *bla*_{KPC} plasmid had occurred between *K. pneumoniae* and *E. cloacae* within a patient from the original outbreak (4); long-read genome sequencing showed discrete genetic contexts for the two *bla*_{KPC} alleles, indicating independent acquisition. Long-read genome sequencing also proved critical for determining the precise genomic context of *bla*_{KPC} alleles in cases where more than one *bla*_{KPC} gene was present in a single isolate.



Context. Long-read sequencing can resolve complete plasmids from bacterial genome assemblies. Plasmid comparisons can help to track the dissemination of antimicrobial-resistance genes among pathogenic bacteria in a clinical setting. By contrast, short-read sequencing will generally produce collapsed repeats at repeated locations such as insertion sequences (black) that are dispersed throughout the plasmid backbone (white), making the context of antibiotic-resistance genes (pink, blue, yellow) difficult to discern.

carbapenemase-resistant *Enterobacter cloacae* strains harbored *bla*_{KPC} on the same plasmid (pKPC-47e), triggering increased surveillance measures. Shared carbapenemase-encoding plasmids were found in three different bacterial genera epidemiologically linked with hospital environments (sinks). Of five patients who presented with carbapenemase-resistant *Klebsiella* sp., long-read genome sequencing associated one of these patients to the original 2011 outbreak, indicating nosocomial transmission. Long-read genome sequencing unequivocally ruled out a link with the 2011 outbreak in the remaining cases, despite epidemiological evidence suggesting otherwise. Carriage of shared plasmids was observed between a patient isolate and isolates of other bacterial species present in the ward environment. For several cases,

Plasmids play a key role in disseminating antimicrobial-resistance genes among pathogenic bacteria. Resistance genes often aggregate in modular clusters (5) that enable resistance to persist even in the absence of direct selection. Some insertion sequences (IS) (6), such as IS26, sandwich resistance genes into a composite transposon that can mobilize elsewhere. Other elements, such as *ISEcp1*, mobilize adjacent genes, leading to multiple copies of resistance genes within the genome. The multiplicity of insertion sequences and other repetitive elements is a major confounding factor in standard short-read genome assembly, which can lead to the fragmentation of chromosome and plasmid sequence assemblies. In this sense, plasmids may be viewed as the “dark matter” of short-read bacterial genome assemblies, with many

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large-scale genomic studies conspicuously avoiding the complexities of plasmid structure. Genomic comparisons such as that described by Conlan *et al.* reveal how the dynamism in the structure and arrangement of resistance elements can only be realized by “closing” plasmid genomes with long-read sequencing (see the figure).

Traditional Sanger sequencing is the gold standard for the analysis and assembly of complete plasmid sequences from antibiotic-resistant strains of bacteria. This approach may suffer from the need to isolate or subclone individual high molecular weight plasmids before sequencing (7), which is often technically difficult, time-consuming, and costly, and may be intractable for multiple plasmids. Short-read sequencing technologies can affordably produce an assembly of a bacterial genome that contains nonrepetitive sequences typically in hundreds of “contigs” separated by “collapsed repeats” indicative of multiple copies of the same sequence located in several different locations within the genome. These repeats are often mobile elements such as insertion sequences that may be found in multiple copies on plasmids, thus making it difficult to assemble plasmid sequences.

Cataloging the collection of antibiotic-resistance genes in any particular bacterium is relatively straightforward, but determining how these genes fit together within plasmids, which is critical for understanding how these elements spread in clinical settings, can be more difficult. By contrast, the genome sequences produced through long-read sequencing offer a complete picture of the plasmid content of a bacterium, including the number, position, and context within mobile elements of every acquired antibiotic-resistance gene. Long-read genome assembly offers clear advantages for the resolution of complete plasmid sequences that can discriminate plasmid diversity, antimicrobial-resistance gene context, and multiplicity. Such information will enhance our understanding of plasmid carriage, transfer, epidemiology, and evolution.

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10.1126/science.1260471

NANOMATERIALS

Making strong nanomaterials ductile with gradients

Microstructures that increase metal crystallite size from nanoscale with surface depth are both strong and ductile

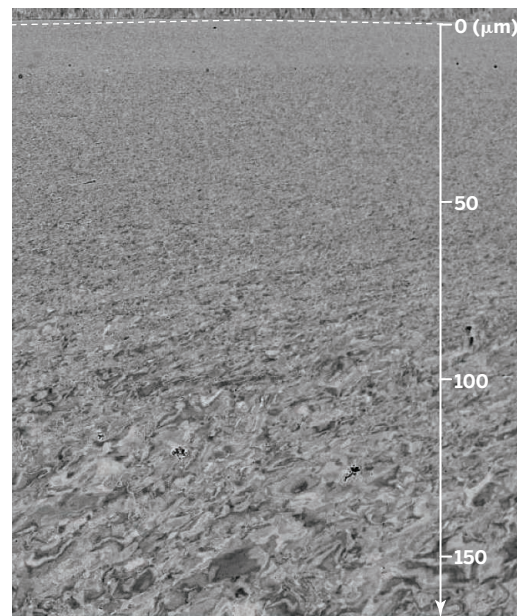
By K. Lu

Steels can be made stronger, tougher, or more resistant to corrosion either by changing composition (adding in more carbon or other elements) or by modifying their microstructures. An extreme microstructural route for strengthening materials is to reduce the crystallite size from the micrometer scale (“coarse-grained”) to the nanoscale. Nanograined aluminum or copper (Cu) may become even harder than high-strength steels, but these materials can be very brittle and crack when pulled (deformed in tension), apparently because strain becomes localized and resists deformation. However, nanograined metals can be plastically deformed under compression or rolling at ambient temperature, implying that moderate deformation can occur if the cracking process is suppressed. Tremendous efforts have been made to explore how to suppress strain localization in tensioned nanomaterials and make them ductile. Gradient microstructures, in which the grain size increases from nanoscale at the surface to coarse-grained in the core, were recently discovered to be an effective approach to improving ductility (1–4).

One advantage of metals in structural applications is that they “signal” their impending failure—they can deform and crack to some extent before they completely fail. However, when a piece of fully nanograined copper is pulled, catastrophic failure occurs immediately when the load exceeds its yield strength (the point at which permanent deformation begins), just like most ceramics and other normal fragile materials. Such tensile brittleness is an Achilles’ heel of nanomaterials that hinders their technological applications; for example, they cannot be strengthened by work hardening. The microscopic origin appears to be early necking (decrease in cross section) induced by strain localization prior to activation of plastic deformation mecha-

nisms of the extremely fine grains that induces cracking. By applying surface plastic deformation onto a bulk coarse-grained metal, a distinctive microstructure is generated from the strain gradient: a nanograined layer (several tens of micrometers thick) covers the coarse-grained substrate with a graded variation of grain size in between (see the first figure).

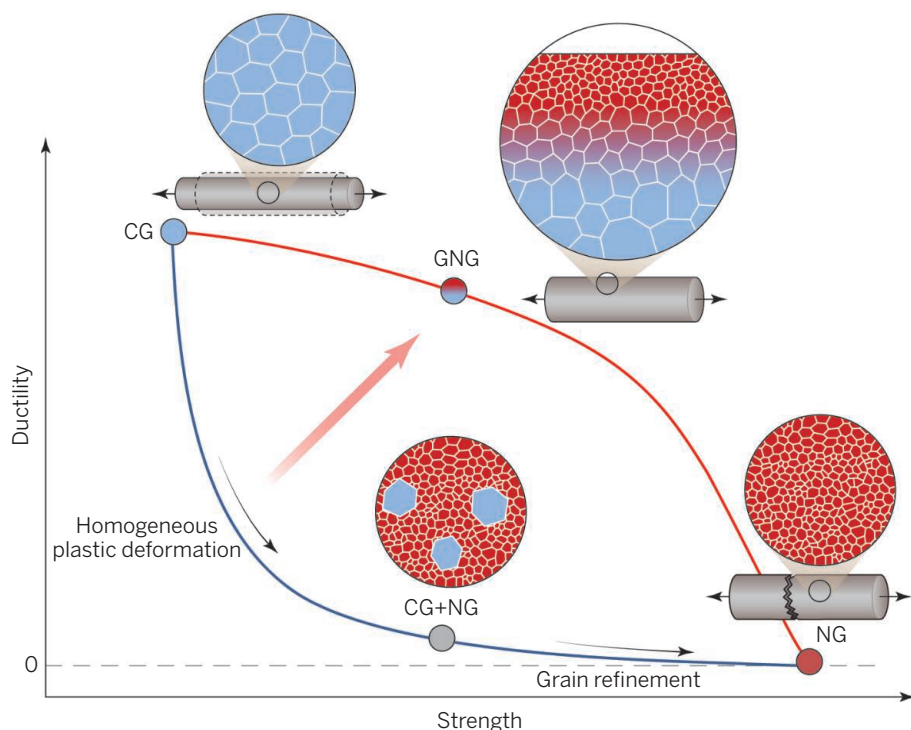
Tensile tests of the heterogeneously structured Cu cylinder (pulling the sample along the long axis) showed that the top



Gradient nanograined structure. After a surface mechanical grinding treatment to copper, grain sizes are about 20 nm in the topmost treated surface (outlined by dashed line) and increase gradually to the microscale with depth.

nanograined layer and the coarse-grained core can be elongated coherently by as much as ~60% before failure—comparable to that in conventional Cu, but the sample’s yield strength is doubled (1). Almost no tensile elongation was observed in the nanograined layer as it was removed from the substrate. Evidently, the observed extraordinary tensile

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Strength-ductility synergy. The strength of a metal is increased at an expense of ductility for homogeneous plastic deformation of coarse-grained (CG) metals or homogeneous refinement to nanosized grains (NG), and follows a typical “banana-shaped” curve (blue line). Similar strength-ductility trade-offs occur for random mixtures of coarse grains with nanograins (CG+NG). However, strength-ductility synergy is achieved with gradient nanograin (GNG) structures (red line).

ductility of the nanograin skin resulted from the ideal confinement of the gradient microstructure. Comparable tensile ductility of gradient-structured nanomaterials with that of the coarse-grained counterparts was observed recently in a number of engineering alloys (2–4).

When a homogeneous-grained material is under tension, the onset of plastic deformation in different grains occurs almost simultaneously. Because adjacent grains cannot deform in concert and displacements across grain boundaries are not matched, intergranular stress and strain localization may develop that create voids or cracks at the grain boundaries. For a material with a grain-size gradient, the onset of plastic deformation occurs initially in coarse grains and propagates gradually into smaller ones with increasing loads. The orderly plastic deformation releases intergranular stress between neighboring grains of different sizes so that strain localization is suppressed. At higher loads, such a strain delocalization process takes place progressively in finer and finer grains until it reaches the topmost nanograin layer. Effective suppression of strain localization and early necking enable the nanograin skin to elongate concurrently with other parts of the sample, and its plastic deformation mechanisms are activated.

Deformation of the nanograin Cu is dominated by a mechanically driven grain boundary migration with concomitant grain coarsening and softening (1). Meanwhile, deformed coarse grains are hardened by dislocation slip and accumulations, providing work hardening of the global sample. Hence, both hardening and softening occurs simultaneously in the gradient microstructure, and the dominating deformation mechanisms change gradually from dislocation slip into grain boundary migration as grains become smaller. In a critical submicroscaled region, neither hardening nor softening is induced as the two mechanisms are balanced, corresponding to the strain-induced saturation structures (5). The gradient microstructure allows various plastic deformation mechanisms of largely different microstructures to be activated concurrently. This balance does not exist in homogeneous nanograin structures, nor in random mixtures of nanograins and coarse grains.

The extraordinary tensile ductility of the gradient nanograin surface layer, which is several times stronger than the coarse-grained structure, leads to a strength-ductility synergy, as opposed to the traditional trade-off between strength and ductility. In homogeneously deformed or homogeneous nanograin metals, or random mixtures of

nanograins and coarse grains (6), the overall strength gain comes at a loss of ductility leading to a “banana-shaped” curve, as shown in the second figure. Gradient nanostructuring avoids this ductility loss, and the use of even smaller nanograins or thicker gradient skin (7) may further upbow the strength-ductility line. Exceptionally superior strength-ductility combinations were discovered in a number of gradient nanograin or gradient nanotwinned materials (2–4). The enhanced ductility in gradient nanograin interstitial-free steel sheets was alternatively explained by an extra strain hardening induced by a macroscopic strain gradient and a change in stress states (2).

The strain delocalization of gradient microstructures also greatly enhances fatigue resistance after cyclic loading and unloading in several gradient nanograin materials (8). In homogeneous nanograin or submicrograined materials, resistance to fatigue crack growth is reduced relative to that in coarse grains, and the low-cycle, strain-controlled fatigue properties become even worse. A gradient nanostructured skin covering a coarse-grained substrate is actually optimal for enhancing fatigue resistance. Fatigue crack initiation would be suppressed by the hard-and-deformable gradient nanograin skin while the coarse-grained interior is effective in arresting the crack propagation. The highly deformable gradient nanograin surface layer eliminates the deformation-induced surface roughening that is frequently seen in tension or drawing of metals, which suppresses surface cracking and facilitates subsequent mechanical processing (1).

Quantifying correlations between gradient microstructures and properties is vital for optimizing global properties of the hierarchical nanostructured materials. The development of processing techniques for stabilizing nanostructures via proper alloying (9), grain boundary modifications, or both to enlarge the microstructure gradient is challenging and critical for exploration of more properties and functionalities. ■

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ACKNOWLEDGMENTS

Supported by Ministry of Science & Technology of China grant 2012CB932201 and National Natural Science Foundation of China grants 51231006 and 5126113009.

10.1126/science.1255940

Pursuit of the common good

Religious institutions may mobilize public opinion and action

By Partha Dasgupta¹ and
Veerabhadran Ramanathan^{2,3*}

Humanity is at a crossroads. Do we continue trends of preceding decades that lift people out of poverty and extend life spans, but in the process run down the planet's natural capital? Solutions to this profound problem will require greater cooperation among people. The rise of market fundamentalism and the drive for growth in profits and gross domestic product (GDP) have encouraged behavior that is at odds with pursuit of the common good. Finding ways to develop a sustainable relationship with nature requires not only engagement of scientists and political leaders, but also moral leadership that religious institutions are in a position to offer.

So it was significant that the Vatican convened a workshop earlier this year at which scholars (including the authors) from the humanities and the social and natural sciences reflected on issues at the nexus of poverty, population, consumption, and environment (1). Given the secular nature of the issues and the requisite solutions, scholars' religious beliefs were not a criterion for participation (2). Hosted jointly by the Pontifical Academies of Sciences and of Social Sciences, the workshop held one of the broadest and most interdisciplinary discussions to date on those issues. Such discussions are critical, as the United Nations General Assembly convenes later this month with a focus on a "transformative post-2015 development agenda."

OPPOSED PERSPECTIVES. The 20th century saw enormous increases in global income and improvements in human health. But this involved a massive increase in exploitation of Earth's resources (3). The Millennium Ecosystem Assessment reported in 2005 that 15 of 24 ecosystem services investigated were degraded or being exploited at unsustainable rates. The matter is now worse (4). Environmental problems are manifest at scales from the global, such as climate change, to the local, such as declines in availability of fresh water and forest prod-

ucts in villages in the poor world. High fertility rates in the poorest regions exacerbate pressure on local systems and contribute to the persistence of poverty.

Unsustainable consumption, population pressure, poverty, and environmental degradation are intricately linked (5), but this is appreciated neither by development economists (6, 7) nor by national governments who permit GDP growth to trump environmental protection in their policies.

Because the socioecological processes giving rise to this state of affairs aren't self-correcting (5), there is urgent need for collective action from the community level to the international level. Studies on resource allocation in nonlinear systems have shown that Adam Smith's famous "invisible hand" cannot, even in theory, be expected to come to the rescue (8, 9). Natural and social scientists have done their part in documenting the irreversible environmental damages

(albeit with large uncertainties) that we have inflicted and in spelling out specific mitigation actions (1). The transformational step may well be a massive mobilization of public opinion by the Vatican and other religions for collective action to safeguard the well-being of both humanity and the environment.

Among topics discussed at the Vatican, we develop three that we believe are central to disrupting destructive feedbacks between unsustainable consumption, population, poverty, and environment: well-being, wealth, and natural capital; stabilizing climate change; and universal access to energy.

WELL-BEING, WEALTH, AND NATURAL CAPITAL. Discourses on economic growth, equity, and poverty alleviation should include the role natural capital plays in our lives. The first step would be for governments to take inventory of their nations' stocks of assets and keep the accounting current on a regular basis. Most countries don't have inventories of natural capital stocks, nor are effective institutions in place for governing their use. Wide varieties of natural capital, such as ecosystems, are thereby freely available. Methodologies



A woman using a mud oven for cooking in Mukteshwar in the Kumaon Hills of Uttarakhand, India.

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are available to show how national accounts should incorporate a nation's portfolio of assets and provide estimates of its true wealth (10). The social worth (or "shadow price") of ecosystem services that are needed for the accounts can be estimated by designing institutions that make beneficiaries pay for services they enjoy or by uncovering the role ecosystems play in production. The estimates depend on, among other things, the model describing the operations of the economy, the choice of rates at which future costs and benefits are discounted, and social attitudes toward uncertainty. Pilot studies show that such accounts can indeed be prepared (10). They show also that wealth estimates should be presented as bands, not as precise figures. The move away from feigned precision that characterizes national accounts to reasoned bands should prove salutary when governments deliberate economic policies.

STABILIZING CLIMATE CHANGE. Anthropogenic climate change raises questions about the responsibilities we have to one another and to nature. Some 1 billion people are responsible for 50% of greenhouse gas emissions; a further 3 billion people for 45%; while the bottom 3 billion, who do not have access to affordable fossil fuels, are responsible for a mere 5% (11, 12). Although we all will soon be affected by climate change, it is the latter 3 billion who will, tragically, experience the worst consequences. Not only is their direct reliance on natural capital disproportionately large, they are also far less able to afford protection from extreme weather events.

A number of actions need to be taken now, particularly by the 1 billion responsible for the bulk of the emissions. Emissions of carbon dioxide (CO₂), the dominant contributor to global warming, have to be halved by mid-century and eliminated before the end of the century. Decarbonization involves moving to sources of "clean" energy, but the process would be eased if the efficiency with which energy is used were raised. Reducing waste would help. For example, some 3 billion tons of CO₂ (8 to 9% of total annual emission) are released in producing food that is wasted (13).

Humanity needs also to reduce emissions of four short-lived climate pollutants (methane, ozone, black carbon, and hydrofluorocarbons), that are currently responsible for a third of the heat energy added to the planet (11). The underlying cause of some of that pollution is acute poverty. The majority of the 3 billion at the bottom of what we may call the "energy pyramid," use firewood, dung, and crop residues for cooking and kerosene for lighting (14). This increases the

atmospheric concentration of black carbon ("soot") and ozone. Scalable technologies that reduce these emissions are available off the shelf [(11) and references therein], e.g., cleaner-burning cook stoves to replace rudimentary mud stoves and solar lamps to replace kerosene lamps for the three billion without access to fossil fuels (Fig. 1). Be-

"Adam Smith's famous 'invisible hand' cannot, even in theory, be expected to come to the rescue"

cause the lifetimes of these pollutants range from weeks to a decade, the mitigation effect would be almost immediate. The Vatican and other religions have vast networks of voluntary organizations that can have a major impact on distribution of clean technologies in rural areas of Asia, Africa, and South America.

UNIVERSAL ACCESS TO ENERGY. Some 1.5 billion people among the bottom 3 billion do not have access to electricity for lighting (14). In those households, women and children typically collect fuel for cooking and fetch water for drinking, meaning they can ill afford time for education or work outside the home. Four million premature deaths yearly are attributable to indoor smoke from cooking and kerosene lighting [(15) and references therein]. Lack of access to facilities that promote reproductive health contributes to keeping fertility rates high, maintaining pressure on local ecosystems and aiding perpetuation of poverty. These features of poverty should be set against the aspirations that underlie Sustainable Development Goals being discussed within the UN system. If the bottom billion were to rely on fossil fuels for their well-being, CO₂ emissions would rise by about 10 billion tons a year from the current 35 to 40 billion tons (11). But there are alternative technologies such as high-efficiency biomass, biogas or electric stoves, and solar lamps that could drastically reduce emissions of toxic pollutants (11). Minigrids for electricity powered by solar photovoltaic cells, agriculture waste, and biogas can provide energy for pumping water for irrigation and small-scale industries (14). Unfortunately the poor cannot afford them. But the strong link between poverty, air pollution, public health, and climate change makes energy access a strong contender for collective action. The Vatican and other religions can take a decisive role by mobilizing public opinion and

public funds to meet energy needs of the bottom 3 billion who struggle with preindustrial-era technologies.

PURSuing THE COMMON GOOD. Mitigation measures, including capture of atmospheric carbon, will require large investment and huge commitment from communities, charities, national governments, and international bodies. But the risks that a runaway change in Earth's climate system or significant further losses in biodiversity will produce devastating damage are not negligible. Involvement of religious institutions with issues discussed at the Vatican workshop could go a long way toward lessening risks to humanity originating at the poverty-population-consumption-environment nexus. The statement issued by contributors to the workshop (16) urged that, over and above institutional reforms and policy changes that are required, there is a need to reorient our attitude toward nature and, thereby, toward ourselves. In convening the workshop and in statements that followed (17), Pope Francis displayed deep concern over our relationship with nature and raised the profile of the issues that stem from it. It can only be hoped that such moral leadership will mobilize people to act upon them. ■

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ACKNOWLEDGMENTS

The authors thank Bishop M. Sanchez Sorondo, Chancellor of the Pontifical Academies of Science and Social Sciences for hosting the workshop and for help in preparing this article.

10.1126/science.1259406

Ode to fine wines and rocks

By Lawrence D. Meinert

Geologist Charles Frankel's *Land and Wine: The French Terroir* is not so much a scientific exposé as it is a beautifully described love triangle involving wine, rocks, and French history. With stories of Charles the Fat (839 to 888 CE), Philip the Bold (1342 to 1404), and Joan of Arc (1412 to 1431), each a contributor to the development of different wine regions, one cannot help but be entranced by this delightful interplay of history, wine, and the geologic evolution of the European continent. For example, it is noted that some of the terroirs (i.e., the geology, geography, and climate that affect the crops) of Beaujolais were already famous in Roman times, such as the hill of Brouilly, named for Brulius—an officer of Julius Caesar's army who received vine land from the emperor in exchange for his loyal service. Does one need to know about the connection between a fine bottle of Brouilly and Julius Caesar to enjoy the wine? No, but it could be argued that life is richer when we understand such history, and this is an area in which *Land and Wine* excels.

The book's 12 chapters cover most of the more famous wine regions of France, but, in an interesting departure from normal organizational schemes such as geography or grape varieties, the chapters are organized by the geological age of the rocks in each region. These rocks began about 500 million years ago with the mica schists of Anjou, through the first dinosaurs in the layered sedimentary beds of the Alsace appellation, to the demise of dinosaurs in the rocks of Languedoc, and last the relatively recent glacial outwash cobbles that underlie the storied vineyards of Bordeaux and some parts of the Rhone Valley.

Even though the author states that the book is not a travelogue, it in fact would be a fascinating accompaniment to a trip to the vineyards of France, adding another dimension to one's appreciation of the region.

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Chapters can be read in isolation or in any order. Although I have been to several of these wine regions, reading this book made me want to go back to them, armed with new insight and ready to taste many of the wines described in each chapter.

Frankel provides numerous interesting historical anecdotes that spice up the many descriptions of wine and geology. In the chapter on the famous Corton appellation of Burgundy, he quotes the writer and philosopher Voltaire (1694 to 1778) as confessing to his wine supplier, "I serve to my guests a decent Beaujolais wine, but I drink in secret your good wine from Corton." Frankel goes on to state that Voltaire "consumed the equivalent of four bottles a day" for "health reasons" but also notes, with an implied smile, that Voltaire lived to be 84. Perhaps this is the enological equivalent of "an apple a day keeps the doctor away?"

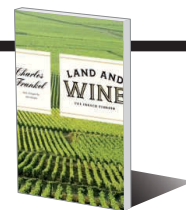


The author has a particular fascination with paleontology and liberally sprinkles the names of bedrock fossil shells such as *Campanile giganteum*, dinosaurs such as *Ampelosaurus ataxis* (Latin for dinosaur of the vines from the Aude area), and giant pterodactyls with 5-m wingspans among the wines and vineyards. This interest is reflected in some of the author's other books, such as (1).

Frankel states that most background information for the book has been gathered from websites of the various wine regions and individual wine estates. Five pages of URLs for websites are listed in the back of the book, in contrast to only a quarter page of more-standard published references on wine and geology. Even though many scientists might regard this approach as light on scholarship, it seems to work for the wine regions discussed because of the author's personal expertise with the geology and wines of France. This reliance on websites is more apparent regarding interpretations of terroir, climate,

Land and Wine The French Terroir

Charles Frankel
University of Chicago
Press, 2014. 263 pp.



and plant physiology, where some mythology and misstatements of facts creep in.

A notable example of wine myths is the repeated assertion that grapevine roots transport minerals, elements, and various soil constituents into the grapes and ultimately into the wines. For example, "these ... pebbles—along with their parent bedrock—give Savennières wine its celebrated mineral flavor," Le Clos du Papillon has "a lingering mineral note of 'warm schist,'" and "flint in the soil translates into spicy, smoky aromas."

Actually what is occurring is that climate and bedrock, plus soil characteristics such as porosity and permeability, combine to affect grapevine physiology in ways that result in certain wine flavors being produced. For example, terroir factors that lead to unripe grapes will result in tart, acidic wines, which wine writers sometimes refer to as "bracing minerality." But it is a simple fact that neither minerals nor acids are transported by the roots to the grapes to the wine (2). The scientific basis of the effect of climate, environment, and terroir on wine is well treated in many widely available texts [e.g., (3–5)], and these usefully augment the author's more-poetic travelogue through the vineyards of France.

A rather amusing sidelight of the book is the translation of many of the elegant-sounding place names that appear on esteemed wine bottles such as Les Brûlés, Vignes Derrière, Vers la Roche, La Côte des Monts Damnés, and Les Croux, which lose some of their luster in translation to English—respectively, "The Burned Ones," "Vines at the Back," "Toward the Rock," "The Hillslope of the Damned Mountains," and "The Chalky Ones." Overall, *Land and Wine* will add a new dimension to anyone interested in wine and terroir.

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10.1126/science.1259718

CONSERVATION ECOLOGY

Understanding and saving big predators

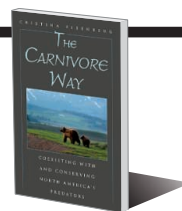
By Matthew E. Gompper

The public is fascinated by large carnivores because they seem dangerous, beautiful, intelligent, and secretive. Furthermore, they are rare and, like dinosaurs, no longer even a minor threat to the vast majority of North America's human population. This was not always the case; when Lewis and Clark set out across North America a mere two centuries ago, wolves and grizzly bears were a serious health hazard. How quickly things change. These days, when the public thinks about big carnivores in North America, typically the focus is one of trying to maintain or restore these species.

Ecologists are fascinated by large carnivores for all of these same reasons but also because studying large carnivores is one of the few realms in which one gains insights into the ecological importance of not just a species or a population but the actual individual animal. Because of the combined

The Carnivore Way
Coexisting with and
Conserving North
America's Predators
Cristina Eisenberg

Island Press, 2014. 325 pp.



communities changes, often fundamentally.

This narrative is a central underpinning of Cristina Eisenberg's new book, *The Carnivore Way*. Writing for both the researcher and the general public, Eisenberg uses her very personal experiences with large predators in western North America to establish the ecological basis for conserving these creatures. In the introduction, she writes "In the late 1990s, when wolves recolonized our valley, ... deer and elk that used to stand around complacently mowing aspens and shrubs down to ankle height had to stay wary and on the move to avoid being eaten by wolves. And within five years, unbrowsed aspens and shrubs had filled our meadow." Her observations are supported by extensive studies in a handful of (mainly) wolf-dominated sites, such as Yellowstone, Grand Teton, and Isle Royale national parks in the United States. The simplicity of these ecosystems in terms of numbers of species allows impacts of the loss

or return of large predators to be relatively easily measured, and the picture derived from these measures is impressive. Ungulate, bird, butterfly, and plant communities change. Fluvial and biogeochemical processes are altered.

It still is not clear if these insights are fully generalizable across the globe, including regions of high biodiversity such as the tropics, where the potential for buffering perturbations to food webs may be high, or to regions where interannual abiotic variation may overwhelm the top-down role of large predators in driving ecosystem func-

tion. Eisenberg's own research suggests nuance, and she attempts to impart this nuance. Nonetheless, the evidence from western North America, where Eisenberg focuses her attention, is such that the ecological importance of top predators has become something of a default framework for most researchers and the general public. "Large carnivores can help slow extinction because they increase biodiversity, thereby creating more resilient ecosystems." The null hypothesis has seemingly become that large apex predators, through the top-down pressures they directly (by killing) or indirectly (by creating fear) impart, are the reason natural systems appear as they do and why researchers and conservationists alike should strive to aid the persistence of these taxa, not just in a few scattered locales but across vast landscapes.

Eisenberg is a fine writer. She centers her attention on the linked rugged landscapes that run from the Sierra Madre Occidental of Mexico, along the Rocky Mountains of the United States and Canada, and into Alaska. This is the author's "Carnivore Way," although clearly the phrase can mean something more. The book focuses on six species (gray wolves, grizzly bears, wolverines, lynx, jaguars, and cougars) and asks how carnivores inhabiting these regions live and die as they interact with increasing human populations and how we might overcome human-carnivore conflicts and gain, as the book jacket describes, a "peaceful coexistence." Examining each species separately, Eisenberg is able to deftly touch on diverse themes: border fences; species life histories; ranching, hunting, and trapping; reintroduction efforts; drought and fire; public land availability and management; ranging and dispersal tendencies; and environmental laws and legal structures. The book, like its focal species, roams widely.

The author is at her best when telling stories. By discussing how individual animals use their landscape, Eisenberg paints a picture of their trials and tribulations and how they make a living in an increasingly human-dominated environment. Collectively we gain a better understanding of the scale at which carnivore ecologists should be working. Yet the very wide-ranging nature of these animals means that in some ways Eisenberg's geographic Carnivore Way is a figment of human perspective. It is a glorious, rugged landscape. But these species once roamed far beyond it. Wolves and cougars were common in eastern deciduous forests, and grizzly bears once inhabited the Great Plains. These landscapes are the next frontier in carnivore conservation.



(and interrelated) effects of their size, ranging habits, physiology, and diet, the presence of an individual large carnivorous animal is important—through its land use, or shifts in the whims of its behavior, the individual animal can have a drastic effect on a community. Having a handful of sea otters or wolves on the landscape can result in actual landscape change. Lose those carnivores, and the structure of the remaining plant and animal

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10.1126/science.1256065

LETTERS

Edited by Jennifer Sills

Island outlook: Warm and swampy

IN HIS IN DEPTH News story “Warming may not swamp islands” (1 August, p. 496), C. Pala argues that “coral reefs supporting sandy atoll islands will grow and rise in tandem with the sea,” based largely on studies that showed stable Pacific-island area over recent decades (1–4). He suggests that recent land losses are driven mostly by bad choices and that islanders are being affected “for the same reason as millions of people on the continents: because they live too close to shore.” We disagree with these conclusions.

Pala bases his arguments on (i) evidence that reefs can build vertically at 10 to 15 mm/year, a rate far exceeding the anticipated rate of sea-level rise, and (ii) sequential air photographs that document stable island areas despite rising sea level. An analysis of the early literature (i.e., pre-1999) revealed 22 articles reporting much lower reef-accretion rates—0.60 to 7.89 mm/year, averaging 3.54 (see supplementary materials). More recent reviews similarly conclude that rates of 10 to 15 mm/year are much too high for Holocene reef building (5–8). Recent declines in coral cover can only slow accretion in the future.

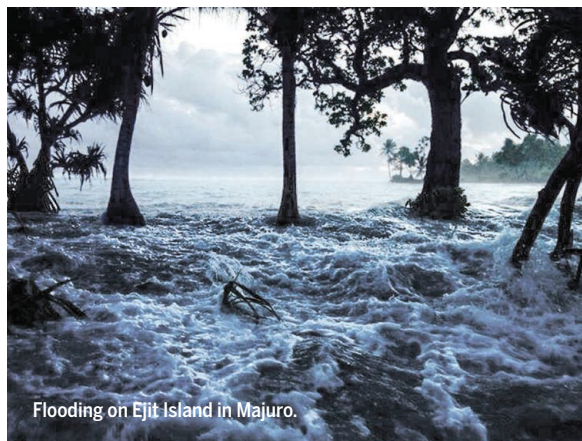
Naomi Biribo has noted that “wide-spread erosion along the ocean and lagoon shorelines is primarily due to human activities” (3). However, the same paper also attributes 90% of the increase in island area on South Tarawa to reclamation projects (i.e., no inherent ability to keep pace with sea level naturally). Island area may not provide an adequate measure of either changing sediment volume or the susceptibility of the island to flooding, erosion, or drowning. It is possible that erosion of sand from higher marginal areas and redistribution to lower shorelines elsewhere on the island can increase island area, even in the face of declining sediment volume.

Pala’s sources describe islands building seaward 4800 to 4500 years ago (3, 9),

a pattern opposite to the island retreat that is occurring today. If rising sea level is to be discounted, then a mechanism to explain this recent reversal must be identified. Also, fewer corals making calcium carbonate and stronger storms removing at least some of it will likely reduce sediment availability.

We also take exception to laying the sole blame at the feet of islanders who have occupied these fragile islands for millennia. Human communities living on atoll islets often depend on thin aquifers, agroforestry, and freshwater wetland taro production. Saltwater intrusion and flooding destroys otherwise sustainable food and water sources (10), making islands unlivable long before drowning.

Certainly the situation has been exacerbated by poor choices, and politics is rarely the best lens for viewing natural phenomena. Adapting to either sea-level rise or more frequent inundation often



Flooding on Ejit Island in Majuro.

involves ill-placed engineering solutions that require substantial volumes of sand and rock that would be better left in place to protect coastlines and contribute to island sediment budgets. The meteorological instability that comes with rising temperatures, the likely increase in erosion and storm surge, islands constantly retreating from the sea, dwindling groundwater supplies, decreasing rainfall, and rising sea level will all have disproportionate impacts on populations that are least responsible for the global carbon emissions that are at the heart of these changes.

Dennis Hubbard,^{1*} Eberhard Gischler,² Peter Davies,³ Lucien Montaggioni,⁴ Gilbert Camoin,⁵ Wolf-Christian Dullo,⁶ Curt Storlazzi,⁷ Michael Field,⁷ Charles Fletcher,⁸ Eric Grossman,⁹ Charles Sheppard,¹⁰ Halard Lescinsky,¹¹ Douglas Fenner,¹² John McManus,¹³ Sander Scheffers¹⁴

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1461/DC1
Table S1
References (11–32)

Lab animal protection overdue

WHEN THE INSTITUTE of Medicine (IOM) concluded in 2011 that most current use of chimpanzees in biomedical research was “unnecessary,” it highlighted systemic deficiencies in the oversight of experiments on animals (1). Specifically, harmful and invasive experiments on chimpanzees continued to be reviewed, funded, approved, and conducted at government and private facilities—all of which were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)—despite the studies’ irrelevance to human health and/or the availability of alternative research methods. It was only when

the public, activists, and Congress raised questions that the problem was discovered and rectified, leading to the retirement of most federally owned chimpanzees, the discontinuation of most federally funded biomedical experiments on chimpanzees, and a complete overhaul of the process by which federally funded experiments on chimpanzees are reviewed. Oversight and accreditation from AAALAC certainly did not protect these chimpanzees from painful and unnecessary experiments at laboratories across the country.

In his In Depth News story “Animal welfare accreditation called into question” (29 August, p. 988), D. Grimm describes a study led by Justin Goodman, the director of the Laboratory Investigations Department at People for the Ethical Treatment of Animals (PETA). Goodman *et al.*'s study shows that AAALAC-accredited laboratories are more likely to violate animal welfare laws than nonaccredited laboratories. The results lend credence to the idea that AAALAC may not deserve the designation as the “gold standard” for laboratory animal care.

This new analysis, the IOM report, and other studies like them [e.g., (2, 3)] highlight the need for oversight mechanisms that are truly independent, unbiased,

and transparent, none of which apply to AAALAC in its current incarnation.

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Prenatal prevention

THE EDITORIAL “A focus on child development” (K. L. Silver and P. A. Singer, 11 July, p. 121) rightly calls attention to the final United Nations report on Sustainable Development Goals and the need to address the developmental origin of health and disease in the successor to the Millennium Development Goals. Silver and Singer highlight the postnatal risks to the world's children and the need to ensure “that children who survive reach their full potential.” However, a child is vulnerable from the time of conception.

Some organ functions and noncommunicable disease risks are determined during prenatal development (1). This is particularly true for brain development, where exposures to substances such as methylmercury and ethanol during pregnancy have led to clear cognitive and behavioral abnormalities that may even be present at low levels of exposure (2).

As Silver and Singer point out, “society only reaps the full benefits...if that child becomes a productive individual as an adult.” In terms of lost income associated with neurobehavioral deficits, current exposures to environmental pollutants are causing societal losses in terms of many billions of dollars annually. The worldwide loss due to lead alone has been estimated to be at least 1% of the GDP (3). Prevention of this “chemical brain drain” would be a highly rewarding investment.

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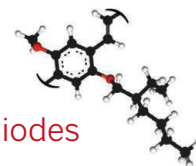
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RESEARCH

How spin comes into play in carbon-based light-emitting diodes

Malissa et al., p. 1487



IN SCIENCE JOURNALS

Edited by **Melissa McCartney** and **Margaret Moerchen**



CLIMATE CHANGE

Rings of ocean upwelling

Coastal upwelling along the coast of California has become more variable than during nearly any period in the past 600 years. Black *et al.* used a 576-year tree ring record to construct a record of wintertime climate along the California coast. Because wintertime climate depends heavily on coastal upwelling, they were able to determine that upwelling variability has increased more over the past 60 years than for all but two intervals during that time. The apparent causes of the recent trend appear to be unique, resulting in reduced marine productivity and negative impacts on fish, seabirds, and mammals. — HJS

Science, this issue p. 1498

Growth rings in blue oaks like this one provide a climate record

NUCLEAR CHEMISTRY

A carbonyl compound that tips the scales

Life is short for the heaviest elements. They emerge from high-energy nuclear collisions with scant time for detection before they break up into lighter atoms. Even *et al.* report that even a few seconds is long enough for carbon to bond to the 106th element, seaborgium (see the Perspective by Loveland). The authors used a custom apparatus to direct the freshly made atoms out of the hot collision environment and through a stream of carbon

monoxide and helium. They compared the detected products with theoretical modeling results and conclude that hexacarbonyl $\text{Sg}(\text{CO})_6$ was the most likely structural formula. — JSY

Science, this issue p. 1491;
see also p. 1451

IMMUNOTHERAPY

Cancer immunotherapy expands T cell attack

Patients with advanced melanoma can survive longer after treatment with the immunotherapy drug ipilimumab. Kvistborg *et al.* show how

this therapeutic antibody exerts its beneficial effects. Although directed against an immune inhibitor, the drug unexpectedly failed to increase the magnitude of immune T cell responses. Rather, the patients' T cells targeted a greater variety of antigens. This increased breadth of the T cell response suggests that ipilimumab works by activating T cells directed against previously untargeted tumor-related antigens, rather than by boosting preexisting immune responses. If so, similar strategies could be successful in battling other cancers. — ACC

Sci. Transl. Med. **6**, 254ra128 (2014).

QUANTUM GASES

Aligning a magnetic atomic gas

When a bunch of fermions get together, they obey the Pauli exclusion principle: No two fermions can be in the same quantum state. The fermions populate the available states, starting from those lowest in energy. The boundary between the empty and filled states is called the Fermi surface (FS). For cold gases of fermionic atoms in the lab, the FS is usually spherical. Now, Aikawa *et al.* observe the FS squishing in a gas of Er atoms, which behave

like tiny magnets and align with their magnetic field environment. The squishing reflects the very directional interactions between the Er atoms. — JS

Science, this issue p. 1484

SOCIAL SCIENCE

The file drawer is full. Should we worry?

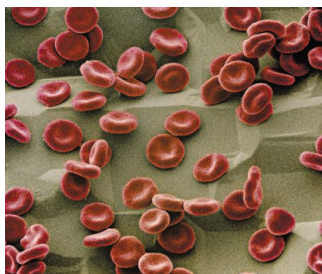
Experiments that produce null results face a higher barrier to publication than those that yield statistically significant differences. Whether this is a problem depends on how many null but otherwise valid results might be trapped in the file drawer. Franco *et al.* use a Time-sharing Experiments in the Social Sciences archive of nearly 250 peer-reviewed proposals of social science experiments conducted on nationally representative samples. They find that only 10 out of 48 null results were published, whereas 56 out of 91 studies with strongly significant results made it into a journal. — GJC

Science, this issue p. 1502

CORD BLOOD EXPANSION

Human adult stem cell expansion

Transfused blood saves lives. Despite the widespread use of this critical resource, it is difficult to increase blood cell numbers outside of the body. By screening thousands of small compounds, Fares *et al.* identify a molecule that expands human stem cell numbers in cord blood. The researchers generate many variations of that molecule and show that one such compound



Cord blood is valued for stem cells

provides even greater human blood cell expansion. If researchers can provide increased numbers of stem cells and progenitor cells, cord blood should find even greater use in the clinic. — BAP

Science, this issue p. 1509

NEUROMUSCULAR DISEASE

Building connections by gene therapy

Voluntary movement requires a molecular conversation between nerves and muscles. This conversation occurs at the neuromuscular junction, a structure where nerves and muscle physically connect. People with diseases characterized by muscle weakness have aberrantly small neuromuscular junctions. Arimura *et al.* used gene therapy to enlarge the neuromuscular junction, which made muscles stronger. Studying mouse models of two distinct neuromuscular disorders, they used an adenovirus vector to deliver *DOK7*, a gene required for formation of the neuromuscular junction. The therapy improved the mice's motor activity and life span. — PAK

Science, this issue p. 1505

SEPSIS

One protein smothers other deadly ones

Sepsis is a condition in which the body responds to infection with inflammation, which then spirals out of control. During sepsis, dead cells release histones, proteins normally found in the nucleus, which can kill nearby healthy cells. Daigo *et al.* discovered that a protein found in the circulation called pentraxin 3 interacted with histone proteins. Pentraxin 3 bundled the histones into aggregates that no longer killed healthy cells. When the researchers injected pentraxin 3 into mice with sepsis, the mice had less inflammation and survived. Thus, pentraxin 3 might be an effective therapy against sepsis in humans. — JFF

Sci. Signal. **7**, ra88 (2014).

IN OTHER JOURNALS

Edited by **Melissa McCartney** and **Jesse Smith**



Default choices drive taxi tipping

BEHAVIOR

Biting into Big Data in the Big Apple

Default options are an effective means of influencing consumer choices: With opt-out choices as defaults, people donate more organs and set aside more money for retirement. But how do people respond when faced with short-term choices in everyday life? Haggag and Paci analyzed 13 million taxi rides taken in New York City in 2009. They find that most customers press one of the default option buttons (for example, a \$3 tip for a ride under \$15) that specify the amount to tip the driver—enough to raise the average amount tipped across the full sample. Alternatively, some riders opt instead to leave no tip at all. — GJC

Am. Econ. J. Appl. Econ. **10**, 1257/app.6.3.1 (2014).

CATALYSIS

Simpler coating of silica surfaces

Organic coatings often are applied to silica surfaces found in optics, catalysts, and drug delivery systems in order to improve their functional properties. These coatings typically are formed by reacting surficial hydroxyl groups with organosilicon compounds. However, that approach requires either stringent reaction conditions or long reaction times. Moitra *et al.* show that silica can be coated in

a few minutes with hydrosilanes, using a fluoro-organoborane catalyst. Hydrosilanes can bear a wide variety of functional groups, making this a versatile method. — PDS

J. Am. Chem. Soc. **136**, 11570 (2014).

ANTIBIOTIC RESISTANCE

Clone wars: Let's pick out the superbugs

Since the 1960s, group B streptococci (GBS) have lost genetic diversity, yet they cause more virulent neonatal infections and

show high rates of tetracycline resistance. The puzzle is that tetracycline is not used to treat GBS. Nevertheless, since the 1950s, most GBS clones were wiped out by the collateral effects of the widespread use of tetracycline and other antibiotics, even though they were not the treatment target. Da Cunha *et al.* show that a few GBS clones survived by virtue of their persistence or virulence, and these clones also acquired mobile genetic elements carrying a mixture of antibiotic resistance genes, including those for tetracycline resistance. — CA

Nat. Comm. 10.1038/ncomms5544 (2014).

SIGNALING BONE

Signaling back and forth for cartilage

Skeletal bones provide body structure and protect inner tissues, but if they form in the wrong place, major problems result. Bone development begins with the aggregation of mesenchymal cells and their association with cartilage cells, which are then replaced by bone. Signaling molecules called Wnts limit expression of the SOX9 gene, which is needed for cartilage development. Too much SOX9 makes inappropriate cartilage. Kumar *et al.* show that Wnts limit cartilage development in the limb by chemically modifying the Sox9 gene and the histone protein that surrounds part of the gene. Another cell signal, called FGF, promotes cartilage development by blocking chemical modification. — BAP

Cell Rep. 10.1016/j.celrep.2014.07.038 (2014).

IMMUNOLOGY

Gut bugs may boost flu shots' effects

Why do some people get the flu after the flu shot? The answer may be in the gut. While studying gene expression and the strength of the immune response in people infected with influenza vaccine, Oh *et al.* found

a surprise: The gene that codes for the protein toll-like receptor 5 (TLR5) which is linked to gut bacteria, was also linked to strong immune response. The team gave flu vaccine to mice, some engineered to lack gut microbes and some to lack the gene for TLR5. Seven days later, the mice had significantly reduced levels of vaccine-specific antibodies in their blood as compared with control mice, suggesting that bacterial signaling boosts the vaccine's effect. — KS

Immunity 10.1016/j.immuni.2014.08.009 (2014).

ANIMAL COGNITION

Getting by with a little help from eel friends

Collaboration is an essential component of human behavior. Many other species are known to collaborate as well. For example,

coral trout collaborate with moray eels, recruiting the eels to flush out prey hidden in crevices. Vail *et al.* show that the trout chose to recruit eels under the appropriate conditions (i.e., when prey were hidden). Further, after their first experience with particular eel collaborators, the trout more often chose those eels that were more willing to join in. These results suggest that the cognitive ability to assess appropriate collaborators and conditions is more widespread than previously believed, and likely due more to ecological need than shared ancestry. — SNV

Curr. Biol. 10.1016/j.cub.2014.07.033 (2014).

PHYSICS

Magnetism leads to superconductivity

Some materials become perfect conductors of electricity—superconductors—when they are

cooled to low enough temperatures. In a superconducting material, electrons pair up with each other to form Cooper pairs, a counterintuitive phenomenon because we usually think of electrons as mutually repulsive. The electrons in these pairs must be “glued” together with a mediating interaction, but the nature of the glue is known reliably only for the simplest, conventional kind of superconductors. Van Dyke *et al.* provide strong evidence that magnetic interactions mediate the pairing in the unconventional superconductor CeCoIn₅. They used a scanning tunneling microscope to measure the electronic structure of the material, and then used those measurements to extract the material's magnetic interactions and calculate its superconducting properties. The results agreed well with independent experiments. — JS

Proc. Natl. Acad. Sci. USA 111, 11663 (2014).



Geyser remnant of the Heise volcanic super-eruption

VOLCANOLOGY

Reservoir assembly drives super-eruptions

Volcanic super-eruptions are hundreds to thousands of times larger than nearly any volcanic eruption that has occurred in recorded human history. Woltzlaw *et al.* looked for clues about what triggers these unimaginable events in minerals that formed in volcanic rocks. They analyzed zircons from the last super-eruption of the now-extinct Heise volcanic field and found that erupted magma was stored underground for a surprisingly short time, only about 5000 years. Furthermore, magma production occurred in distinct batches, forming thin sheetlike structures called sills. When many sills located at different depths connect with each other, the magma quickly becomes more buoyant, which may trigger these massive eruptions. — BG

Geology 10.1130/G35979.1 (2014).

ALSO IN SCIENCE JOURNALS

Edited by Melissa McCartney and Margaret Moerchen

GENE REPRESSION

Establishing memory of gene repression

Although cells in the body contain the same DNA content, they can display widely varying form and function among tissues. This comes about by differential gene regulation and by establishing a type of gene expression memory that is passed down during cell division to daughter cells. Gaydos *et al.* report that in nematodes, both sperm and oocytes transmit a memory of chromatin repression to embryos in the form of modified histones. During DNA replication, modified histones are passed to daughter chromatids to provide chromatin memory for a few cell divisions. Histone-modifying enzymes replenish histone modifications and provide long-term chromatin memory. — BAP

Science, this issue p. 1515

DNA BIOCHEMISTRY

Regulating DNA building blocks

The building blocks of DNA, deoxynucleotide triphosphates, are synthesized by the enzyme ribonucleotide reductase (RNR). Its central role in DNA synthesis and its aberrant expression in tumors have made it an important anticancer target, but nucleoside analog inhibitors of RNR can have adverse side effects. Arnaoutov and Dasso identify IRBIT (IP₃-receptor-

binding protein released with inositol 1,4,5-trisphosphate) as an RNR-binding protein. IRBIT regulates the activity of RNR by stabilizing the inactive form bound to deoxyadenosine triphosphate in the low-affinity A site. In turn, phosphorylation of IRBIT regulates its RNR-binding activity. IRBIT provides a new target for RNR inhibition that might avoid some of the side effects of current drugs. — GR

Science, this issue p. 1512

QUANTUM SIMULATION

Studying magnetism with cold atoms

Physicists have used cold atomic gases to simulate quantum phenomena that we normally associate with much more complex systems, such as solid-state materials. Because the properties of these gases are so tunable, it is also possible to create conditions that otherwise do not exist in nature. Zhang *et al.* studied an exotic type of magnetism in an array of pancake-shaped atomic clouds of ⁸⁷Sr. They prepared the atoms' nuclei in different combinations of 10 quantum states. They then shone pulses of light at the atoms to deduce the properties of the atoms' interactions. The interactions were independent of the atoms' nuclear states—a hallmark of an unusual symmetry that theorists predict may lead to interesting collective effects. — JS

Science, this issue p. 1467

ORGANIC ELECTRONICS

Organic semiconductors go out for a spin

Magnetism is a commonly observed phenomenon in the macroscopic world, but its origins lie in the quirky quantum-mechanical property of electrons and certain nuclei known as spin. Recent research has sought to leverage and expand the role of spin in the operation of electronic devices. Malissa *et al.* used a highly sensitive spectroscopic technique to probe, and ultimately manipulate, the subtle effects of spin interactions on the current that flows through organic light-emitting diodes (OLEDs) (see the Perspective by Bobbert). They pinpointed coupling between the spins of the current carriers and the hydrogen nuclei in the hydrocarbon-based material making up the device. — JSY

Science, this issue p. 1487;
see also p. 1450

MOOD REGULATION

A pathway that controls our mood

A brain area called the lateral habenula is involved in negative motivation and may thus play a role in depression. Shabel *et al.* investigated synaptic transmission in a brain pathway to the lateral habenula that transmits disappointment signals. Surprisingly, they found the simultaneous release of two

antagonistic substances, glutamate and GABA, from individual nerve cells. In an animal model of depression, GABA release was reduced in this pathway. A widely used antidepressant drug, however, increased GABA co-release. These results reveal an unusual synaptic mechanism that affects lateral habenula activity. This mechanism may be instrumental for regulating the emotional impact of disappointment. — PRS

Science, this issue p. 1494

STRUCTURAL BIOLOGY

A foreign-DNA-destroying machine

Bacteria have an adaptive immune system, called CRISPR, that identifies invading viruses through their DNA or RNA sequences and cuts them up (see the Perspective by Zhang and Sontheimer). Jackson *et al.* and Mulepati *et al.* have determined the structure of the large protein complex, called Cascade, that targets the invading nucleic acids and does the cutting. The seahorse-shaped structure reveals how the 11 subcomponents of Cascade assemble into the final protein complex. The structure also shows how Cascade presents the short CRISPR-derived RNAs so that they can bind and target foreign DNA. — GR

Science, this issue p. 1473 and p. 1479;
see also p. 1452

REVIEW SUMMARY

CELL BIOLOGY

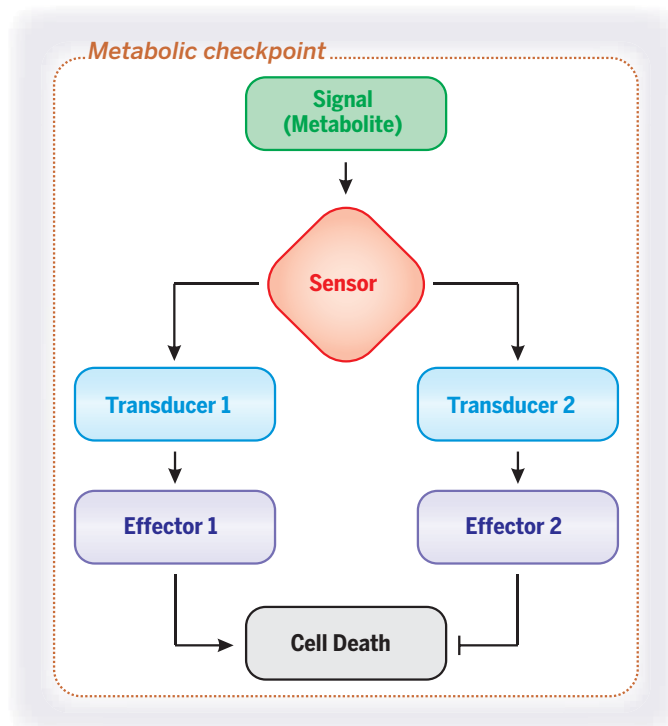
Metabolic control of cell death

Douglas R. Green,^{1*} Lorenzo Galluzzi,^{2,3,4} Guido Kroemer^{2,3,4,5,6*}

BACKGROUND: For several decades, intermediate metabolism and signal transduction have been considered two independent entities. On one side stood the catabolic and anabolic reactions that provide cells with the energy and building blocks required for life. On the other side were the cascades of transcriptional events and posttranscriptional modifications that control nearly all cellular processes, including regulated variants of cell death such as apoptosis and regulated necrosis. This somewhat simplistic perception has been instrumental for the detailed characterization of several metabolic circuitries and signaling pathways. However, owing to this theoretical construction, the intimate cross-talk between metabolism and cell death has been largely disregarded until recently, even though organelles with major metabolic functions, such as mitochondria, were shown to play critical roles in regulated cell death nearly 20 years ago.

ADVANCES: It is now clear that metabolism and cell death are deeply intertwined at several levels. Many proteins that mediate vital metabolic functions also have key activities in the transduction of cell death-regulatory signals. An example is holocytochrome c, which not only shuttles electrons between respiratory complex III and IV [hence required for oxidative phosphorylation and mitochondrial adenosine triphosphate (ATP) synthesis] but also promotes the lethal activation of caspases in response to mitochondrial outer membrane permeabilization. Conversely, several proteins that were originally characterized for their capacity to regulate cell death have been found to control metabolic cir-

cuitries. This applies to various antiapoptotic members of the Bcl-2 protein family, which play a critical role in the regulation of mitochondrial outer membrane permeabilization in apoptosis and influence intracellular Ca^{2+} fluxes (hence affecting



Metabolic control of cell death. Cells are provided with multiple metabolic checkpoints that sense specific metabolic variables and generate a biological response through one or more transducers and effectors. Such basic checkpoints are highly interconnected, making up a complex network that controls cell fate in response to metabolic perturbations.

many metabolic activities), the efficacy of mitochondrial ATP synthesis (by binding to the F₁F₀ ATP synthase), and autophagic flux (by engaging in physical interactions with beclin-1). Moreover, several metabolic intermediates, including (but not limited to) ATP, acetyl-coenzyme A (CoA), oxidized nicotinamide adenine dinucleotide (NAD⁺), NAD phosphate (NADP⁺), and reactive oxygen species, are intimately involved in signal transduction cascades that influence the propensity of cells to commit to die.

OUTLOOK: We propose the existence of several “metabolic checkpoints,” that is, refined molecular mechanisms that sense a panel of metabolic variables and emit one or more signals controlling cell fate. Most often, these checkpoints react to metabolic imbalances by activating an organelle-specific or cellwide adaptive response that, at least initially, attempts to reestablish homeostasis. However, when metabolic perturbations are excessively severe or protracted in time, metabolic checkpoints become capable of initiating apoptotic or necrotic forms of regulated cell death. Accumulating evidence indicates that metabolic checkpoints of this type are in

place to monitor the ATP/ADP, acetyl-CoA/CoA, NAD⁺/NADH, and NADP⁺/NADPH ratios, as well as the abundance of lipid products, glycosylated proteins, and reactive oxygen species. Perturbations of any such metabolic variables (signals) are detected by specific systems (sensors) and converted into vital or lethal stimuli, which are dispatched to components of the cell death-regulatory machinery (targets) through one or more signaling nodes (transducers). Metabolic checkpoints therefore integrate various “bits” of information into a biological response that inhibits or promotes cell death. It is tempting to speculate that a detailed understanding of metabolic checkpoints might allow for the development of novel pharmacological approaches that block or stimulate cell death by inducing specific metabolic alterations. Such interventions may be useful for the treatment of pathologies involving unwarranted cell death, such as ischemic, infectious, and neurodegenerative disorders, as well as pathological states in which the cellular

demise is intrinsically blocked, as seen in autoimmune diseases and neoplastic conditions. ■

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REVIEW

CELL BIOLOGY

Metabolic control of cell death

Douglas R. Green,^{1*} Lorenzo Galluzzi,^{2,3,4*} Guido Kroemer^{2,3,4,5,6†}

Beyond their contribution to basic metabolism, the major cellular organelles, in particular mitochondria, can determine whether cells respond to stress in an adaptive or suicidal manner. Thus, mitochondria can continuously adapt their shape to changing bioenergetic demands as they are subjected to quality control by autophagy, or they can undergo a lethal permeabilization process that initiates apoptosis. Along similar lines, multiple proteins involved in metabolic circuitries, including oxidative phosphorylation and transport of metabolites across membranes, may participate in the regulated or catastrophic dismantling of organelles. Many factors that were initially characterized as cell death regulators are now known to physically or functionally interact with metabolic enzymes. Thus, several metabolic cues regulate the propensity of cells to activate self-destructive programs, in part by acting on nutrient sensors. This suggests the existence of “metabolic checkpoints” that dictate cell fate in response to metabolic fluctuations. Here, we discuss recent insights into the intersection between metabolism and cell death regulation that have major implications for the comprehension and manipulation of unwarranted cell loss.

All living things metabolize nutrients as a source of energy and building blocks for their components to survive, and ultimately all living things die. When we apply these simple concepts to cells, the notion that the biochemistries of metabolism and cell death intimately interact is obvious. In this Review, we take a largely mammal-centric view of the interplay between metabolic and cell death pathways.

The survival of animal cells critically relies on an energy source (be it extrinsic or, if necessary, provided by the autophagic, lysosomal, or proteasomal degradation of cellular components). In the absence of such a source, cells lose the control of plasma membrane channels and are doomed to passive necrosis (which can also occur upon excessive physical damage to the plasma membrane). This said, our discussion focuses on those forms of cellular demise that require an active engagement of the cell. In this regard, it is useful to consider that such a participation can be of two types: (i) cellular “suicide,” in which cells engage molecular circuitries that have (presumably) evolved to precipitate their demise (i.e., apoptosis and some forms of regulated necrosis); and (ii) “subversion,” in which a cellular process drives cell death when disrupted, although (at least presumably) it did not evolve as a cell

death mechanism (1). The latter is akin to a sabotage: Dislodging a railway tie can destroy a train only if the train is moving. This dichotomy can help in understanding how there can be so many forms of cell death (supplementary text), although from some perspectives (e.g., the design of anticancer therapies and the control of pathological cell death) such a distinction may not be of overwhelming concern.

Apoptosis as well as regulated necrosis are accompanied by a sudden loss of metabolic functions that, once a threshold of deterioration has been trespassed, seal the cell's irreversible fate (2). Beyond the cell death-associated loss of essential bioenergetic functions, metabolism and its derangement have a major impact on the avoidance, initiation, and execution of cell death at multiple levels. Here, we consider how metabolic signals are sensed and transduced to have an impact on active cell death pathways.

Metabolic checkpoints in cell fate: Signals, sensors, transducers, and effectors

Nutrient and oxygen supplies, activation (reflecting extracellular signals or intracellular events, such as oncogenic mutations), as well as the differentiation state of a cell influence the metabolic pathways engaged for the production of energy and biomaterials. In response to changes in these conditions, cells either adapt or die. Extending a classical convention referring to the cell cycle and DNA damage response (3), we propose the existence of metabolic checkpoints that dictate the consequences of such alterations on cell fate. Metabolic checkpoints can be defined as molecular mechanisms that regulate cellular functions in response to metabolic fluctuations and comprise four components: signals, sensors, transducers, and effectors (4). In our discussion of the

metabolic control of cell death, we consider these in terms of either the signal that promotes downstream events (perhaps through different sensors) or the sensor that coordinates one or more signals. Although this nomenclature is admittedly arbitrary, we suggest that the checkpoints we propose are useful starting blocks to probe how different metabolic processes feed into the cell fate decision, engaging processes that promote active death (Fig. 1).

Major metabolic signals that arise as a consequence of changes in nutrient availability or intracellular metabolic pathways include the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, the acetyl-coenzyme A (acetyl-CoA)/CoA ratio, the ratios of oxidized and reduced nicotinamide adenine dinucleotide (NAD^+/NADH) and NAD phosphate ($\text{NADP}^+/\text{NADPH}$), as well as the amounts of lipid products, glycosylated proteins, and reactive oxygen species (ROS). For illustrative purposes, we distinguish these signals from second messengers, such as cyclic adenosine monophosphate (cAMP), phosphoinositides, and ion (including Ca^{2+}) fluxes. However, the frontier between metabolism and signaling may be less defined than previously thought (5). Specific sensors directly interact with these metabolic cues to initiate downstream events, thereby affecting signal transducers, including those involved in cell death regulation. Of note, for a sensor to be considered so, it must possess a substrate affinity for the signal that allows it to function in physiological (or pathophysiological) conditions. Our consideration of sensors within metabolic checkpoints attempts to take this concept into account, but at least in some cases this has not been formally determined. We discuss specific examples below.

The mitochondrial checkpoints: MOMP, MPT, and mitochondrial dynamics

Mitochondria are central to the control of cell life and death and are fundamentally involved in metabolism because they are responsible for energy production through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (fueled by glycolysis, glutaminolysis, β -oxidation, and other sources), as well as for the synthesis of lipids, pyrimidines, heme moieties, some amino acids, and other biomolecules. Moreover, mitochondria are the major intracellular source of ROS. As such, they are under extensive metabolic control, as is their biogenesis and removal.

Mitochondria control cell fate in four fundamental ways: (i) through mitochondrial outer membrane permeabilization (MOMP), leading to apoptosis; (ii) through the mitochondrial permeability transition (MPT), leading to regulated necrosis; (iii) by providing an energy supply; and (iv) by participating in the synthesis of several products, including lipid precursors, iron-sulfur clusters, and nucleotides (Fig. 2). Cells that have been depleted of mitochondria through an artificial widespread wave of mitophagy are resistant to apoptosis (6). However, despite assertions that a nonapoptotic form of cell death, necroptosis (supplementary text), is executed

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by mitochondrial alterations, cells lacking the vast majority of their mitochondria remain sensitive to this form of cellular demise (6). In contrast, mitochondria can precipitate other forms of necrosis via the MPT.

Mitochondria are the only cellular source of holocytochrome c, which is normally sequestered in the mitochondrial intermembrane space, where it operates as an essential electron shuttle of the respiratory chain. In response to some lethal stimuli, however, mitochondria can undergo MOMP, a process that is executed and regulated by Bcl-2 family proteins (Fig. 2). Upon MOMP, holocytochrome c and other mitochondrial proteins are released into the cytosol, where they cooperate with cytosolic factors to promote the activation of caspases. This function of holocytochrome c is regulated by its redox state. Oxidized holocytochrome c does not activate caspases (7), and some cells can survive MOMP owing to an enhanced rate of glycolysis (7, 8). The survival of cells undergoing MOMP is facilitated by the enforced expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enabling the regeneration of the mitochondrial pool from a small pool of organelles that resisted permeabilization (8, 9). The demise of cells in which MOMP does not (or cannot) activate caspases

occurs by energetic catastrophe (10) or upon the release of mitochondrial factors that trigger caspase-independent cell death mechanisms, such as apoptosis-inducing factor (AIF) (11).

The continuous fission and fusion of the mitochondrial network (generally referred to as mitochondrial dynamics) is crucial for the biogenesis of these organelles as well as for the segregation and autophagic removal of dysfunctional mitochondria, thereby playing a key role in the control of intracellular homeostasis (12, 13). Fission relies on the small guanosine triphosphatase (GTPase) dynamin 1-like (DNM1L, best known as DRP1), which operates by interacting with mitochondrial fission factor (MFF) on the outer mitochondrial membrane (OMM) to constrict both mitochondrial membranes and eventually divide them (14). Fusion requires the interaction of mitofusin 1 (MFN1) and MFN2, two OMM proteins, with optic atrophy 1 (OPA1), which is found at the inner mitochondrial membrane (IMM). Fusion (but not fission) of the IMM depends on the transmembrane potential ($\Delta\psi_m$) generated by the respiratory chain. Thus, $\Delta\psi_m$ dissipation favors mitochondrial fragmentation (15). Mitochondrial dynamics are highly sensitive to metabolic cues. In line with this notion, mitochondria form a hyperfused network characterized by an increased

amount of IMM cristae in conditions of low nutrient availability. This is presumed to increase the efficiency of the respiratory chain while restricting the autophagic removal of mitochondria. Conversely, mitochondria generally fragment in cells exposed to excess nutrients, a situation that presumably decreases the bioenergetic efficacy of the organelles and favors the removal of lipids and other potentially toxic molecules (16).

Mitochondrial dynamics reportedly influence the propensity of the organelles to undergo MOMP, but the precise mechanisms remain partially understood. MOMP has been suggested to preferentially occur at fission sites (17), which are also points of contact with the endoplasmic reticulum (ER) (18). Studies in cells (19) and isolated mitochondria (20) revealed that at least one of the proapoptotic Bcl-2 family members BAX or BAK1 is necessary for mitochondrial fusion through interaction with MFN1 and MFN2, a function that is lost upon the induction of MOMP (21). Along similar lines, the antiapoptotic protein BCL-X_L appears to regulate mitochondrial fusion, either by interacting with MFN2 (22) or by sequestering the active forms of BAX and BAK1 (23). Conversely, silencing or inhibiting DRP1 dampens BAX- or BAK1-dependent MOMP (17, 24, 25), but this effect may be unrelated to the

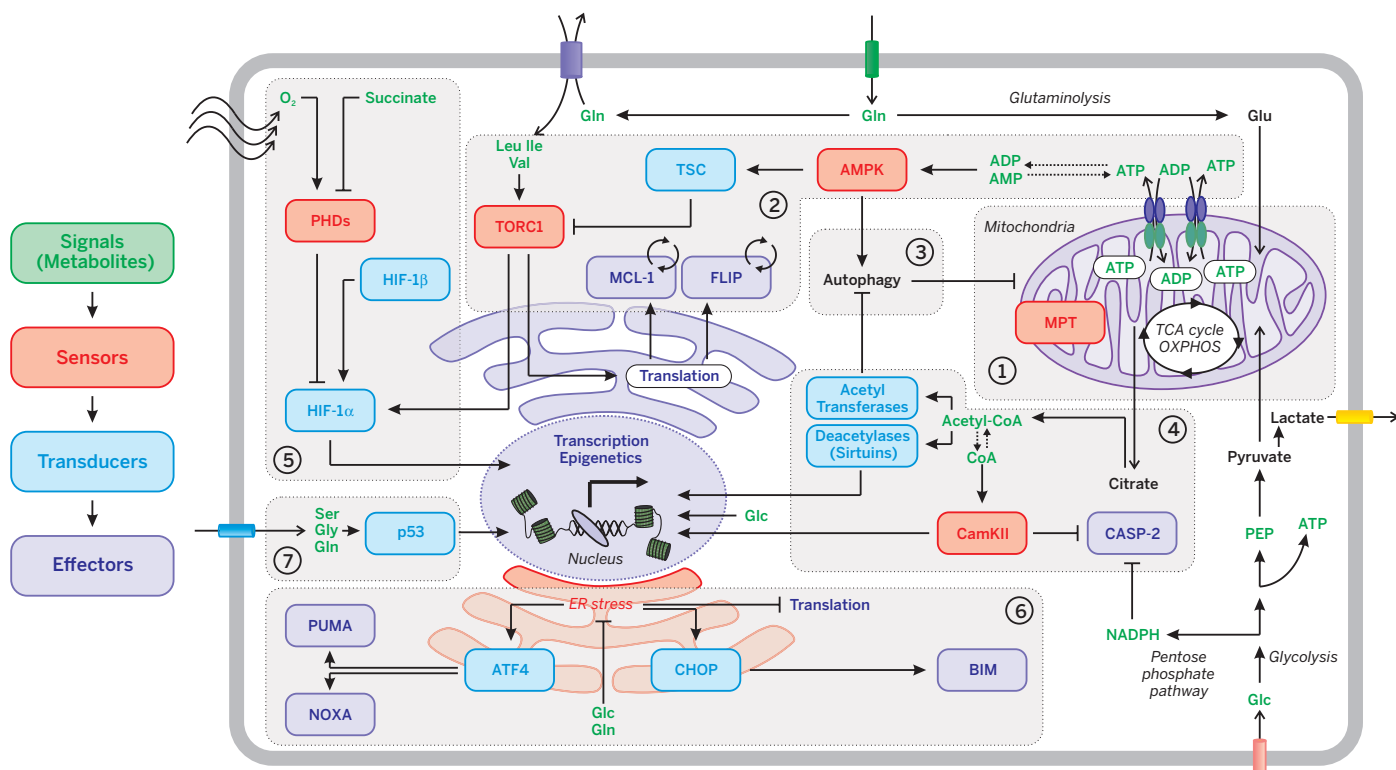


Fig. 1. Metabolic checkpoints in cell death regulation. Several metabolic checkpoints are in place to convert metabolic perturbations (signals), which are detected by specific systems (sensors), into vital or lethal stimuli that are dispatched to components of the cell death-regulatory machinery (effectors) through one or more signaling nodes (transducers). These include (but are not limited to) the mitochondrial checkpoint, in part impinging on the so-called MPT (1); the AMPK-TORC1 checkpoint, which is based on the very short half-life of antiapoptotic proteins such as FLIP_L and MCL-1 (2); the autophagy

checkpoint, which is extensively interconnected with other checkpoints (3); the acetyl-CoA/CoA checkpoint, which controls cell death through both transcriptional and posttranslational mechanisms (4); the HIF-1 checkpoint, integrating signals about oxygen availability and TCA cycle proficiency (5); the ER stress checkpoint, which operates by altering the abundance of multiple BH3-only proteins (6); as well as the p53 checkpoint, detecting the availability of non-essential amino acids and converting it into an adaptive or lethal response (7). Glc, glucose; OXPHOS, oxidative phosphorylation; PEP, phosphoenolpyruvate.

role of DRP1 in the regulation of mitochondrial dynamics. It has indeed been suggested that DRP1 may favor MOMP by increasing the tension of the OMM (25). Thus, alterations in nutrient availability or other metabolic conditions affecting mitochondrial dynamics may influence the propensity of cells to undergo MOMP-driven apoptosis. Conversely, shifts in the mutual interactions between pro- and antiapoptotic Bcl-2 family members as induced by other metabolic checkpoints (see below) may affect mitochondrial dynamics, hence affecting their bioenergetic efficacy. Intriguingly, the ability of BAX to control mitochondrial dynamics has been proposed to lower the threshold for MPT, hence affecting regulated variants of necrosis (26). This possibility, however, remains to be explored in detail.

The ATP synthasome is a multiprotein complex that produces ATP and exports it from mitochondria in exchange for ADP and inorganic phosphate (27). The ATP synthasome is composed of the F_1F_0 -ATP synthase and two mitochondrial solute-carrier (SLC) family members: adenine nucleotide translocase (ANT), which exists in three isoforms (official names are SLC25A4, SLC25A5,

and SLC25A6 but best known as ANT1, ANT2, and ANT3, respectively) and exchanges ADP for ATP, and the inorganic phosphate carrier (PIC, officially named SLC25A3), which mediates the mitochondrial uptake of phosphate. Beyond their function in ATP synthesis, the components of the ATP synthasome, individually or together, may regulate cell death as driven by the MPT, a sudden increase in the permeability of the IMM to solutes <1.5 kD. This occurs in response to increased intracellular Ca^{2+} concentrations or oxidative stress and results in $\Delta\psi_m$ dissipation, uncoupling of the respiratory chain, influx of H_2O and ions, osmotic swelling of the mitochondrial matrix, and consequent mechanical breakdown of the OMM (28).

The concentration of Ca^{2+} ions required for induction of the MPT is increased by ~twofold upon addition of cyclosporine A (CsA) or knock-out of the gene coding for cyclophilin D (CYPD, official name is peptidyl-prolyl isomerase F, PPIF), a CsA-inhibitable enzyme of the mitochondrial matrix. CYPD can be immunoprecipitated with ANT in cells responding to MPT inducers, and this interaction can be blocked by CsA (28). Therefore, CYPD appears to bind the ATP synthasome

to form the so-called permeability transition pore complex (PTPC), the supramolecular entity that is responsible for the MPT (Fig. 2). In stringent conditions of coimmunoprecipitation, CYPD interacts with the oligomycin sensitivity-conferring protein (OSCP) but not with other subunits of the F_1F_0 -ATP synthase lateral stalk (29). Among various proteins that have been suggested to compose the PTPC, including ANT and voltage-dependent anion channel (VDAC, an OMM-embedded protein), so far only CYPD has been ascribed with a critical role in MPT (30). Partial depletion of PIC also fails to affect MPT. Conversely, the knockdown of either OSCP or the c subunit of F_0 alters the propensity of mitochondria to undergo MPT *in vitro* and *in cellula* (29, 31). These observations suggest a major role for the F_1F_0 -ATP synthase in MPT.

ANT also interacts with proteins that are not involved in the ATP synthasome, including VDAC (Fig. 3). In turn, VDAC (which constitutes a preferential channel for the export of mitochondrial ATP across the OMM) binds cytosolic hexokinase II (HKII). This interaction provides HKII (which catalyzes the initial, ATP-consuming step

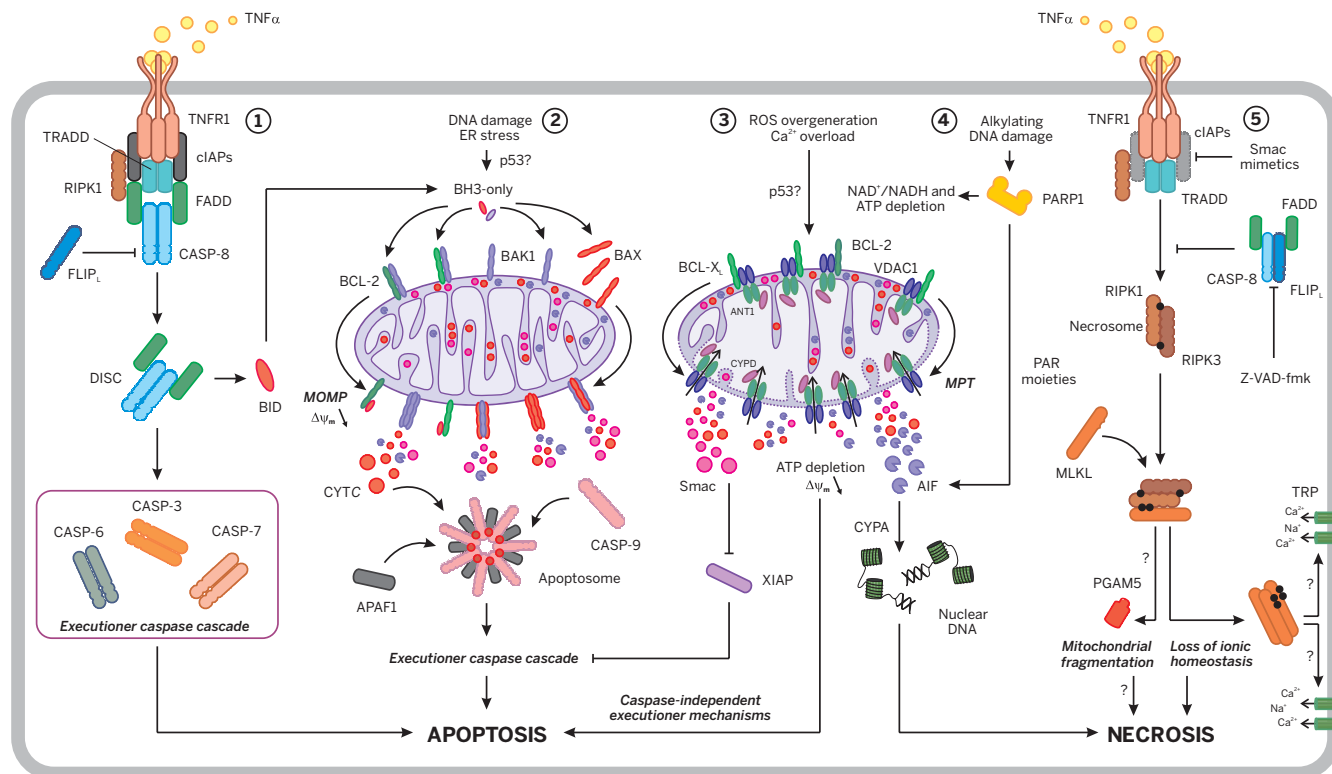


Fig. 2. Major signal transduction cascades leading to active cell death.

The ligation of death receptor such as tumor necrosis factor receptor 1 (TNFR1) generally results in the assembly of a supramolecular complex at the inner leaflet of the plasma membrane known as a death-inducing signaling complex (DISC), which is under the control of FLIP_L. By promoting the activation of CASP-8, the DISC directly ignites the caspase cascade that is responsible for apoptotic cell death (1). Similarly, the executioner caspase cascade can be set off upon MOMP (2) or the so-called MPT (3), owing to the release into the cytosol of caspase activators as well as to the engagement of caspase-independent mechanisms. Death receptor-elicited apoptosis and MOMP are not entirely disconnected, as in some cells CASP-8 cleaves BID to generate a MOMP-promoting factor. In some settings, MPT can trigger necrotic forms of

cell death, at least in part owing to the release of AIF, a mitochondrial protein with latent endonucleolytic activity that is also involved in the PARP1-dependent lethal cascade elicited by alkylating DNA damage (4). Of note, the ligation of TNFR1 in the presence of Z-VAD-fmk (a pan caspase inhibitor), alone or combined with a Smac mimetic, drives a specific instance of regulated necrosis known as necroptosis (5). Necroptosis, which is inhibited by a supramolecular complex involving FADD, CASP-8, and FLIP_L, obligatorily relies on the formation of MLKL oligomers that relocate to the plasma membrane and disrupt ionic homeostasis. CYPD, cyclophilin D (official name, peptidylprolyl isomerase A); CYTC, holocytochrome c; TRADD, TNFRSF1A-associated via death domain; TRP, transient receptor potential cation channel; XIAP, X-linked inhibitor of apoptosis.

of glycolysis) with a privileged access to mitochondrial ATP. Moreover, the binding of HKII to VDAC reduces the propensity of cells to undergo lethal MPT (32). It is an open conundrum whether this effect is direct or involves fluctuations in the local gradients of ATP, ADP, inorganic phosphate, and protons, all of which regulate MPT (28).

Rapid openings (“flickering”) of the PTPC may avoid a potentially toxic accumulation of Ca^{2+} ions in the mitochondrial matrix, which may explain why the myocardium of *Ppif*^{-/-} mice ages with an accelerated kinetics (33). Conversely, widespread (affecting most mitochondria within a cell) and irreversible MPT has been linked to necrosis. Indeed, several organs from *Ppif*^{-/-} mice are relatively protected against ischemia-reperfusion damage, which is mostly necrotic. Such a phenotype is even more pronounced when *Ppif*^{-/-} mice are crossed with mice lacking the pronecrotic kinase receptor-interacting protein kinase 3 (RIPK3) or treated with the anti-necrotic agent necrostatin-1 (34), supporting the notion that CYPD and RIPK3 induce necrosis via mutually independent signaling pathways (6).

The mitochondrial checkpoint also influences the propensity of cells to die because it regulates the activation of caspase-1 by the nucleotide-binding domain and leucine-rich repeat-containing (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome (35). A number of clinically relevant stimuli, including monosodium urate crystals (associated with gout) (36), calcium pyrophosphate dihydrate crystals (associated with pseudogout) (36), and cholesterol crystals (associated with atherosclerosis) (37), activate the NLRP3 inflammasome through a mechanism that relies on mitochondrial ROS and VDAC1 (38). This may reflect the role of VDAC1 in mitochondrial metabolism. Of note, the activation of other inflammasomes, such as those orchestrated by absent in melanoma 2 (AIM2) and NLR family, caspase-associated recruitment domain (CARD) containing 4 (NLRC4), is independent of VDAC1 but may require VDAC2 (38). How the NLRP3 inflammasome is activated remains unknown. In some cases, this indeed occurs independently of ROS (39) and may rely on the translocation of cardiolipin (CL), a mitochondrion-restricted lipid, from the IMM to the OMM (40). Thus, alterations in mitochondrial functions, including ROS output, can have direct effects on the activation of the inflammasome and hence on inflammation and cell death.

The AMPK-TORC1 checkpoint

The target of rapamycin complex 1 (TORC1) is a major regulatory node that controls cell growth, proliferation, and autophagy through the kinase activity of mammalian target of rapamycin (mTOR). TORC1 is activated on the surface of lysosomes when amino acids, especially leucine, are available. Several signal transducers can inhibit mTOR, including glycogen synthase kinase 3 (GSK3), which operates only when AKT1 is inactive (e.g., upon growth factor withdrawal), and 5' AMP-activated protein kinase (AMPK), which

responds to declining ATP levels (owing to the adenylate kinase-dependent conversion of ADP into AMP) by phosphorylating (and thus activating) the mTOR-inhibitory protein tuberous sclerosis 2 (TSC2) (41), as well as by phosphorylating (and thus inactivating) regulatory associated protein of mTOR, complex 1 (RPTOR), a component of TORC1 (42). AMPK is activated even by mild perturbations of ATP synthesis, acting as a primary sensor of mitochondrial stress in concert with other mitochondrial checkpoints.

Besides regulating TORC1, AMPK controls a variety of metabolic processes. In particular, AMPK inhibits the synthesis of fatty acids, sterols, and ribosomal RNA while promoting catabolic processes such as glycolysis and fatty acid oxidation (43). In conditions of limited amino acid or growth factor availability, the activity of TORC1 is reduced independently of AMPK (44). Conversely, the inhibition of glycolysis by glucose deprivation, 2-deoxyglucose (45), or caloric restriction (46) promotes the apoptotic demise of many cell types upon the AMPK-induced inhibition of TORC1. In both settings, TORC1 inhibition causes the arrest of several anabolic processes, including protein translation. As a result, the abundance of the short-lived antiapoptotic Bcl-2 family member MCL-1 decreases, sensitizing the cell to mitochondrial apoptosis (46). In line with this notion, an unbiased approach identified MCL-1 as a target for the chemotherapy-enhancing effects of the TORC1 inhibitor rapamycin (47).

Because glucose uptake by plasma membrane transporters relies on growth factor receptor signaling, the withdrawal of growth factors also promotes apoptosis (48). This effect reflects the activation of GSK3, which phosphorylates and hence further destabilizes MCL-1. In line with this notion, cells expressing a MCL-1 variant that cannot be phosphorylated by GSK3 or exposed to GSK3 inhibitors resist growth factor withdrawal-induced cell death (49–52). Therefore, this metabolic checkpoint controls cell fate at least in part by influencing the abundance of MCL-1. Of note, in cells lacking the proapoptotic Bcl-2 proteins BAX and BAK1, the apoptotic response to growth factor withdrawal is substantially delayed and viability is preserved owing to the activation of the autophagic machinery (discussed below) (48).

Another short-lived protein is CASP8 and FADD-like apoptosis regulator (best known as cFLIP), a caspase-like protein that limits the proapoptotic activity of caspase-8 (as initiated by death receptors) and cooperates with caspase-8 to inhibit necroptosis (which manifests upon death receptor ligation in the presence of caspase inhibitors) (53). Glucose deprivation sensitizes cells to death receptor-induced apoptosis (45, 54), a phenomenon that may involve alterations in the abundance of both cFLIP and MCL-1. Indeed, cells lacking BAX and BAK1 (which are expected to be resistant to declining amounts of MCL-1) can succumb to caspase-8-dependent apoptosis in response to glucose deprivation (55), perhaps as a consequence of alterations in the amounts of cFLIP. Moreover, although glucose deprivation affects the expression of c-FLIP, this may be

independent of AMPK, at least in some cases (56). Thus, other metabolic checkpoints (as outlined below) are likely to influence cFLIP expression.

The overexpression of MYC, which occurs in some cancers, can cause an imbalance in ATP/ADP ratios and hence promote AMPK activation. In line with this notion, the increased sensitivity of MYC-expressing cells to the proapoptotic activity of a number of agents reportedly relies on this kinase (57). However, these effects of MYC overexpression were ascribed to the phosphorylation-dependent stabilization of a cytosolic pool of the tumor suppressor p53, resulting in the activation of BAK1 (57). Conversely, the roles of TORC1 and MCL-1 (which inhibits BAK1) in this setting were not examined.

Given the profound effects of the AMPK-TORC1 checkpoint on protein translation and the abundance of MCL-1, many metabolic conditions that result in TORC1 inhibition are expected to sensitize cells to death. These include amino acid deprivation, which—among many other effects (discussed below)—inhibits the catalytic activity of TORC1 in an AMPK-independent manner. Last, AMPK also promotes, whereas TORC1 inhibits, autophagy, as discussed in the next section.

The autophagy checkpoint

Macroautophagy (herein referred to as autophagy) is a major mechanism for the maintenance of intracellular homeostasis that can be dramatically up-regulated by nutrient deprivation and organellar damage (12, 58). Thus, autophagy is controlled by several metabolic cues, including the ATP/ADP ratio (by the AMPK checkpoint, see above) and the availability of acetyl-CoA, which affect the enzymatic activity of several acetyltransferases, notably E1A binding protein p300 (EP300) (59). The ability of autophagy to inhibit or promote cell death is an area of intense investigation (60, 61). Interestingly, permeabilized mitochondria are rapidly sequestered by autophagic vacuoles and degraded by mitophagy, a process that favors the maintenance of intracellular homeostasis (12, 13). Moreover, part of the mitochondrial network elongates and is hence spared from autophagic degradation in the course of adaptive responses to stress (62). These observations indicate that the mitochondrial and autophagy checkpoints are intimately interconnected.

Autophagy limits the cytotoxicity of tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10, best known as TRAIL) in cells that either do not (type I) or do (type II) require the mitochondrial machinery to succumb to death receptor-induced apoptosis. In contrast, FAS ligand (FASLG, also known as CD95L) promotes apoptosis upon the autophagic degradation of protein tyrosine phosphatase, nonreceptor type 13 (also known as FAP-1), which results in an increased expression of FAS (also known as CD95) on the cell surface (63). Autophagy can also promote cell death by degrading the antioxidant enzyme catalase, resulting in increased ROS production (64).

Type II cell death (which should not be confounded with type II cells, see above) has been defined as a cell death subroutine accompanied

by autophagy, although whether or not the autophagic machinery truly contributes to (rather than accompanies) cell death cannot be determined by morphological parameters alone (65). Type II death often manifests with large vacuoles that may be enlarged lysosomes or other structures. A form of nonapoptotic cell death that has recently been dubbed autosis is promoted by starvation (and therefore is likely to involve the AMPK-TORC1 checkpoint), hypoxia, ischemic insults, and autophagy-promoting peptides (66). Because autosis critically relies on several factors that are essential for autophagy, it may qualify as bona fide autophagic cell death (2). Similarly, *HRAS* induces the demise of several cell types through a pathway that manifests with type II morphological features and depends on essential autophagic mediators as well as the BH3-only protein phorbol-12-myristate-13-acetate-induced protein 1 (best known as NOXA) (67). This cell death subroutine can be inhibited by antiapoptotic Bcl-2 proteins, most likely because they sequester the critical autophagic factor beclin-1 (BECN1). NOXA as well as other BH3-only proteins promote autophagy by releasing BECN1 from inhibitory interactions with BCL-2 and its homolog, BCL-X_L (67, 68). It is therefore possible that the complex interactions between Bcl-2 family members and components of the autophagic machinery control not only apoptosis but also autophagic cell death.

How autophagy may promote cell death is unclear. Cells lacking BAX and BAK1 (which are unable to undergo MOMP) survive growth factor deprivation for long periods of time in an autophagy-dependent manner. These cells manifest increasing degrees of atrophy and eventually succumb without displaying overt features of type II cell death (48). It is possible, but remains to be formally demonstrated, that (at least in some settings) autophagy promotes cell death by favoring lysosomal membrane permeabilization. Lysosome-disrupting agents can actually provoke a nonapoptotic form of cell death (69), leaving this possibility open.

The acetyl-CoA/CoA checkpoint

The acetyl CoA/CoA ratio is dynamically regulated in response to metabolic functions. In mammalian cells, acetyl-CoA is mainly generated by glycolysis, and a large fraction of the intracellular acetyl-CoA pool is sequestered within mitochondria. In this setting, the extramitochondrial demand for acetyl-CoA is satisfied by a cytosolic variant of citrate lyase, which generates acetyl-CoA from citrate (an intermediate of the TCA cycle actively exported by mitochondria). Alternatively, acetyl-CoA can be produced from acetate by acetyl-CoA synthetase. A nuclear isoform of citrate lyase is required for the acetylation of histones and other proteins (70). Similarly, the deacetylating activity of sirtuins, which affects hundreds of proteins, is under a strict metabolic control.

From our perspective, acetylation and deacetylation regulate cell death by virtue of specific effectors. During the synthesis of fatty acids,

acetyl-CoA is consumed and CoA is produced. CoA acts directly on Ca²⁺/calmodulin-dependent protein kinase II (CamKII) to reduce its requirement for Ca²⁺ ions, thus effectively activating it (71).

One target of the enzymatic activity of CamKII is caspase-2, which upon phosphorylation is sequestered and kept inactive by 14-3-3ζ (72). Because the phosphorylation of caspase-2 requires

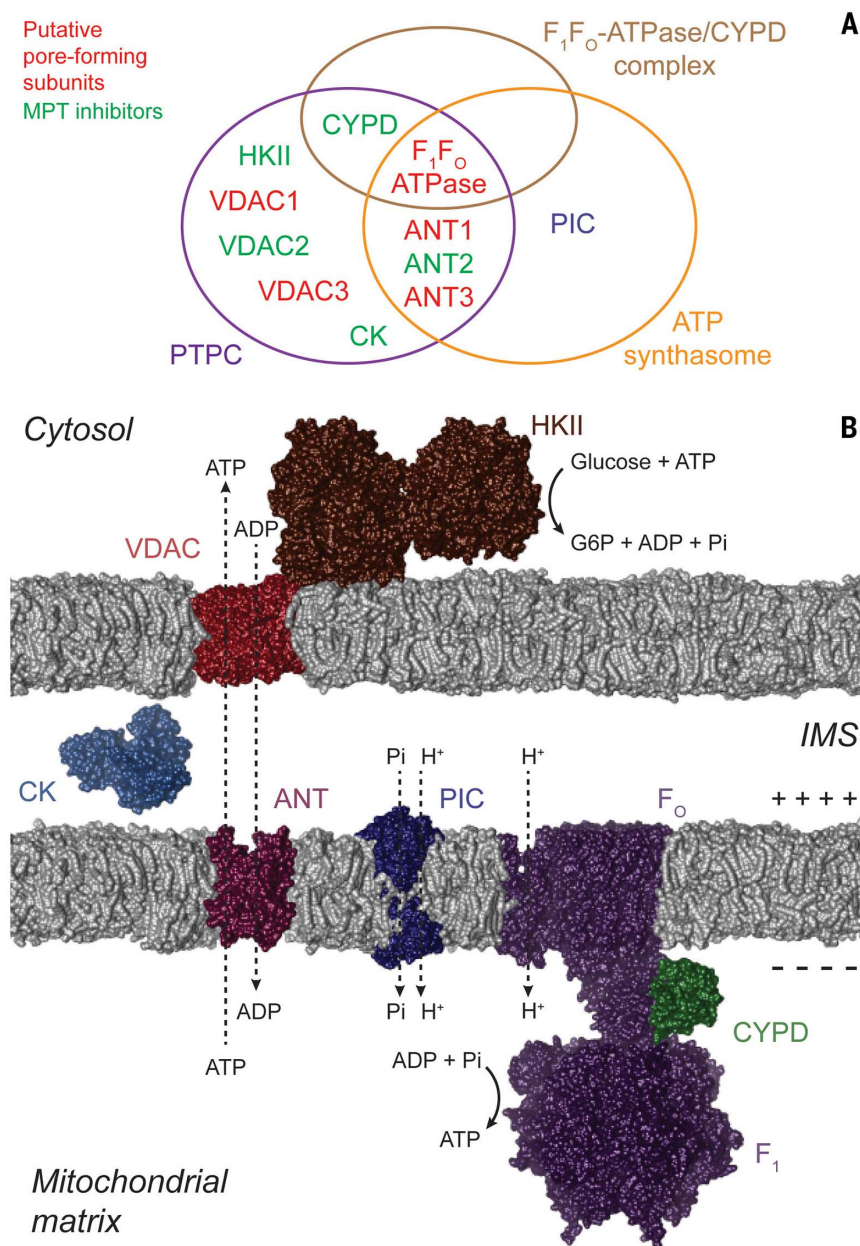


Fig. 3. The ATP synthasome and its interacting partners. (A and B) The term “ATP synthasome” refers to the supramolecular complex composed of the F₁F₀-ATP synthase, ANT, and PIC. The F₁F₀-ATP synthase harnesses the electrochemical gradient generated by the respiratory chain across the IMM to catalyze the synthesis of ATP, whereas PIC and ANT ensure the availability of inorganic phosphate and ADP, respectively. The ATP synthasome interacts with CYPD, a protein of the mitochondrial matrix, and the VDAC1-HKII complex, which is assembled at the OMM. On one hand, these interactions allow ATP molecules produced by the F₁F₀-ATP synthase to be channeled to the mitochondrial surface and support the HKII-mediated conversion of glucose into glucose-6-phosphate (G6P). On the other hand, they place the ATP synthasome in a privileged position to contribute to (or regulate) the so-called PTPC, the supramolecular entity that mediates the MPT. CK, creatine kinase (official name is creatine kinase, mitochondrial 1, ubiquitous) IMS, intermembrane space. Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by National Institute for General Medical Sciences grant P41-GM103311).

NADPH, a product of the pentose phosphate pathway (PPP), caspase-2 is released from 14-3-3 ζ when the flow through the PPP is limited, resulting in the sensitization of cells to various stress signals (72). Sirtuin 1 (SIRT1) is one of the enzymes that deacetylate 14-3-3 ζ , thus counteracting this effect (73). Declines in the abundance of acetyl-CoA also result in the inhibition of the acetyltransferase EP300, hence promoting autophagy (59).

An unbiased screen in *Drosophila* cells identified the human homolog of Ardl1, which functions in irreversible N-terminal acetylation, as an essential regulator of caspase activation (74). N-terminal acetylation can promote protein degradation by the N-end rule pathway (75). Intriguingly, the antiapoptotic protein BCL-X_L appears to inhibit the production of acetyl-CoA (and hence limit N-acetylation) in a BAX- and BAK1-independent but citrate- and acetate-sensitive manner (76). Remarkably, the antiapoptotic effects of BCL-X_L are partially inhibited by citrate and acetate, suggesting that N-acetylation also regulates apoptosis.

The HIF-1 checkpoint

The α chain of hypoxia-inducible factor 1 (HIF-1) is regulated by specific proline hydroxylases (PHDs), which can target it to ubiquitin-dependent degradation. PHDs function to split molecular oxygen (O₂) into two atoms, one of which hydroxylates its target (either of two highly conserved HIF-1 α proline residues) and the other oxidizes α -ketoglutarate (α -KG) into succinate. Hypoxia or high amounts of succinate inhibit the activity of PHDs, whereas increased concentrations of α -KG stimulate it (77). When the functions of PHDs are compromised, HIF-1 α accumulates and forms transcriptionally active heterodimers with HIF-1 β .

Prolonged hypoxia (78, 79) as well as an increased availability of succinate (which occurs, for example, in individuals bearing inactivating loss-of-function mutations in succinate dehydrogenase or fumarate hydratase) (77) promotes a HIF-1-dependent state of resistance to apoptosis. This effect reflects the ability of HIF-1 to drive the expression of MCL-1 (80) and BCL-X_L (81), as well as many enzymes involved in glycolysis, including HKII (82). An increased glycolytic flux exerts cytoprotective effects, especially in hypoxic conditions, as it expands the pool of antioxidants generated by the PPP (5). Last, HIF-1 stimulates mitophagy and inhibits mitochondrial biogenesis (83, 84). This may affect cell survival directly (by limiting the mitochondrial involvement in apoptotic and nonapoptotic cell death, see above) or indirectly, upon engagement of other checkpoints.

The ER stress checkpoint

The accumulation of unfolded proteins in the ER triggers a stress response that inhibits protein synthesis and activates several transcription factors (85). The ER stress response can originate from alterations in protein synthesis (e.g., during viral infection), degradation (e.g., when lysosomal proteases are defective), or folding (e.g., when the availability of chaperones is limited or in the presence of Ca²⁺, ROS, or increased temper-

atures). From our perspective, the ER stress response represents a checkpoint that promotes cell survival or cell death in a context-dependent manner.

Because protein glycosylation is critical to the normal processing of proteins in the ER and Golgi apparatus, defects in this process provoke ER stress responses. Protein glycosylation requires nucleotide sugars and hexosamines, which are synthesized from glucose (or other sugars) and various amino acids, including glutamine. Thus, glucose and amino acid deprivation can trigger ER stress. Along similar lines, an ER stress response can be elicited by hypoxia, because oxygen is required for the formation of disulfide bonds as well as for oligosaccharide modifications. Another source of ER stress is represented by dysregulated TORC1 activity (as caused by the absence of TSC2). In these conditions, cell survival is highly dependent on unsaturated lipids that sustain the expansion of the ER during the stress response (86).

In the course of ER stress, the eukaryotic translation initiation factor 2 α (eIF2 α) kinase 3 (best known as PERK) phosphorylates eIF2 α , restricting the translation of a majority of proteins while stimulating the synthesis of activating transcription factor 4 (ATF4) and DNA damage-inducible transcript 3 (best known as CHOP), which are necessary and sufficient for the death of cells exposed to tunicamycin, an inhibitor of protein glycosylation (87). Along similar lines, the cytotoxic response to glutamine deprivation of neuroblastoma cells overexpressing the MYC family proto-oncogene *MYCN* depends on ATF4 (88). ER stress as induced by ROS overgeneration also relies, at least in part, on the PERK-mediated induction of CHOP. Nonetheless, the cytotoxic activity of thapsigargin, which depletes ER Ca²⁺ stores, appears to be enhanced in PERK-deficient cells (89). These observations indicate that PERK may regulate cell death in opposing ways, depending on the initiating stimulus and perhaps other cell-intrinsic or -extrinsic variables.

It is likely that the ER stress machinery promotes cell survival by limiting protein synthesis, favoring the production of chaperones, enhancing protein degradation in the ER, stimulating the expression of amino acid transporters (which support glycosylation), hence globally inhibiting the accumulation of additional unfolded proteins in this compartment. When this is insufficient to reestablish homeostasis, the cells die, a process that is exacerbated when eIF2 α cannot be inhibited by PERK (87). The ER stress appears to promote apoptosis by inducing changes in the abundance of Bcl-2 proteins. ATF4 transactivates genes encoding the BH3-only proteins BCL2-binding component 3 (best known as PUMA) and NOXA (88), whereas CHOP stimulates the expression of BCL2-like 11 (best known as BIM) (90). BIM is further stabilized by the ER stress-dependent activation of protein phosphatase 2A (PP2A), resulting in the inhibition of antiapoptotic Bcl-2 proteins coupled to the activation of their proapoptotic counterparts and MOMP. Another, poorly understood proapoptotic Bcl-2 pro-

tein, BOK, which structurally resembles BAX and BAK1, may be important in the response of some cells to tunicamycin (91). The molecular mechanisms whereby BOK is activated by ER stress remain to be elucidated. Of note, the accumulation of unfolded proteins within the ER can also cause the production of ROS by luminal NADPH oxidases that are activated by local protein disulfide isomerases and PPP-derived NADPH (87). In this setting, ATF4 and CHOP can further exacerbate ROS production and hence contribute to cell death.

Although ER stress-induced apoptosis appears to proceed predominantly through the mitochondrial pathway, a number of studies suggest that caspases other than caspase-9 and caspase-3 are linked to cell death as induced by ER stress. These include caspase-2 (perhaps acting upstream of the mitochondrial pathway) (92), caspase-4 (93), and caspase-12 (94). However, caspase-12 is expressed in only a subset of humans (95), and murine *Casp12*^{-/-} cells (96) as well as human cells lacking caspase-12 or caspase-4 (97) are fully susceptible to ER stress-induced apoptosis. The ER protein B cell receptor-associated protein 31 (BCAP31) can interact with pro-caspase-8 during the ER stress response and apparently activate it in a FADD-independent manner (98). In line with this notion, cells lacking the BCAP31-interacting molecule cell death-inducing p53 target 1 (CDIPT1) are resistant to the ER stress-induced activation of caspase-8 and consequent cell death (99). How these pathways contribute to the metabolic control of ER stress-induced cell death has not been fully resolved.

p53 and the nonessential amino acid checkpoint

The availability of amino acids in the extracellular milieu influences not only the activity of TORC1 but also protein glycosylation, as discussed above. In addition, several nonessential amino acids can promote cell survival (or death) by alternative mechanisms. Rapidly dividing and transformed cells can actually be “addicted” to these amino acids, which include (at least in some circumstances) glutamine, serine, glycine, arginine, and asparagine (5).

Serine and glycine are interconvertible and serve as precursors for single-carbon folate metabolism. Strikingly, the overexpression of several enzymes that participate in serine-glycine metabolism promotes oncogenic transformation, an effect that depends on their catalytic activity (100, 101). In this scenario, protein kinase C ζ (PKC ζ), which represses the activity of many of these enzymes, acts as a tumor suppressor (102). Malignant cells as well as activated T lymphocytes consume large amounts of glutamine, representing a major source of carbon for the TCA cycle as well as for the synthesis of hexosamines, lipids, and polyamines (103). Glutamine is also important for uptake of other amino acids, because its concentration gradient drives specific antiporters expressed at the cell surface.

When cells are deprived of serine or glycine, the enzymes of the serine-glycine synthetic pathway

accumulate and p53 is temporarily stabilized, resulting in the transactivation of multiple target genes, including cyclin-dependent kinase inhibitor 1A (*CDKN1A*), coding for the cell cycle inhibitor p21^{Cip1} (104). Glutamine deprivation appears to trigger a similar signaling pathway (105). Cells in which p53 functions are compromised (such as many cancer cells) succumb to serine-glycine or glutamine deprivation (104, 105), suggesting that p53 actually suppresses (rather than activates) cell death in these settings. Whether the engagement of p53 by amino acid deprivation might also promote cell death upon the transactivation of proapoptotic p53 targets such as *PUMA* and *NOXA* is currently unexplored.

Some neoplasms lack the enzymes that generate arginine and asparagine, that is, argininosuccinate synthetase and asparagine synthetase, respectively, thereby depending on external supplies for survival (5). The successful use of bacterial L-asparaginase (which degrades extracellular asparagine) in patients with acute lymphoblastic leukemia indicates that such a metabolic liability can be harnessed for therapeutic purposes. However, it seems that the cytotoxic effect of L-asparaginase stems (at least in part) from its ability to catabolize not only asparagine but also glutamine, hence activating the AMPK-TORC1 checkpoint (106).

Besides regulating cell cycle progression and cell death, p53 controls the expression of several proteins that are directly or indirectly involved in metabolism (107). These p53 targets not only include enzymes of the serine-glycine transformation pathway but also encompass TP53-induced glycolysis and apoptosis regulator (TIGAR, officially named C12orf5), which diverts the glycolytic flux toward the PPP (hence promoting nucleotide synthesis and the scavenging of ROS by the glutathione system) (108); glutaminase 2, which converts glutamine into glutamate, providing NH₃⁺ for amino acid synthesis (109); SCO2, which regulates mitochondrial respiration (110); signal transducers implicated in the AMPK-TORC1 checkpoint, such as the $\beta 1$ subunit of AMPK and TSC2 (111); as well as several components of the autophagic machinery (112). Thus, p53 sustains cell survival in response to amino acid deprivation, as it does in conditions of limited DNA damage. Conversely, p53 may promote cell death when the effects of amino acid deprivation are severe, although this possibility has not yet been explored.

Several mechanisms may account for the impact of nonessential amino acids on p53 stabilization. In response to glutamine deprivation and the ER stress-dependent production of ROS, PP2A dephosphorylates (and hence inactivates) an E3 ubiquitin ligase that normally inhibits p53 (105). Importantly, amino acid deprivation also causes a disruption in ribosome biogenesis, mainly because of the inhibition of ribosomal RNA (rRNA) synthesis coupled to the ongoing production of ribosomal proteins. Accumulating rRNA-free ribosomal proteins promote the stabilization of p53 by interfering with its interaction with MDM2 (113). Thus, when the availability of amino acids is limited, the p53 checkpoint may be transiently

activated to promote survival and permanently engaged to stimulate cell death (if homeostasis cannot be reestablished). The p53 system is highly interconnected with various other metabolic checkpoints, including those orchestrated around the MPT and autophagy (114–116). Moreover, p53 appears to participate in the regulation of organismal homeostasis in a non-cell-autonomous manner, by both immunological (117) and metabolic circuitries (118). In particular, p53 has been proposed to control the synthesis of the adenosine A2b receptor, hence regulating the cellular sensitivity to the stress-related extracellular accumulation of adenosine (118). Thus, the p53 checkpoint may be sensitive to metabolic fluctuations of both the intracellular and extracellular microenvironments.

Lipid synthesis as a metabolic checkpoint

Lipoapoptosis, that is, the apoptotic death of cells exposed to lipids, is thought to provide a major etiological contribution to multiple diseases that develop when long-chain fatty acids (LCFAs) accumulate in nonadipose tissues, including non-alcoholic fatty liver disease, diabetes mellitus, and cardiovascular disorders. LCFAs such as palmitate can induce a lethal ER stress response (see above) (119), whose execution may rely on caspase-2 (120). Intriguingly, the ribonuclease endoplasmic reticulum to nucleus signaling 1 (best known as IRE1 α), which is usually activated upon ER stress, cleaves microRNA precursors that normally inhibit the translation of caspase-2 (121), corroborating the existence of a link between LCFA-induced ER stress and caspase-2 activation. Additionally, palmitate activates potentially lethal kinases such as c-Jun N-terminal kinase 1 and GSK3 (122).

The accumulation of cholesterol and its oxidation products also can have cytotoxic consequences. The cardiac damage inflicted by ischemia-reperfusion damage is accompanied by an increase in cholesterol uptake by mitochondria and the generation of auto-oxidized oxysterols, a phenomenon that can be avoided by ligands of translocator protein (TSPO), an OMM protein that interacts with VDAC1 and has been suggested to regulate MPT (123).

Cardiolipin (CL) is responsible for the positive curvature of the IMM and the proper function of several IMM enzymes. The CL molecules present at contact sites between the IMM and OMM reportedly facilitate the insertion into the latter of several Bcl-2 family members (124). In addition, CL usually adsorbs a large fraction of holo-cytochrome c to the IMM. Thus, besides facilitating MPT (see above) (125), the cell death-associated oxidation of polyunsaturated CL species favors the release of holo-cytochrome c from mitochondria upon MOMP (126). Recently, OMM-exposed CL has been shown to constitute a signal for the autophagic removal of permeabilized mitochondria (127), hence playing a major role in the maintenance of intracellular homeostasis.

Sphingolipid metabolism also has a prominent role in the regulation of cell death. Ceramide is

generated from sphingosine or sphingomyelin by the enzymatic activity of ceramide synthase or various sphingomyelinases, respectively (128). Ceramide can trigger distinct apoptotic pathways depending on the subcellular compartment in which it accumulates. In response to ionizing radiation, acidic sphingomyelinase is activated, catalyzing the generation of ceramide on the plasma membrane of endothelial intestinal cells, where it coalesces to form microdomains that transmit apoptotic signals (129). Ceramide that accumulates in mitochondria can trigger MOMP either by stimulating the insertion of BAX into the OMM (130) or by forming lipid pores that are under the control of proteins from the Bcl-2 family (131).

A physical and functional interaction between the ER and mitochondria regulates MOMP as it affects sphingolipid metabolism. In this setting, two products of the hydrolysis of sphingomyelin (which is mediated by ER and OMM enzymes), namely, sphingosine-1-phosphate and *trans*-2-hexadecenal, promote the pore-forming activity of BAK1 and BAX, respectively, whereas no role for ceramide other than as a precursor was identified (132). In line with this notion, the inhibition of the sphingomyelin pathway at any point upstream of the synthesis of *trans*-2-hexadecenal efficiently preserved cell survival in response to stress. Although it is not known how the ER and mitochondria precisely interact to regulate sphingolipid metabolism, it is interesting to note that palmitate stimulates sphingomyelin hydrolysis, implying that some of its cytotoxic effects may stem from direct MOMP stimulation.

Additional contributions of lipid metabolism to apoptosis are likely. The mitochondrial enzyme 3-oxoacyl-acyl carrier protein synthase (OXSM) participates in stress-induced apoptosis in human cervical carcinoma cells (74). OXSM is required for the extension of long-chain fatty acids and may have a role in the synthesis of lipoleic acid, a potentially lipotoxic antioxidant. This said, how OXSM precisely contributes to apoptosis remains to be determined.

The PARP checkpoint

Poly(ADP-ribose) polymerases (PARPs) make up a large family of enzymes that catalyze the addition of poly(ADP-ribose) moieties (an NAD⁺-dependent reaction also known as ADP-ribosylation) to multiple substrates, hence regulating a plethora of cellular processes, notably the repair of single and double-strand DNA breaks (133, 134). Thus, in the presence of extensive DNA damage, PARP1 hyperactivation may deplete the intracellular stores of NAD⁺, NADH, and ATP, resulting in a bioenergetic catastrophe often associated with necrotic cell death (135). Conversely, in the course of apoptosis, caspase-3 proteolytically inactivates PARP1, hence avoiding such a rapid drop in intracellular ATP concentrations leading to regulated necrosis. In line with this idea, cells that are unable to undergo MOMP because they lack BAX and BAK1 respond to an otherwise proapoptotic stimulus (alkylating DNA damage) by undergoing PARP1-dependent necrosis, in particular in conditions of intense proliferation

(which are associated with an increase in glycolytic flux) (136). Conversely, quiescent cells (i.e., interleukin-3-dependent *Bax*^{-/-}*Bak1*^{-/-} cells deprived of growth factors or mouse embryonic fibroblasts at confluence), which mainly obtain energy from oxidative phosphorylation, are resistant to such treatment (136). Thus, the metabolic status of the cell (notably the availability of NAD⁺, NADH, and ATP) determines the subroutine by which cells die in response to (at least some) stimuli.

Hyperactivation of PARP1 may also promote nonapoptotic cell death by favoring the mitochondrial release of AIF (137), an NADH-consuming enzyme endowed with a latent endonuclease activity previously implicated in a MOMP- and calpain-dependent, but caspase-independent, form of apoptosis (11). Intriguingly, AIF appears to be released by mitochondria in response to PARP1 hyperactivation upon the binding of poly(ADP-ribose) moieties rather than as a result of calpain cleavage (138). How this would lead to the desorption of AIF from the IMM, in which it is stably inserted through a transmembrane domain, remains to be clarified. Irrespective of this unresolved issue, BCL-2 appears to suppress PARP1-dependent cell death by two mechanisms, namely, by inhibiting the mitochondrial release of AIF (11), and by directly blocking the enzymatic activity of PARP1 (139). Genetic ablation of *Parp1* as well as a hypomorphic mutation in *Aifm1* (which results in decreased AIF expression) exert cytoprotective effects in several paradigms of acute cell loss in vivo, including various models of cerebral ischemia, supporting the potential pathophysiological relevance of this pathway, which has been named parthanatos (135).

Other metabolic checkpoints

Several metabolic enzymes may have roles in signaling that are controlled by the availability of their substrates. Although these have not been directly linked to cell death pathways, it is possible that such enzymes may represent metabolic checkpoints in cell fate decisions. This may account for the transforming activity of enzymes of the serine-glycine transformation pathway, although this has not been formally established. Two other examples involve glycolytic enzymes, namely, the M2 isoform pyruvate kinase, muscle (PKM2) and GAPDH.

PKM2 catalyzes the last step of glycolysis as it converts phosphoenolpyruvate into pyruvate. The glycolytic intermediate fructose-1,6-bisphosphate shifts the equilibrium between (enzymatically inactive) PKM2 dimers and (enzymatically active) PKM2 tetramers toward the latter. Conversely, by diverting the glycolytic flux toward the PPP, TIGAR (which operates as a fructose-2,6-bisphosphatase) favors PKM2 inactivation (5). The dimeric state of PKM2, which predominates over its tetrameric counterpart in many tumors, is also favored by direct interactions with oncogenic kinases (including RAF1 and SRC). Intriguingly, PKM2 physically interacts with, and thus modulates the transcriptional activity of, β catenin (140). In addition, dimeric PKM2 can operate as a protein kinase, thereby promoting

the functions of signal transducer and activator of transcription 3 (STAT3), which control the synthesis of multiple antiapoptotic proteins, including BCL-2, BCL-X_L, and MCL-1 (141).

GAPDH converts glyceraldehyde-3-phosphate (G3P) into 1,3-bisphosphoglycerate. Under limited G3P availability, GAPDH binds specific mRNAs, such as that coding for interferon- γ , and inhibits their translation (142). GAPDH also functions in a complex with other proteins to inhibit the translation of additional mRNAs in response to interferon- γ (143). Moreover, a nuclear pool of GAPDH promotes transcriptional events through interactions with promyelocytic leukemia (PML) and OCT1 (144, 145). In one study, GAPDH inhibited caspase-independent cell death by promoting the expression of the essential autophagic factor ATG12, thereby stimulating mitophagy (8). GAPDH may accumulate at mitochondria in the course of apoptosis, thereby interacting with VDAC1 and promoting MPT (146). Except for the example cited above, however, the extent to which glycolysis and G3P contribute or modulate the extraglycolytic functions of GAPDH is unknown. It remains possible that the metabolic state of the cell influences the ability of GAPDH to regulate cell death pathways. These and additional checkpoints based on metabolic enzymes may also function in noncanonical ways to control cell fate decisions in response to metabolic cues, although this remains to be formally demonstrated. Irrespective of such a possibility, the extraglycolytic functions of PKM2 and GAPDH strongly corroborate the idea that metabolism and signaling are more intimately interconnected than previously thought.

Another possible metabolic checkpoint might involve Bcl-2 family members. These proteins have a number of functions that may affect metabolism independently of their ability to control MOMP. For instance, BCL-2 and perhaps other members of the family physically interact with the inositol 1,4,5-trisphosphate receptor on the ER membrane, hence controlling reticular Ca²⁺ fluxes (147). The release of Ca²⁺ ions from reticular stores provides an important control on mitochondrial Ca²⁺ concentrations, which regulate a number of enzymes involved in the TCA cycle and in the electron transport chain (148). Proapoptotic Bcl-2 proteins also promote mitochondrial fusion (19) in a manner that is regulated by their antiapoptotic counterparts (23, 149). This is relevant, because mitochondrial dynamics have an important role in preserving a metabolically efficient pool of mitochondria in response to stress (62). In addition, MCL-1 and BCL-X_L appear to operate in the mitochondrial matrix or at the IMM, respectively, to promote respiratory functions and ATP production (150, 151). Last, as mentioned above, (i) various members of the Bcl-2 protein family participate in the regulation of autophagy, mostly because they control the activity of BECN1, and (ii) BCL-X_L appears to inhibit protein N-acetylation. Although such observations indicate that Bcl-2 proteins regulate various metabolic circuitries, the extent to which they do so is unclear. T lymphocytes lacking BAX and BAK1 exhibit activation defects that have been linked

to a metabolic derangement (152), indicating that (at least in some circumstances) the metabolic activity of Bcl-2 proteins has a pathophysiological relevance.

If Bcl-2 proteins indeed represent an important hub for the regulation of cellular metabolism, we may ask how the detection of metabolic alterations feeds into this process. It is plausible that the mechanisms by which metabolic sensors impinge on the cell death-regulatory activity of Bcl-2 proteins also control the ability of this protein family to influence metabolic responses. As such, the interplay of metabolism and cell fate decisions may be “hardwired” through Bcl-2 proteins. It is an intriguing possibility that such interactions may have been ancestral, predating their role in cell death. In this context, it is interesting to note that the two Bcl-2 proteins identified so far in *Drosophila* probably do not regulate cell death but rather exert metabolic functions (153).

Conclusions, implications, and speculations

The relations between metabolism and cell death regulation are profound and intricate. On one hand, both apoptosis and necrosis can be initiated by metabolic perturbations (signals), mainly owing to the existence of refined detection systems (sensors) that are wired to the cell death machinery (effectors) through one or more signaling node(s) (transducers) (Fig. 1). Thus, small metabolites and metabolic by-products (e.g., ATP/ADP, NADH/NAD⁺, and ROS), as well as various enzymes involved in metabolic circuitries (e.g., holocytochrome c, AIF, and GAPDH), can influence cell fate decisions. On the other hand, several proteins originally characterized for their cell death-regulatory activity (e.g., Bcl-2 family members and p53) have a major metabolic functions (table S1). Thus, any distinction between metabolism and signaling has now decayed.

Two additional considerations can be made in this respect. First, because many factors that regulate or execute apoptosis and necrosis have roles unrelated to cell death, including roles in metabolism (154), these systems may have evolved to serve vital functions and only later may have acquired the ability to control cellular demise. Second, because many major regulators of cell death, including proteins and small metabolites, are also involved in steady-state metabolism, each of these molecules appears to operate in the context of a possible transition between physiological conditions, adaptive responses to stress (aimed at the reestablishment of homeostasis), and the activation of the cellular demise (when homeostasis cannot be restored). How the transition between the vital, adaptive, and lethal functions of each of these components is achieved remains unexplored. Possibly the entire “network” built up at the interface between metabolism and cell death regulation may at some stage collapse as a result of an irreversible switch in the activity of one (or a few) of its components, provoking a domino effect. In this setting, multiple Bcl-2-like proteins may operate as central checkpoints that

either interrupt (antiapoptotic members) or amplify (proapoptotic members) the transition from homeostasis to death. Once these potential “firebreaks” have been trespassed, the fall is irreversible and cell death cannot be avoided.

How the various metabolic checkpoints are interconnected has just begun to emerge. It will be interesting to determine whether these systems are organized in a hierarchical manner and, if so, which are the most critical ones. Based on current knowledge, the checkpoints organized around MOMP, MPT, p53, and the Bcl-2 protein family stand out as prominent candidates for this title. A detailed understanding of the interplay between metabolism and cell death regulation is expected to drive the development of therapeutically relevant agents that either promote or inhibit cell death as they modulate metabolic circuitries (table S2). This knowledge will undoubtedly expand our armamentarium of anticancer agents as well as of drugs for avoiding (or at least minimizing) unwarranted cell death as it occurs in the course of ischemic insults, neurodegeneration, and various other clinical settings, including a large panel of genetic diseases in which specific metabolic alterations result in the pathological loss of postmitotic cells (table S3).

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ACKNOWLEDGMENTS

We thank O. Kepp (INSERM, France) for critical help with figures and E. Gottlieb (Beatson Institute For Cancer Research, UK). G.K. is supported by the Ligue contre le Cancer (équipe labellisée), Agence Nationale de la Recherche, Association pour la recherche sur le cancer, Cancéropôle Ile-de-France, AXA Chair for Longevity Research, Institut National du Cancer, Fondation Bettencourt-Schueller, Fondation de France, Fondation pour la Recherche Médicale, the European Commission (ArtForce), the European Research Council, the LabEx Immuno-Oncology, Les sites de recherche intégrée sur le cancer (SIRIC) Stratified Oncology Cell DNA Repair and Tumor Immune Elimination, the SIRIC Cancer Research and Personalized Medicine, and the Paris Alliance of Cancer Research Institutes. D.R.G. is supported by grants from the NIH and the American Lebanese and Syrian Associated Charities.

SUPPLEMENTARY MATERIALS

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Supplementary Text
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10.1126/science.1250256

RESEARCH ARTICLES

QUANTUM SIMULATION

Spectroscopic observation of $SU(N)$ -symmetric interactions in Sr orbital magnetism

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$SU(N)$ symmetry can emerge in a quantum system with N single-particle spin states when spin is decoupled from interparticle interactions. Taking advantage of the high measurement precision offered by an ultrastable laser, we report a spectroscopic observation of $SU(N \leq 10)$ symmetry in ^{87}Sr . By encoding the electronic orbital degree of freedom in two clock states while keeping the system open to as many as 10 nuclear spin sublevels, we probed the non-equilibrium two-orbital $SU(N)$ magnetism via Ramsey spectroscopy of atoms confined in an array of two-dimensional optical traps; we studied the spin-orbital quantum dynamics and determined the relevant interaction parameters. This study lays the groundwork for using alkaline-earth atoms as testbeds for important orbital models.

Symmetries play a fundamental role in the laws of nature. A prominent example is the $SU(3)$ symmetry of quantum chromodynamics, which governs the behavior of quarks and gluons. When generalized to large N , $SU(N)$ is expected to generate exotic many-body behaviors emerging from the increased degeneracy and strict conservation laws. Owing to the strong decoupling between the electronic-orbital and nuclear-spin degrees of freedom ($I, 2$), alkaline-earth atoms and others with similar structure prepared in the two lowest electronic states (clock states with zero electronic angular momenta) are predicted to exhibit nuclear spin (I) independence for their interatomic collisional parameters. This property directly leads to a $SU(N \leq 2I + 1)$ symmetry for the interaction physics (3–6). Thanks to this symmetry, in addition to their use as ideal time-keepers (7) and quantum information processors (8–11), alkaline-earth atoms are emerging as a unique platform for the investigation of high-energy lattice gauge theories (12); for testing of orbital models used to describe transition metal oxides, heavy fermion compounds, and spin liquid phases (13); and for the observation of exotic

topological phases (6, 14). Progress toward these goals includes the production of quantum degenerate alkaline-earth gases (15–17), imaging of individual spin components (18), control of interactions (16, 19, 20), and study of many-body spin dynamics (21).

Thus far, only indirect evidence for $SU(N)$ symmetry exists (22), including inference from suppressed nuclear spin-relaxation rates (18), reduced temperatures in a Mott insulator for increased numbers of spin states (23), and the changing character of a strongly interacting one-dimensional fermionic system as a function of N (24). Furthermore, these observations are limited to the electronic ground state. The corresponding ground-state s -wave scattering parameter a_{eg} has been determined from photoassociation (25) and rovibrational spectroscopy (26), but the excited state-related scattering parameters remain undetermined.

Here, we report a spectroscopic observation of $SU(N)$ symmetry and two-orbital $SU(N)$ magnetism in an ensemble of fermionic ^{87}Sr atoms at microkelvin temperatures and confined in an array of two-dimensional (2D) disc-shaped, state-insensitive optical traps (27). The trapping potentials are approximately harmonic, with an axial (Z) trapping frequency ν_Z of ~ 80 kHz and a radial (X - Y) frequency ν_R of ~ 600 Hz; the slight anharmonicity makes the spacings between the energy levels uneven. Axial and radial degrees of freedom are decoupled during the initial lattice loading and cooling. Under typical temperatures ($1 \mu\text{K} < T_R < 7 \mu\text{K}$, $T_Z \sim 2 \mu\text{K}$), atoms are cooled to the motional ground state along the Z direction. In contrast, the radial modes are thermally populated. The $SU(N)$ symmetric spin degree of freedom is encoded in the 10 nuclear spin states

with quantum number m_I (Fig. 1A) and the pseudo-spin $\frac{1}{2}$ orbital degree of freedom in the two lowest electronic (clock) states (1S_0 and 3P_0 , henceforth $|g\rangle$ and $|e\rangle$, respectively). Under typical atomic occupancies ($\lesssim 20$ atoms per disc), temperatures, and trap volume ($\propto T_R$), the mean interaction energy per particle is at least two orders of magnitude smaller than the single-particle vibrational spacing along any direction. The spectral resolution available with a laser of 1×10^{-16} stability (28) enables us to accurately probe these interactions while addressing individual nuclear spin levels.

 $SU(N)$ spin-orbital Hamiltonian in energy-space lattice

Under our operating conditions, the atomic interactions are insufficiently energetic to transfer atoms between the initially populated, slightly anharmonic motional eigenmodes. To first-order approximation, atoms remain frozen in these quantized motional levels and the quantum dynamics takes place only in the internal degrees of freedom (spin and orbital) (21, 29, 30), in a way analogous to localized atoms in real-space lattice trapping potentials. This approximation greatly simplifies the modeling of our system. Here, the large energy gap between the interaction energy and the single-particle vibrational spacing, along with the anharmonicity and nonseparability of the optical trapping potential provided by the Gaussian laser beam profile, lead to an energetic suppression of mode-changing collisions (31). Moreover, the s -wave and p -wave (Fig. 1B) interactions, which are responsible for the dynamics, provide nonlocal interactions when viewed within the energy-space lattice, as they couple atoms without being overly sensitive to the thermally populated motional levels. The decoupling between motional and internal degrees of freedom, combined with the sub-hertz spectral resolution of the stable laser, allows us to probe spin lattice models with effective long-range couplings in a nondegenerate Fermi gas, as schematically illustrated in Fig. 1C. This system thus paves the way for studies of quantum orbital magnetism beyond the ultracold regime.

Spin models with long-range interactions have been implemented in dipolar gases (32) or trapped ionic systems (33), but our system is further enriched by $SU(N)$ symmetry and holds potential for addressing important open questions about many-body dynamics in spin-orbital models under the co-presence of large degeneracy (13, 34, 35) and long-range interactions. By performing Ramsey spectroscopy with various nuclear spin mixtures, we determine the nuclear spin independence of the s -wave and p -wave interactions. Furthermore, we probe the non-equilibrium dynamics of the orbital coherence, and the results are well reproduced by a two-orbital $SU(N)$ spin lattice model in quantized motional eigenenergy space.

Interactions between two ^{87}Sr atoms are governed by Fermi statistics with an overall wave function antisymmetrization under exchange in the motional, electronic, and nuclear spin degrees of freedom (Fig. 1B). Consider a pair of interacting atoms (j and k) occupying two of the quantized

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eigenmodes of the trapping potential, $\mathbf{n}_j$ and $\mathbf{n}_k$. If the atoms are in a nuclear spin symmetric state, they experience s -wave interactions only if their electronic state is antisymmetric: $(|eg\rangle - |ge\rangle)/\sqrt{2}$. We denote the elastic scattering length characterizing those collisions as a_{eg}^- . They can collide via p -wave interactions in three possible electronic symmetric configurations $\{|gg\rangle, |ee\rangle, (|eg\rangle + |ge\rangle)/\sqrt{2}\}$, corresponding to the p -wave elastic scattering lengths b_{gg} , b_{ee} , and b_{eg}^+ , respectively. In contrast, if the two atoms are in an antisymmetric nuclear spin configuration, they experience s -wave collisions under these three electronic symmetric configurations, with the corresponding scattering lengths a_{gg} , a_{ee} , and a_{eg}^+ , respectively. Similarly, p -wave interactions occur in $(|eg\rangle - |ge\rangle)/\sqrt{2}$, corresponding to the scattering length b_{eg}^- . These eight parameters characterize elastic collisions at ultralow temperatures, and $SU(N)$ symmetry predicts them to be independent of the nuclear spin configuration.

The Hamiltonian that governs these interactions, between atoms j and k , can be written in terms of orbital-spin $1/2$ operators $\hat{T}_j^{x,y,z}$ acting on the j -atom's electronic state, $\{|e, g\rangle$, and in terms of nuclear-spin permutation operators $\hat{S}_n^m(j) = |\mathbf{n}_j\rangle\langle\mathbf{m}|$, acting on the j -atom's nuclear spin levels, $n, m \in \{1, 2, \dots, N\}$ as

$$\hat{H}_{j,k} = (\hat{P}^+ \hat{H}^+ + \hat{P}^- \hat{H}^-) \quad (1)$$

and

$$\hat{H}^\pm = J_{j,k}^\pm \vec{T}_j \cdot \vec{T}_k + \chi_{j,k}^\pm \hat{T}_j^z \hat{T}_k^z + C_{j,k}^\pm \left(\frac{\hat{T}_j^z + \hat{T}_k^z}{2} \right) + K_{j,k}^\pm \hat{\mathbb{I}} \quad (2)$$

(3I), where $\hat{\mathbb{I}}$ is the identity matrix, and

$$\hat{P}^\pm = \frac{\left[\hat{\mathbb{I}} \pm \sum_{n,m=1}^N \hat{S}_n^m(j) \hat{S}_m^n(k) \right]}{2} \quad (3)$$

are nuclear spin projector operators into the symmetric triplet (+) and antisymmetric singlet (−) nuclear spin states, respectively. Equation 1 states that if the nuclear spin of the atoms is in (+) or (−), then they interact according to $\hat{H}^+$ or $\hat{H}^-$, respectively. The coupling constants $J_{j,k}^\pm, \chi_{j,k}^\pm, C_{j,k}^\pm, K_{j,k}^\pm$ depend on the scattering parameters a_η and b_η , $\eta \in \{ee, gg, eg^+, \text{ and } eg^-\}$, and the wave function overlap of the radial vibrational modes of the j - and k -atoms in the 2D traps (3I).

The total interaction Hamiltonian is the sum over all pairs of atoms, $\hat{H}^{\text{so}} = \sum_{j>k} \hat{H}_{j,k}^{\text{so}}$, and commutes with all the $SU(N)$ generators, $\hat{S}_n(j)$. $\hat{H}^{\text{so}}$ is thus invariant under transformations from the $SU(N)$ group [i.e., $SU(N)$ symmetric]. This implies that the number of atoms in each of the nuclear spin sublevels is conserved. N is chosen by the initial state preparation of the nuclear spin distribution and can vary from 1 to $2I + 1 = 10$ in ^{87}Sr ($I = 9/2$). In this experiment, we control N via optical pumping through another electronic state.

In addition to elastic interactions, ^{87}Sr atoms exhibit inelastic collisions. Among those, however, only the e - e ones have been observed to give

rise to measureable losses (36); we denote these two inelastic scattering lengths as γ_{ee} and β_{ee} for the s -wave and p -wave, respectively. We

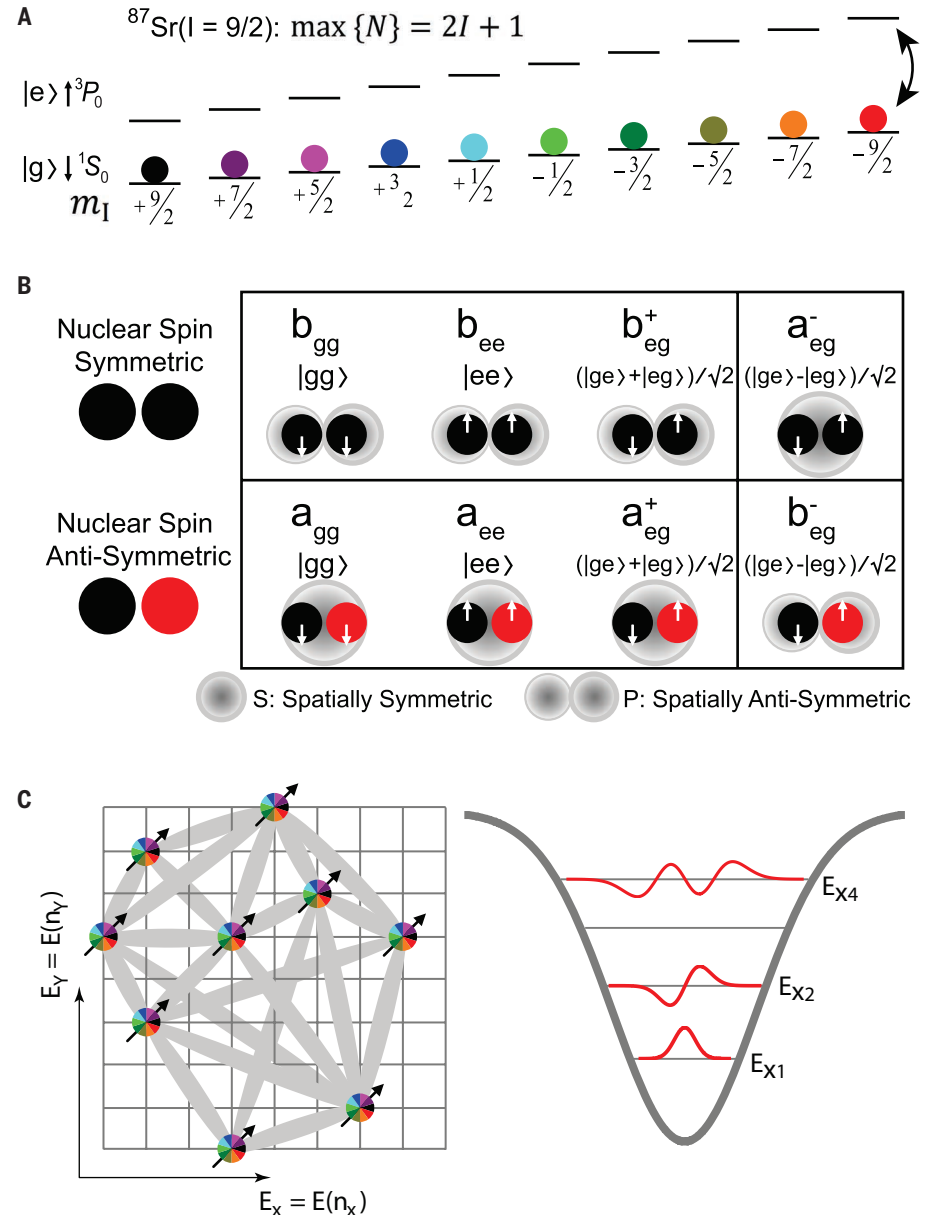


Fig. 1. Diagram of the interacting spin lattice. (A) Energy levels for the two lowest electronic states (1S_0 and 3P_0) of ^{87}Sr atoms in a magnetic field, each with 10 nuclear spin states, depicted by colors. This color scheme is used in all figures to denote the interrogated state. (B) Interactions between two fermionic atoms characterized by four s -wave (a) and four p -wave (b) elastic scattering parameters. The interactions are governed by symmetries in motional states (bottom labels), nuclear spins (left labels), and electronic orbitals (white arrows). (C) Left panel: Schematics of the interacting electronic orbitals (spin- $1/2$ arrows) distributed over a lattice spanned by motional eigenenergies. In our system, the energy levels are populated according to a Boltzmann distribution and the energy spacings are slightly anharmonic; the latter is crucial for freezing atoms in their initially populated motional modes. For simplicity, neither the anharmonic lattice spacing, nor the Boltzmann population of modes are reflected in the figure. The shaded connections illustrate the long-range nature of the interactions in energy space. In our theoretical approach, those interactions are calculated using the matrix element overlap of the corresponding modes (3I). To a first-order approximation, p -wave interactions can be treated collectively and can be assumed to be of all-to-all type. Thus, we can replace the values of the coupling constants with their thermal averages (29). Colored circles show the possibility of preparing statistical mixtures of N nuclear spin states. Right panel: Illustration of a few occupied eigenmodes in our optical trap formed by a Gaussian beam.

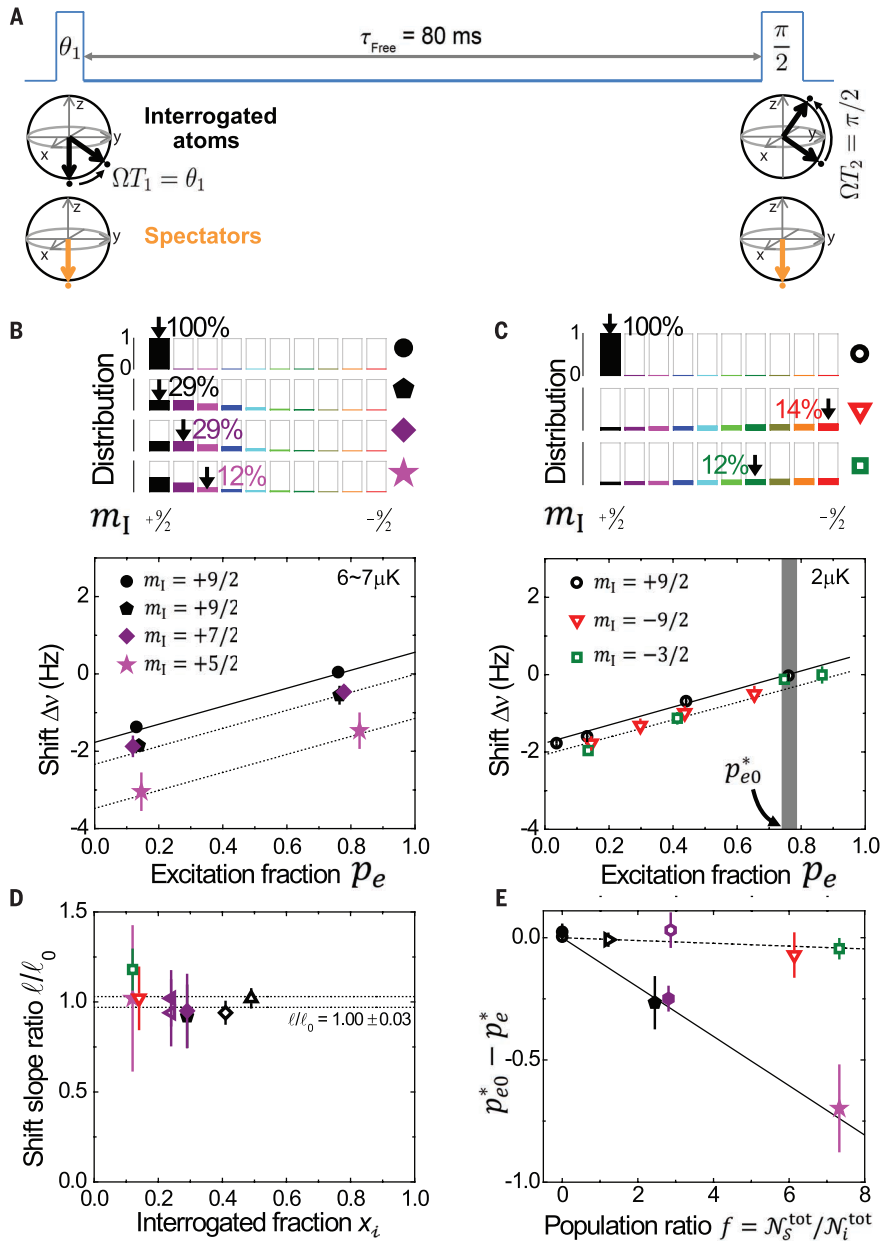


Fig. 2. Nuclear spin independence of interaction effects. (A) Ramsey sequence with an initial pulse of area θ_1 , a final $\pi/2$ pulse, and a free evolution period $\tau_{\text{free}} = 80$ ms. The spectator atoms remain in $|g\rangle$. (B and C) Upper panels: Illustration of the interrogated states (black arrows) and population distributions among various nuclear spin states. Lower panels: Measured density shifts (in symbols) for different nuclear spin configurations at (B) $T_R = 6$ to 7 μK and (C) ~ 2 μK . For consistency, the shifts are scaled for $\mathcal{N}_i^{\text{tot}} = 4000$. The solid and dotted lines show theory calculations for the corresponding x_i and T_R as indicated in the plots, with b_{eg}^- and a_{eg}^- evaluated using the fitting parameter extracted from (E) and other remaining parameters determined from previous measurements (Table 1). The gray band in (C) corresponds to p_{e0}^* , the excitation fraction for zero density shift in a polarized sample. The spectator atoms generate a temperature-dependent density shift, which is independent of p_e of the interrogated atoms and thus manifests as a net offset from the purely polarized density shift. (D) Ratio of the slope of the frequency shift between the spin-mixed and spin-polarized samples. The dotted lines represent the standard error. (E) The difference in the zero-shift excitation fraction between the spin-mixed and spin-polarized samples. The solid and dashed lines are theory fits used to determine b_{eg}^- and consequently a_{eg}^- by the analytic relations between s - and p -wave scattering parameters (31). In (D) and (E), two values of T_R are used: 2.3 ± 0.2 μK (open symbols) and 6.5 ± 0.4 μK (solid symbols). In addition to conditions used for (B) and (C), other spin configurations are studied: open up triangles ($x_i = 49\%$), open diamond (41%), open right triangles (46%), open and solid hexagons (26%), open and solid left triangles (24%), solid pentagons (29%), and solid stars (12%). Error bars represent 1σ multiplied by $\sqrt{\chi_{\text{reduced}}^2}$.

set other inelastic parameters to zero because of their negligible contributions in measurements (36).

Density-dependent frequency shift for nuclear spin mixtures

We first tested $SU(N)$ symmetry in a two-orbital system by measuring the density-dependent frequency shift of the clock transition under various nuclear spin population distributions. We used a Ramsey sequence to measure interactions (21) under an external magnetic field that produces Zeeman splittings much larger than the interaction energy. The sequence starts with all atoms in $|g\rangle$ (Fig. 2A). Only atoms in a particular nuclear spin state are coherently excited and interrogated, whereas atoms in other states (“spectators”) remain in $|g\rangle$. We denote the number of interrogated atoms by $\mathcal{N}_i^{\text{tot}}$, the number of spectator atoms by $\mathcal{N}_s^{\text{tot}}$, and define a population ratio $f = \mathcal{N}_s^{\text{tot}} / \mathcal{N}_i^{\text{tot}}$ and the interrogated fraction $x_i = \mathcal{N}_i^{\text{tot}} / (\mathcal{N}_i^{\text{tot}} + \mathcal{N}_s^{\text{tot}})$. We controlled the fraction of interrogated atoms excited to $|e\rangle$, $p_e = (1 - \cos \theta_1)/2$, by varying the initial pulse area θ_1 , where $0 < \theta_1 < \pi$. After a free evolution time $\tau_{\text{free}} = 80$ ms, a second pulse of area $\pi/2$ was applied for subsequent readout of the electronic orbital distributions. The resonance frequency shift was recorded as a function of the atomic number in the trap, which can be varied in a controlled manner. Interactions between atoms modify the resonance frequency in a density-dependent manner, and the measurement of this frequency gives us information about the interaction parameters.

We operate with highly homogeneous atom-laser coupling such that the orbital excitation is the same for all interrogated atoms. Consequently, in a fully spin-polarized sample, the s -wave interactions are suppressed and the p -wave interactions dominate the free evolution dynamics (21). The s -wave interactions are allowed only when spectator atoms populate other nuclear spin states. The existence of $SU(N)$ symmetry in the scattering parameters results in an independence of the density shift with respect to both the interrogated nuclear spin state and the distribution of spectator atoms among the various spin components. Moreover, in the presence of a large magnetic field (which energetically suppresses excitation of the spectator atoms), the density shift should be only a function of $\mathcal{N}_i^{\text{tot}}$, p_e , and $\mathcal{N}_s^{\text{tot}}$.

In Fig. 2B, we compare the fully spin-polarized case ($m_i = +9/2$) against three other scenarios with different spin mixtures under $T_R = 6$ to 7 μK . The density shift is expected to be a linear function of p_e , arising from the fact that the p -wave interaction among $\mathcal{N}_i^{\text{tot}}$ has a quadratic dependence on the z component of the total orbital spin (21). When scaled to the same number of interrogated atoms ($\mathcal{N}_i^{\text{tot}} = 4000$), the observed density shifts show three features: (i) The linear slope ℓ depends only on $\mathcal{N}_i^{\text{tot}}$, (ii) the offset with respect to the polarized case increases linearly with f , and (iii) both ℓ and the offset are independent of how the atoms are distributed in the nuclear

spin levels. The last point is verified, for example, by measuring the same shifts when interrogating 29% of the total population in either +9/2 or +7/2.

Temperature-dependent SU(N)-symmetric interactions

To determine the temperature dependence for the density shift and for additional confirmation of the observed nuclear spin independence, we interrogated other nuclear spin states, $-9/2$ or $-3/2$, at a lower $T_R \sim 2 \mu\text{K}$, when the distribution across all spin states is nearly even (Fig. 2C). The measured density shifts scaled to $\mathcal{N}_i^{\text{tot}} = 4000$ are again similar to each other, providing further direct experimental evidence for SU($N = 10$) symmetry. At this lower T_R , the slope still depends only on $\mathcal{N}_i^{\text{tot}}$, but there is a smaller offset of the density shift relative to the polarized case when x_i varies. To quantify the T_R dependence, we plotted all measured ratios, l/l_0 , where l_0 is the linear slope for the polarized case. We see that (I) the ratios collapse into a single value independently of f and T_R for fixed $\mathcal{N}_i^{\text{tot}}$, yielding $l/l_0 = 1.00 \pm 0.03$ (Fig. 2D). This result agrees well with the SU(N)-predicted ratio of unity and verifies this symmetry to the 3% level.

We emphasize that the test of two-orbital SU(N) symmetry (at the 3% level) is based directly on the measured interactions that are independent of nuclear spin configurations, and it does not require accurate knowledge of some common-mode system calibrations. For quantum simulations of SU(N) physics, it is important to precisely test this symmetry to a level much below all relevant energy scales. Although our measurement uncertainty (3%) has not reached the ultimate theoretical prediction (0.1%) resulting from a small admixture of the $^3\text{P}_0$ state with higher-lying P states with finite electronic angular momenta ($I=3$), it is already sufficient for realizing a SU(N)-symmetric, unity-filling spin lattice system (3I). Further reduction in our experimental uncertainty can be achieved by enhancing the measurement precision with improved laser stability. We observe that l decreases by only 10% when T_R is raised from 2 μK to 6 μK , verifying its low sensitivity to T_R as expected for p -wave interactions in 2D systems.

We also determined the excitation fraction where the shift is zero for a spin mixture, p_e^* , and compare it to that of a polarized sample, p_{e0}^* (gray bands, Fig. 2C), for various interrogated spin states (colors in Fig. 2E). The difference shows the following features: (II) It collapses onto a single line (for a given T_R of either 2.3 or 6.5 μK) as a function of f , which provides further evidence for the spin independence of the interactions; (III) at $\mathcal{N}_s^{\text{tot}} = 0$ (fully polarized), the two lines cross each other at the origin, as expected from the T_R insensitivity of the p -wave interactions. The proportionality constant of $(p_{e0}^* - p_e^*)$ to f is finite for 6.5 μK (lower line) and decreases to almost zero for $T_R \sim 2.3 \mu\text{K}$ (upper line). This near-zero proportionality constant for $T_R \sim 2.3 \mu\text{K}$ reflects an accidental cancellation

of the spectators' s - and p -wave interaction effects at this temperature.

Experiment-theory agreement and determination of the scattering parameters

In the presence of a large external magnetic field that produces differential Zeeman splittings much larger than the interaction energy, those terms in the Hamiltonian that exchange the population between the occupied spin-orbital levels are energetically suppressed and the populations of different spin-orbital levels are conserved. Hence, the Hamiltonian is dominated by Ising-type interactions that preserve the spin-orbital population. In this regime, the many-body dynamics for a single lattice site with $\mathcal{N}$ atoms can be captured under a collective approximation that replaces the coupling constants with their corresponding thermal averages, $O_{\mathbf{n},\mathbf{n}'}^{\pm} \rightarrow \bar{O}^{\pm}$ (29). For the experimentally relevant case where only $\mathcal{N}_i$ atoms in spin m_i are interrogated and where $\mathcal{N}_s$ atoms in the other spin components remain in $|g\rangle$, the effective many-body Hamiltonian during τ_{free} simplifies substantially. It consists of two parts, $\hat{H}^{\text{so}} = \hat{H}_i + \hat{H}_s$. The first part, $\hat{H}_i = \bar{\chi}^+(\hat{\mathbb{T}}^x)^2 + \bar{C}^+ \hat{\mathbb{T}}^z \mathcal{N}_i$, describes the p -wave interactions between the interrogated atoms (2I, 29), where $\hat{\mathbb{T}}^{\alpha=x,y,z} = \sum_j \mathcal{N}_{m_i}(j) \hat{T}_j^{\alpha=x,y,z}$ are collective orbital operators acting on the $\mathcal{N}_i$ interrogated atoms. The density shift induced by these interactions, $\Delta v^i = \mathcal{N}_i(\bar{C}^+ - \cos \theta_i \bar{\chi}^+)$, with $\bar{\chi}^+ = [(b_{eg}^3 + b_{eg}^3 - 2b_{eg}^3)/2]\langle P \rangle_{T_R}$ and $\bar{C}^+ = [(b_{eg}^3 - b_{eg}^3)/2]\langle P \rangle_{T_R}$, depends linearly on the number of excited atoms $\mathcal{N}_i p_e$. Here, $\langle P \rangle_{T_R}$ corresponds to the thermal average of the p -wave mode overlap coefficients. Assuming a Boltzmann distribution of initially populated radial motional modes, we have $\langle P \rangle_{T_R} \propto (T_R)^0$ (insensitive to T_R) (2I). For a spin-polarized sample, the observed density shifts are well reproduced by theory (solid black lines in Fig. 2, B and C) based on the same p -wave parameters as determined in (2I). The second part, $\hat{H}_s = \mathcal{N}_s \bar{\Lambda} \hat{\mathbb{T}}^z$, describes the interactions between the interrogated and spectator atoms with both p - and s -wave contributions. The related density shift is $\Delta v^s = \bar{\Lambda} \mathcal{N}_s$, with

$$\begin{aligned} \bar{\Lambda} &= \frac{\bar{C}^+ + \bar{C}^- - \bar{J}^+ - \bar{J}^- - \bar{\chi}^+ - \bar{\chi}^-}{2} \\ &= \frac{a_{eg}^+ + a_{eg}^- - 2a_{gg}\langle S \rangle_{T_R} + b_{eg}^{+3} + b_{eg}^{-3} - 2b_{gg}^3\langle P \rangle_{T_R}}{4} \end{aligned} \quad (4)$$

The s -wave thermal average, $\langle S \rangle_{T_R}$, decreases with T_R as $\langle S \rangle_{T_R} \propto (T_R)^{-1}$. So far we have focused on a single lattice site. To compare with the experimental results, we perform averaging over the atom distributions across all lattice sites. Consequently, the density shifts Δv^s and Δv^i become proportional to $\mathcal{N}_s^{\text{tot}}$ and $\mathcal{N}_i^{\text{tot}}$, respectively.

This model fully reproduces the experimental observations as summarized in points (i) to (iii)

and (I) to (III) above and shown in Fig. 2. To quantitatively compare with the experiment, we performed a Poissonian average of the atom number across the array of 2D traps and used the average excitation fraction to account for the two-body e - e losses (2I, 36) during τ_{free} . The capability of the SU(N) spin lattice model to reproduce the experimental observations also enabled us to determine the remaining s - and p -wave scattering parameters. For each of the four channels, $\eta \in \{ee, gg, eg^+, eg^-\}$, the s -wave and p -wave parameters relate to each other through the characteristic length, $\bar{a}_{\eta}$, of the van der Waals potential (37). Thus, after we determined $\bar{a}_{\eta}$ using the available van der Waals C_6 coefficients (3I), only four elastic scattering parameters remained independent. Among those, a_{gg} , b_{ee} and b_{eg}^+ (and thus their respective p - or s -wave counterparts) are known (2I), leaving only one unknown parameter associated with the eg^- channel. Using the theoretical predictions and a single parameter to fit the data in Fig. 2E, we extracted values for a_{eg}^- and b_{eg}^- . Table 1 lists all the scattering parameters determined from the prior and current measurements.

Coherent dynamic spectroscopy

We performed coherent dynamic spectroscopy to explore the development of many-body correlations as a key effect of Ising orbital magnetism, in the combined orbital and nuclear spin degrees of freedom, generated by the p -wave and s -wave interactions. This allowed us to further validate the SU(N) spin-orbital model (Eq. 1) as a description for our system. The many-body correlations that build up during the free evolution manifest as a decay of the e - g orbital coherence in the form of Ramsey fringe contrast $\mathcal{C}(\tau_{\text{free}}) = 2/\mathcal{N}_i^{\text{tot}} \sqrt{\langle \hat{\mathbb{T}}_{\text{tot}}^x \rangle^2 + \langle \hat{\mathbb{T}}_{\text{tot}}^y \rangle^2}$ that we measure as a function of τ_{free} . Here, $\hat{\mathbb{T}}_{\text{tot}}^{x,y}$ is the sum of $\hat{\mathbb{T}}^{x,y}$ over the 2D traps. We extracted a normalized value of $\mathcal{C}$ by comparing the high-atom-number raw contrast against that of the low atom number; this normalization removes single-particle decoherence effects. The decay of $\mathcal{C}$ during the free-evolution period has been shown to be a particularly suitable observable for characterizing the role of interactions during the dynamics (38). For example, contrast measurements in an array of polar molecules pinned in a 3D lattice provided clear signatures of dipolar interactions and their description in terms of a spin exchange model (32, 39).

In the presence of a large magnetic field, the decay of $\mathcal{C}$ has two sources. The first arises from within the interrogated atoms: p -wave elastic interactions, two-body e - e losses, higher-order interaction-induced mode-changing processes, as well as dephasing induced by the distribution of atoms across traps. All these p -wave effects are accounted for in our theory using the same p -wave parameters that were determined in (2I) and then reconfirmed with our density shift measurements in this work (Fig. 2). The second source comes from spectators, which act on the interrogated atoms at a given site as an inhomogeneous and density-dependent effective magnetic field

Table 1. *s*- and *p*-wave scattering lengths in units of the Bohr radius. Here, except for $a_{gg} = 96.2 \pm 0.1 a_0$, all the other scattering parameters are extracted in this work on the basis of current (this work) and previous measurements (21, 36) as well as the analytic relations (37).

Channel	Scattering lengths in units of the Bohr radius (a_0)		Determination method
	<i>s</i> -wave	<i>p</i> -wave	
<i>gg</i>	96.2 ± 0.1	74.6 ± 0.4	<i>s</i> -wave: Two-photon photoassociative spectroscopy (25) and rovibrational spectroscopy (26) <i>p</i> -wave: Analytic relation between <i>s</i> - and <i>p</i> -wave parameters (37)
<i>eg</i> ⁺	169 ± 8	-169 ± 23	<i>s</i> -wave: Analytic relation (37) <i>p</i> -wave: Density shift in a polarized sample (21)
<i>eg</i> [−]	68 ± 22	-42^{+103}_{-22}	<i>s</i> -wave: Density shift in a spin mixture at different temperatures (this work) <i>p</i> -wave: Analytic relation (37)
<i>ee</i> (elastic)	176 ± 11	-119 ± 18	<i>s</i> -wave: Analytic relation (37) <i>p</i> -wave: Density shift in a polarized sample (21)
<i>ee</i> (inelastic)	$\gamma_{ee} = 46^{+19}_{-32}$	$\beta_{ee} = 121 \pm 13$	Two-body loss measurement (36) and analytic relation (37)

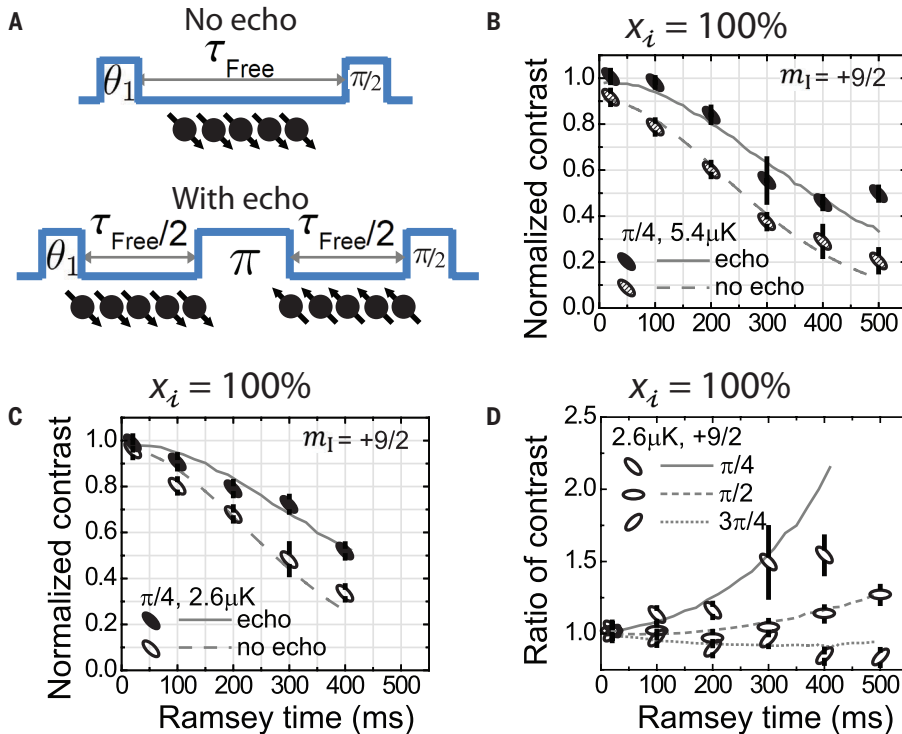


Fig. 3. Evolution of orbital coherence in nuclear spin-polarized samples. (A) Upper panel: Ramsey sequence with varying θ_1 and τ_{free} . Lower panel: Sequence with an echo (π) pulse. The group of circles illustrates the orbital configurations for interrogated atoms (black circles). (B and C) Normalized Ramsey contrasts for $\theta_1 = \pi/4$, $x_i = 100\%$, under two different radial temperatures, $T_R = 5.4 \mu\text{K}$ and $2.6 \mu\text{K}$, respectively. The contrast is normalized by comparing the high-atom-number raw Ramsey fringe contrast, $\mathcal{C}$ (defined in the main text), against the low-atom-number raw contrast. The high total atom numbers (measured at a very short free evolution time, $\tau_{\text{free}} = 20$ ms) are in the range of 2200 to 3100 for Figs. 3 and 4 (day-to-day variation over 4 months). However, for each specific case, the data without and with echo were taken on the same day and their atom numbers are matched to within 3 to 7%. These atom numbers are recorded and serve as inputs to our theory calculation of Ramsey contrast decay. Solid and open symbols denote measurements with and without echo; solid and dashed lines show theory calculations with and without echo, respectively, using a two-orbital model with independently determined parameters (based on measurements shown in Fig. 2 and previous studies, see Table 1). Under the conditions of (B) and (C), the dominant source for contrast decay arises from *p*-wave interactions between the interrogated atoms. (D) Effects of echo, characterized by the ratio of contrast with echo to that without echo, for $\theta_1 = \pi/4$ (antidiagonal ellipse and solid line), $\pi/2$ (horizontal ellipse and short dashed line), and $3\pi/4$ (diagonal ellipse and short dotted line), under $x_i = 100\%$. Error bars represent 1σ multiplied by $\sqrt{\chi^2_{\text{reduced}}}$.

along z , with both *s*- and *p*-wave contributions. The effective magnetic field is static if the atoms are frozen in their motional states, but can vary with time in the presence of higher-order mode-changing processes. The *p*-wave interaction plays a dominant role at high $T_R = 5$ to $6 \mu\text{K}$, whereas the *s*-wave interaction, which has a stronger dependence on the mode distribution, becomes important at lower T_R . We focus first on the nuclear spin-polarized case (Fig. 3) to benchmark our model, and then use various population distributions among nuclear spin states to investigate the interplay between orbital and spin degrees of freedom (Fig. 4).

Two-orbital dynamics in spin-polarized atoms

To understand in detail the orbital dynamics induced by *p*-wave interactions, we first studied a nuclear spin polarized sample ($x_i = 100\%$) for $T_R = 5$ to $6 \mu\text{K}$ and $\theta_1 = \pi/4$ (Fig. 3B). To separate the effects of dephasing and many-body correlation in the contrast decay, we applied a π echo pulse in the middle of the Ramsey sequence (Fig. 3A, lower panel). The π echo pulse modifies the contrast decay in a θ_1 -dependent way because of the enhanced *e-e* loss after the echo pulse (note the number normalization in $\mathcal{C}$) for $\theta_1 < \pi/2$, as well as because the *p*-wave contribution to contrast decay contains both θ_1 -independent and θ_1 -dependent terms. The θ_1 -independent contribution is generated by the term $\bar{C}^+ \bar{T}^z \mathcal{N}_i$. This term is responsible for the density-dependent dephasing between atoms distributed in different 2D traps. The echo pulse removes it as well as other dephasing effects of technical origin. The θ_1 -dependent contribution is generated by the term $\bar{\chi}^+(\bar{T}^z)^2$ in the Hamiltonian and can lead to many-body orbital correlations that are not removable by echo.

For $\theta_1 = \pi/4$, the Ramsey contrast decays more slowly with an echo pulse (Fig. 3B). This positive echo effect can be attributed to the suppressed dephasing from inhomogeneous atomic densities across different 2D traps (θ_1 -independent contribution) and to the faster number loss with echo (as we normalize the contrast against remaining atom number).

In a polarized sample where p -wave interactions dominate, the contrast decay is expected to be insensitive to T_R . This is confirmed with measurements performed at $T_R = 2.6 \mu\text{K}$ (Fig. 3C) that show similar decay behaviors to those at $5.4 \mu\text{K}$ (Fig. 3B). In addition, we find an excellent agreement between the data and the theoretical model that uses temperature-insensitive p -wave parameters. Figure 3D plots the ratio of contrasts with and without echo for different pulse areas and illustrates the positive echo effect in suppressing contrast decay for $\theta_1 = \pi/4$, as well as the negative effect for $\theta_1 = 3\pi/4$ when the echo enhances contrast decay. The negative echo effect can be attributed to both the development of

many-body orbital correlations for $\theta_1 = 3\pi/4$ (2I) and the reduced e - e loss after the echo. All measurements are well reproduced by our spin lattice model.

Spin-orbital SU(N) dynamics in spin mixtures

To investigate the interplay between orbital and spin degrees of freedom, we performed similar spectroscopic measurements in spin-mixed samples (Fig. 4A). We studied the spin-mixed cases under $T_R = 5$ to $6 \mu\text{K}$, with $\theta_1 = \pi/4$ and the interrogated fraction $x_i = 14\%$ and 56% (Fig. 4, B and C, respectively). Here, the data show similar positive effect of an echo pulse in the presence of

spectator atoms. Because p -wave interactions between interrogated atoms are reduced as the interrogated fraction decreases, the overall contrast decay becomes slower. On the basis of the determined scattering parameters, our model predicts that spectator atoms cause almost negligible decoherence effects at this high $T_R = 5$ to $6 \mu\text{K}$ (3I).

When we decreased T_R to $\sim 2 \mu\text{K}$, the rise of the s -wave contribution caused enhanced decoherence effects coming from the spectator atoms. Figure 4D illustrates the influence of spectators for the $x_i = 14\%$ minority case, where contrast decay is clearly faster than in Fig. 4B. The inclusion of off-resonant mode-changing collisions as higher-order corrections is required for temperatures below $5 \mu\text{K}$ to accurately reproduce the experimental observations (3I). These mode-changing collisions can be visualized as relocating pairs of atoms in the energy-space lattice shown in Fig. 1C, analogous to interaction-induced tunneling processes in a real-space lattice. The echo pulse suppresses the part of contrast decay arising from mode-preserving collisions between spectators and interrogated atoms, but it cannot reverse the decay due to mode-changing processes. In Fig. 4D, the measured contrast decay with echo enables us to determine a single parameter characterizing the mode-changing processes (3I).

For a further and independent test of our model, we explored another case with $x_i = 56\%$ and $T_R \sim 2 \mu\text{K}$, such that both the interrogated atoms and spectator atoms have important contributions to the contrast decay. The data were well described by the same theory model (Fig. 4E and fig. S1) without any variation of the predetermined parameters, demonstrating a firm understanding of the system dynamics.

The experimental exploration of exotic SU(N) physics is just starting. The unique capability of precision laser spectroscopy has so far allowed us to explore Ising orbital magnetism at relatively high temperatures. Future studies will explore the full Hamiltonian including the exchange interactions by controlling the atomic density, temperature, and the magnetic field to engineer various spin-spin and spin-orbital dynamics. This will allow expansion of the frontier of emergent many-body quantum physics at increasingly high temperatures, as well as the study of time-resolved dynamics in the SU(N) Kondo lattice and Kugel-Khomskii models (3, 40, 41) in the quantum gas regime.

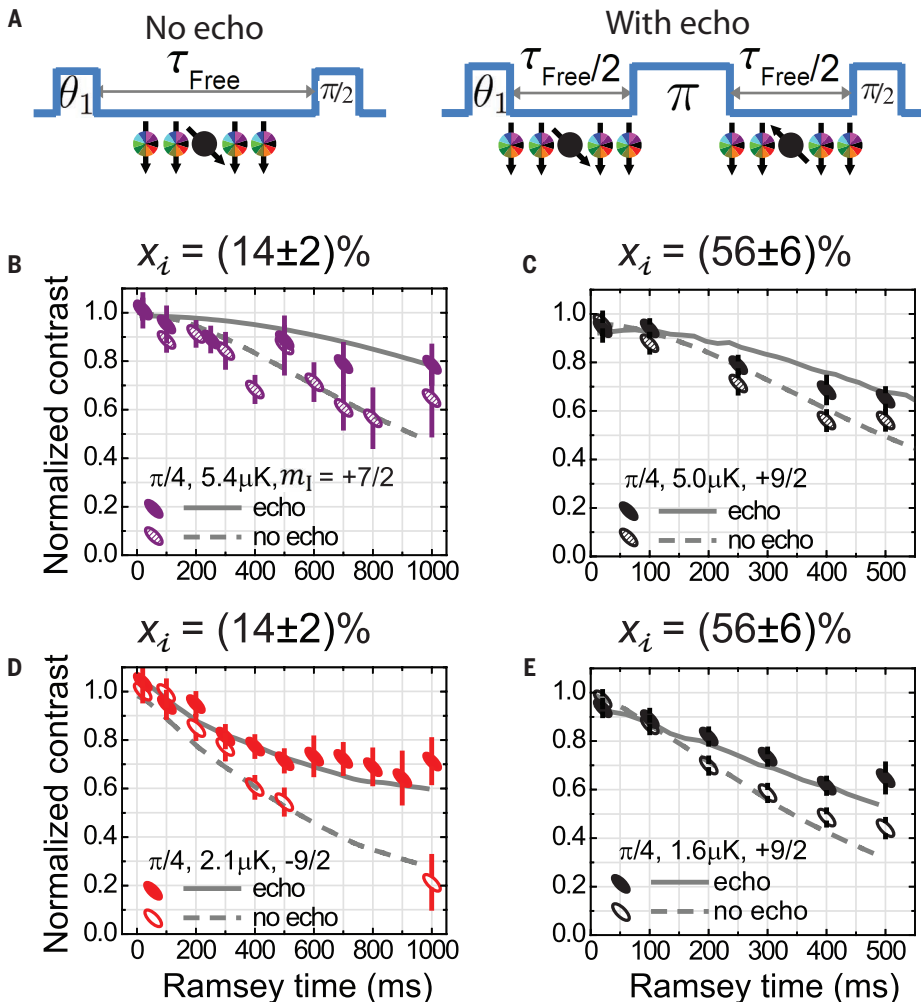


Fig. 4. Evolution of orbital coherence in nuclear spin-mixed samples. (A) Left panel: Ramsey sequence with varying θ_1 and τ_{free} . Right panel: Sequence with an echo (π) pulse. The group of circles illustrates the orbital configurations for interrogated atoms (black circles) and spectator atoms (colored circles). (B and D) Normalized Ramsey contrast for $\theta_1 = \pi/4$, $x_i = 14 \pm 2\%$, under $T_R = 5.4 \mu\text{K}$ and $2.1 \mu\text{K}$, respectively: measurements with echo (solid symbols) and without echo (open symbols). (C and E) Normalized contrast for $\theta_1 = \pi/4$, $x_i = 56 \pm 6\%$, under $T_R = 5.0 \mu\text{K}$ and $1.6 \mu\text{K}$, respectively. Solid and dashed lines show theory calculations with and without echo, respectively, using a two-orbital model with independently determined parameters (based on measurements shown in Fig. 2 and previous studies, see Table 1). In this model, the spectators act as an effective inhomogeneous magnetic field causing dephasing to the interrogated atoms. Without mode-changing collisions, the dephasing is static and removable by echo; with mode-changing collisions, it is assumed and confirmed experimentally that echo does not remove the time-dependent dephasing. The theory uses a single fitting parameter for the mode-changing processes for all plots. Error bars represent 1σ multiplied by $\sqrt{\chi^2_{\text{reduced}}}$.

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ACKNOWLEDGMENTS

We thank P. Julienne, B. Gadoway, T. Nicholson, B. Bloom, and A. V. Gorshkov for technical discussions. Supported by the National

Defense Science and Engineering Graduate Fellowship program and the NSF Graduate Research Fellowship program (M.B.) and by NIST, NSF grant PFC-1125844, Air Force Office of Scientific Research (MURI and single investigator award), Defense Advanced Research Projects Agency (QuASAR), Austrian Science Foundation, SFB FoQus (Foundations and Applications of Quantum Science), European Research Council Synergy Grant (UQUAM), and Simulators and Interfaces with Quantum Systems (SIQS) project. The data described in the paper are archived in a database at JILA.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1467/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 and S2
Tables S1 to S3
References (44–58)

18 April 2014; accepted 11 August 2014
Published online 21 August 2014;
10.1126/science.1254978

STRUCTURAL BIOLOGY

Crystal structure of the CRISPR RNA-guided surveillance complex from *Escherichia coli*

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Clustered regularly interspaced short palindromic repeats (CRISPRs) are essential components of RNA-guided adaptive immune systems that protect bacteria and archaea from viruses and plasmids. In *Escherichia coli*, short CRISPR-derived RNAs (crRNAs) assemble into a 405-kilodalton multisubunit surveillance complex called Cascade (CRISPR-associated complex for antiviral defense). Here we present the 3.24 angstrom resolution x-ray crystal structure of Cascade. Eleven proteins and a 61-nucleotide crRNA assemble into a seahorse-shaped architecture that binds double-stranded DNA targets complementary to the crRNA-guide sequence. Conserved sequences on the 3' and 5' ends of the crRNA are anchored by proteins at opposite ends of the complex, whereas the guide sequence is displayed along a helical assembly of six interwoven subunits that present five-nucleotide segments of the crRNA in pseudo-A-form configuration. The structure of Cascade suggests a mechanism for assembly and provides insights into the mechanisms of target recognition.

Clustered regularly interspaced short palindromic repeat (CRISPR) loci provide the molecular memory of an adaptive immune system that is prevalent in bacteria and archaea (1–5). Each CRISPR locus consists of a series of short repeats separated by non-repetitive spacer sequences acquired from foreign genetic elements such as viruses and plasmids.

CRISPR loci are transcribed, and the long primary transcripts are processed into a library of short CRISPR-derived RNAs (crRNAs) that contain sequences complementary to previously encountered invading nucleic acids. CRISPR-associated (Cas) proteins bind each crRNA, and the resulting ribonucleoprotein complexes target invading nucleic acids complementary to the crRNA guide. Targets identified as foreign are subsequently degraded by dedicated nucleases.

Phylogenetic and functional studies have identified three main CRISPR-system types (I, II, and III) and 11 subtypes (IA to IF, IIA to IIC, and IIIA to IIIB) (6). The type IE system from *Escherichia coli* K12 consists of a CRISPR locus and eight *cas* genes (Fig. 1A). Five of the *cas* genes in this system encode for proteins that assemble with the crRNA into a large complex called Cascade (CRISPR-

associated complex for antiviral defense) (7). Efficient detection of invading DNA relies on complementary base pairing between the DNA target and crRNA-guide sequence, as well as recognition of a short sequence motif immediately adjacent to the target called a protospacer-adjacent motif (PAM) (8–10). Target recognition by Cascade triggers a conformational change and recruits a transacting nuclease-helicase (Cas3) that is required for destruction of an invading target (8, 11–15). However, an atomic-resolution understanding of Cascade assembly and crRNA-guided surveillance has not been available.

To understand the mechanism of crRNA-guided surveillance by Cascade, we determined the 3.24 Å-resolution x-ray crystal structure of the complex (Fig. 1). The structure explains how the 11 proteins assemble with the crRNA into an interwoven architecture that presents discrete segments of the crRNA for complementary base pairing. Overall, the Cascade structure reveals features required for complex assembly and provides insights into the mechanisms of target recognition.

Overview of the Cascade structure

We determined the x-ray crystal structure of Cascade by molecular replacement, using the 8 Å cryo-electron microscopy (cryo-EM) map as a search model (Fig. 1, fig. S1, table S1, and supplementary materials and methods) (12). Initial phases were improved and extended to 3.24 Å by averaging over noncrystallographic symmetry (16). The asymmetric unit contains two copies of Cascade that superimpose with an average root mean square deviation (RMSD) of 1.29 Å for equivalently positioned Cα atoms (fig. S2). Here we focus our description on complex one, but both assemblies consist of 11 protein subunits and a single 61-nucleotide (nt) crRNA that traverses the length of the complex. Nine of the 11 Cas proteins make direct contact with the crRNA, and eight of the nine RNA-binding proteins contain a modified RNA recognition motif (RRM) (Fig. 1, B to D). The 5' and 3' ends of the crRNA are derived from the repeat region of the crRNA and are bound at opposite ends of the seahorse-shaped

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complex. Cas6e binds the 3' end of the crRNA at the head of the complex, whereas the 5' end of the crRNA is sandwiched between three protein subunits (Cas5, Cas7.6, and Cse1) in the tail (Fig. 1C). The head and tail of the complex are connected along the belly by two Cse2 subunits and a helical backbone of six Cas7 proteins (Cas7.1 to Cas7.6). This assembly creates an interwoven ribonucleoprotein structure that kinks the crRNA at 6-nt intervals (Fig. 1D).

Mechanism of RNA recognition by the Cas6e endonuclease

Cas6 family proteins are phylogenetically diverse, but all Cas6 proteins are metal-independent

endoribonucleases that selectively bind and cleave long CRISPR RNA transcripts (Fig. 2A) (7, 8, 17–20). Our structure reveals that the *E. coli* Cas6e protein consists of tandem RRM connected by an eight-residue linker (Fig. 2). Each RRM (also called a ferredoxin-like fold) consists of a conserved β 1- α 1- β 2- β 3- α 2- β 4 arrangement in which the β strands are arranged in a four-stranded antiparallel β sheet and the two helices pack together on one side of the sheet. The β sheets in each of the two RRM face one another, creating a V-shaped cleft along one face of the protein (Fig. 2B). This cleft was initially predicted to bind RNA (21), but a positively charged surface on the opposite face of the protein makes elec-

trostatic contacts with the 3' strand of the crRNA stem-loop (Fig. 2C). In addition, a positively charged “groove-loop” (residues 90 to 119) on the C-terminal RRM domain makes extensive electrostatic contacts with the major groove of the crRNA stem-loop, including base-specific contacts with C49, G48, and G51 (Fig. 2D and fig. S3). Residues at the base of this loop (N91, K94, N99, R102, C112, and I116) make base-specific (G35, U36, and U37) and hydrophobic (A34) contacts with nucleotides at the 5' end of the stem-loop (Fig. 2D and figs. S3 and S4) (22). We expect that other Cas6e proteins make similar contacts, but this portion of the crRNA has not been included in previous studies.

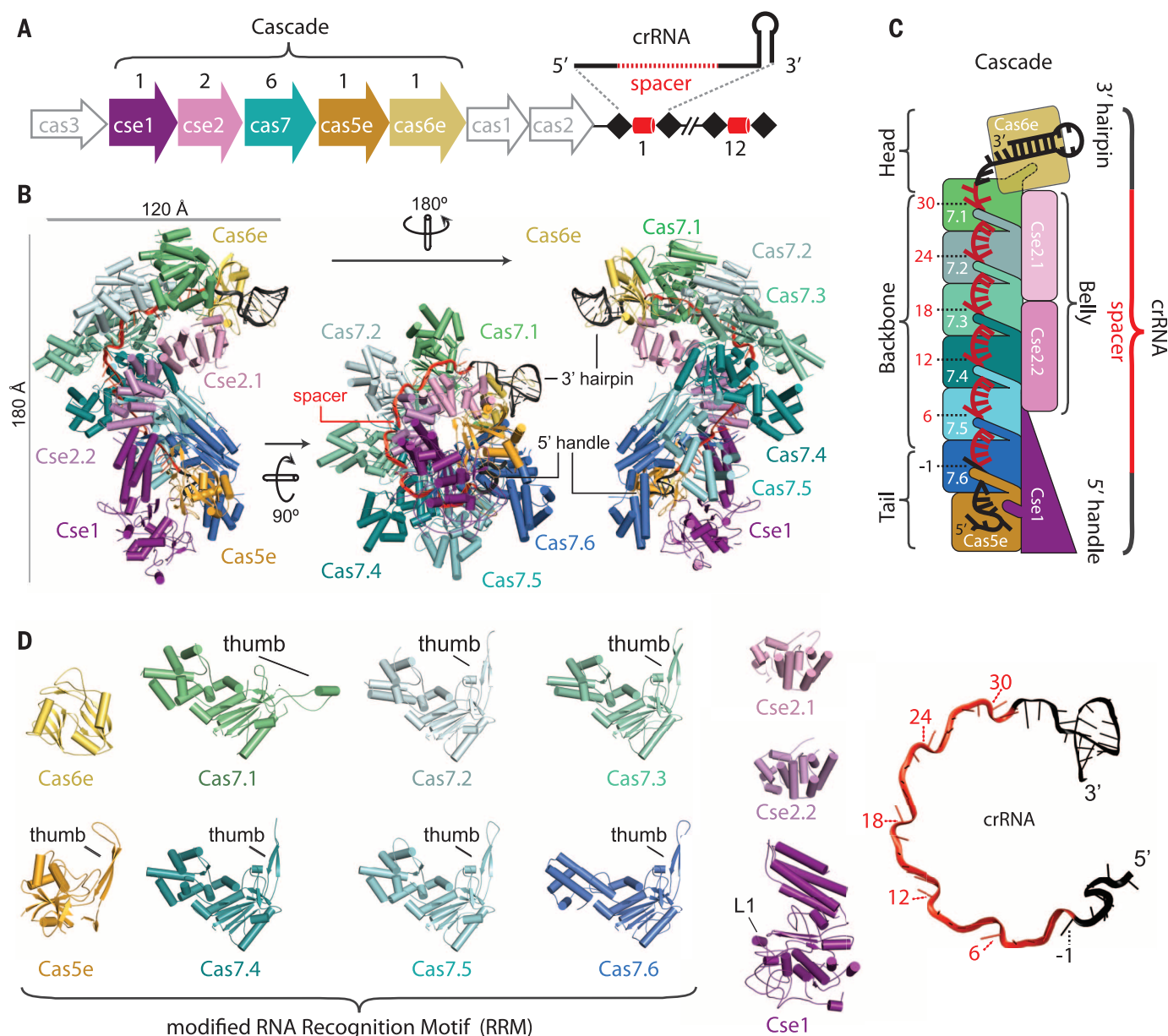


Fig. 1. X-ray crystal structure of Cascade. (A) The type I-E CRISPR-mediated immune system in *E. coli* K12 consists of eight *cas* genes and one CRISPR locus. The CRISPR locus consists of a series of 29-nt repeats (black diamonds) separated by 32-nt spacer sequences (red cylinders). (B) Orthogonal views of the Cascade structure. (C) Schematic of Cascade colored according to (B). Kinked bases are numbered. (D) Cascade consists of an uneven stoichiometry of five different Cas proteins and a single crRNA. The “thumb” of each backbone protein folds over the top of the crRNA, creating a kink in the RNA at 6-nt intervals (–1, 6, 12, 18, 24, and 30).

Precleavage recognition of the CRISPR RNA relies on base-specific interactions within the stem-loop and nucleotides on the 3' side of the stem-loop, which help position the scissile phosphate in the active site (18). After cleavage of the primary CRISPR transcript, Cas6e remains tightly associated with the stem-loop of the mature crRNA (Fig. 3). The V-shaped cleft, opposite the RNA binding face of Cas6e, provides a binding site for a short helix from Cas7.1 that tethers Cas6e to the helical backbone of Cascade (Fig. 3, B to D).

Assembly of the Cas7 backbone

The backbone of Cascade is composed of six Cas7 proteins that oligomerize along the crRNA,

forming an interwoven architecture that presents the crRNA-guide sequence in six discrete segments (Fig. 4A and fig. S3). Each segment consists of a buried nucleotide followed by five solvent-accessible bases that are ordered in a pseudo-A-form configuration by interactions with three different protein subunits (Fig. 4, A to C). The Cas7 protein folds into a structure shaped like a right hand (Fig. 4C and fig. S5) (23, 24). This shape is created by a modified RRM that forms the palm, a helical domain resembles fingers (residues 59 to 181), a 30-amino acid loop takes on the shape of a thumb (residues 193 to 223), and two smaller loops inserted in the RRM form a web between the thumb and

the fingers (Fig. 4C). Unlike most RRM, which bind to RNA using conserved residues positioned on the face of the antiparallel β sheet, our structure reveals a series of interactions with the phosphate backbone that are primarily limited to the first α helix ($\alpha 1$) of the RRM, the web, and the thumb (Fig. 4C and fig. S5). The first α helix of most RRM is positioned on the backside of the β sheet and does not directly contact the RNA. However, in Cas7 the $\alpha 1$ helix is positioned perpendicular to and off to one side of the central β sheet (Fig. 4C). Conserved residues in the $\alpha 1$ helix interact with three consecutive phosphates in a way that introduces two consecutive $\sim 90^\circ$ turns in the backbone of the crRNA (Fig. 4 and figs. S3 and S6). These “chicanes” in the crRNA occur at a regular 6-nt periodicity defined by the distance between $\alpha 1$ helices on adjacent Cas7 subunits. Each chicane is separated by five bases presented to the solvent in pseudo-A-form, whereas the sixth base is flipped out of the helical presentation and covered by the thumb of an adjacent Cas7 molecule (Fig. 4). The position of each thumb is stabilized by electrostatic interactions with the $\alpha 1$ helix on the palm of an adjacent molecule, and mutations in the thumb of homologous Cas7 proteins have been shown to reduce RNA binding affinities (24).

The interwoven arrangement of interlocking Cas7 subunits divides the crRNA into six segments (Fig. 4, A and D). The first five segments consist of a pattern of five ordered nucleotides that are book-ended by thumbs that fold over every sixth nucleotide in the crRNA-guide sequence. This suggests that every sixth nucleotide of the crRNA guide may not participate in target recognition. To test this hypothesis, we determined binding affinity for Cascade to double-stranded DNA (dsDNA) targets that were either 100% complementary to the crRNA guide or mismatched at 6-nt intervals (Fig. 4D and table S2). The equilibrium dissociation constant for a target that contains a PAM (5'-CAT-3') and a target sequence complementary to the crRNA guide is 1.6 nM (Fig. 4D and fig. S7). Mutations in the target that disrupt base pairing at every sixth position (positions 6, 12, 18, 24, and 30) have no measurable defect in target binding, whereas mutations on either side of every sixth position result in major binding defects (Fig. 4D). Mutations at positions 5, 11, 17, 23, and 29 result in binding affinities that are more than two orders of magnitude weaker than targets that are either 100% complementary or mutated at every sixth position. The binding defect is even more pronounced for targets with mismatches at positions 7, 13, 19, 25, and 31 (Fig. 4D).

Complementarity between the crRNA guide and the target is critical at positions 1 to 5, 7 and 8 (9, 10, 25, 26). This portion of the crRNA guide is called the “seed” sequence, and it has been suggested that helical ordering of these bases may explain their importance in target binding. However, the helical arrangement of bases in segment 1 (positions 1 to 5) of the crRNA guide is not substantially different from that in segments 2 to 5 (Fig. 4C). In fact, the ordered nucleotides in

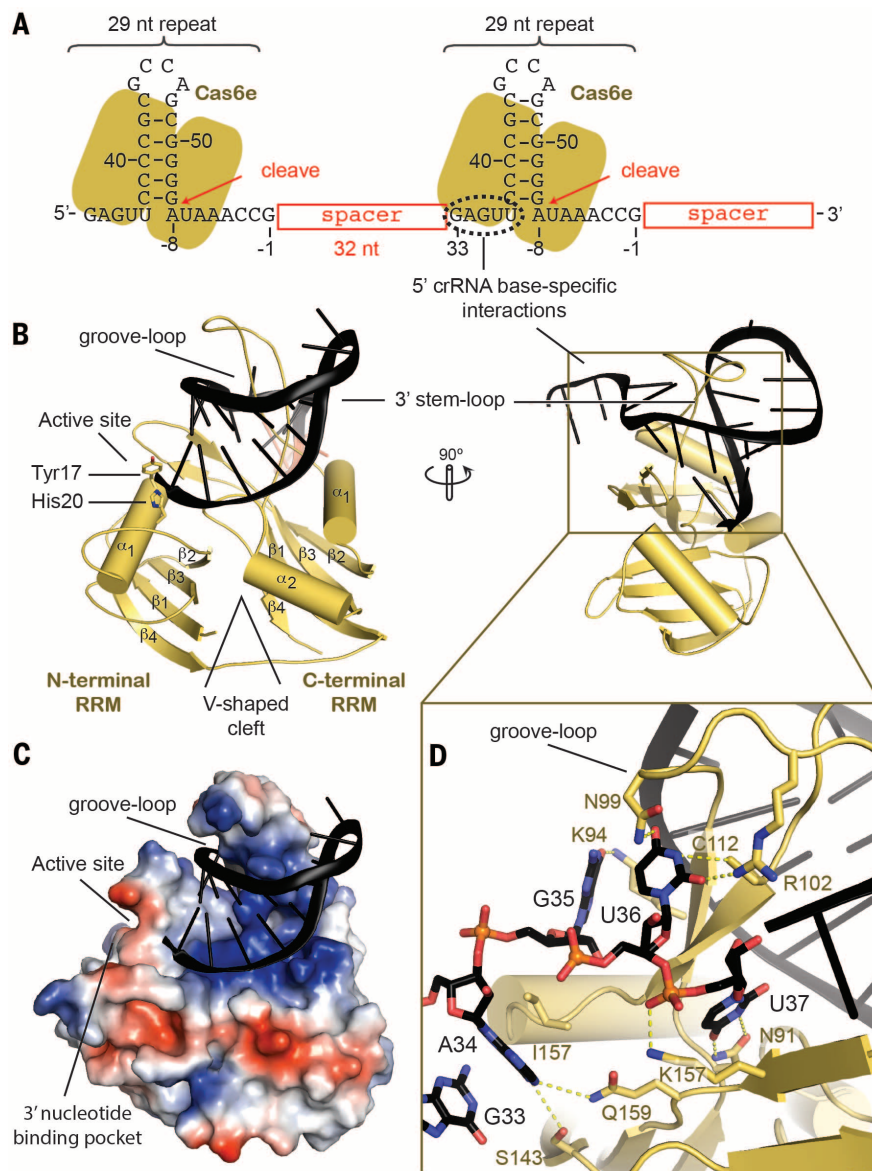


Fig. 2. Mechanism of crRNA recognition by Cas6e. (A) Schematic of Cas6e bound to the stem-loop of the CRISPR RNA repeat. (B) Structure of Cas6e bound to the 3' stem-loop of the crRNA. A β hairpin, referred to as the “groove-loop,” inserts into the major groove of the crRNA stem-loop. Cas6e binding positions the scissile phosphate into the endonuclease active site. (C) Electrostatic surface representation of Cas6e illustrates how the positively charged groove-loop fits into the major groove of the crRNA stem-loop. (D) The groove-loop makes sequence-specific interactions with nucleotides 5' of the stem-loop.

segments 1 through 5 superimpose with an average RMSD of 0.45 Å, suggesting that the importance of the seed in target recognition may have more to do with the location of this sequence relative to the PAM rather than preferential preordering of the bases.

The helical display of each segment is induced by amino acids (T201, L214, W199, and F200) located on the Cas7 thumbs that stack with bases on the 5' and 3' ends of each segment (Fig. 4C and fig. S3). The first two bases in each segment are ordered in an A-form configuration, but the third base is nudged out of ideal A-form by a conserved methionine (M166) that inserts between the third and fourth nucleotides in each segment (Fig. 4C). Many of the amino acids important for ordering the bases in each segment are located on the thumbs that flank segments 1 to 5. Segment 6 is not flanked by a thumb on the 3' end, and the bases in this segment are more flexible (Fig. 4B and fig. S5). Unlike the other five Cas7 subunits, the thumb on Cas7.1 contains a short helix that inserts into the hydrophobic V-shaped cleft of Cas6e, connecting the Cas6e head to the Cas7 backbone (Fig. 3).

In addition to the Cas7 backbone, Cas6e is connected to the body of Cascade via interactions with Cse2 (Fig. 1 and fig. S8). The Cse2 proteins form a head-to-tail dimer that assembles along the belly of Cascade making contacts with the thumb and web of Cas7 proteins (Fig. 1C and fig. S8). Although the Cse2 subunits do not make direct contacts with the crRNA, electrostatic calculations show that both faces of the Cse2 dimer are positively charged, indicating a possible role for Cse2 in stabilizing the bound and displaced strands of the DNA target (fig. S8) (12, 27). Comparison of the two Cascade assemblies in the asymmetric unit reveals that Cse2.1 of assembly two is shifted by 7 Å away from the equivalent position in assembly one and Cas6e is rotated

~16° (fig. S2). This suggests that rotation of the head can influence the position of the Cse2 subunits (12).

Programmed tail assembly

CRISPR RNA processing results in a library of mature crRNAs that have a conserved 8-nt "handle" on the 5' end that is derived from the CRISPR repeat sequence (Figs. 1 and 2). These nucleotides, numbered -8 to -1 according to convention, function as a molecular signal that initiates assembly of Cas7.6, Cas5e, and Cse1. The $\alpha 1$ helix of Cas7.6 introduces a final 5' chicane in the crRNA by interacting with nucleotides that straddle the boundary between the 5' handle and seed sequence (Fig. 5). If the oligomeric assembly of Cas7s were to continue along the crRNA in the 5' direction, then the remaining six nucleotides would be ordered across the web of the next Cas7 subunit. However, these six nucleotides (-8 to -3) are recognized by Cas5e, which may block propagation of Cas7 oligomerization at the 5' end of the crRNA, induce a conformational change in the finger domain of Cas7.6, and provide a platform for the recruitment of Cse1 to the tail (Fig. 5).

The Cas5e protein adopts a "right-handed fist-shape" structure where the thumb arches across the top of the fist (Fig. 5B and fig. S9). The fist is composed of a modified RRM that includes a 50-amino acid insertion between β strands two and three that takes on the shape of a thumb. The Cas5e thumb, which bears no recognizable sequence similarity to the Cas7 thumb, performs a very similar function by folding over the top of the kinked base (nucleotide -1), and positions the first nucleotide of the seed in an A-form configuration (Fig. 5B and fig. S3). However, unlike the straight thumb on Cas7 proteins, the Cas5e thumb arches over the top of the fist and interacts with the finger domain of Cas7.6. Structural alignments of Cas7.6 with the other Cas7 sub-

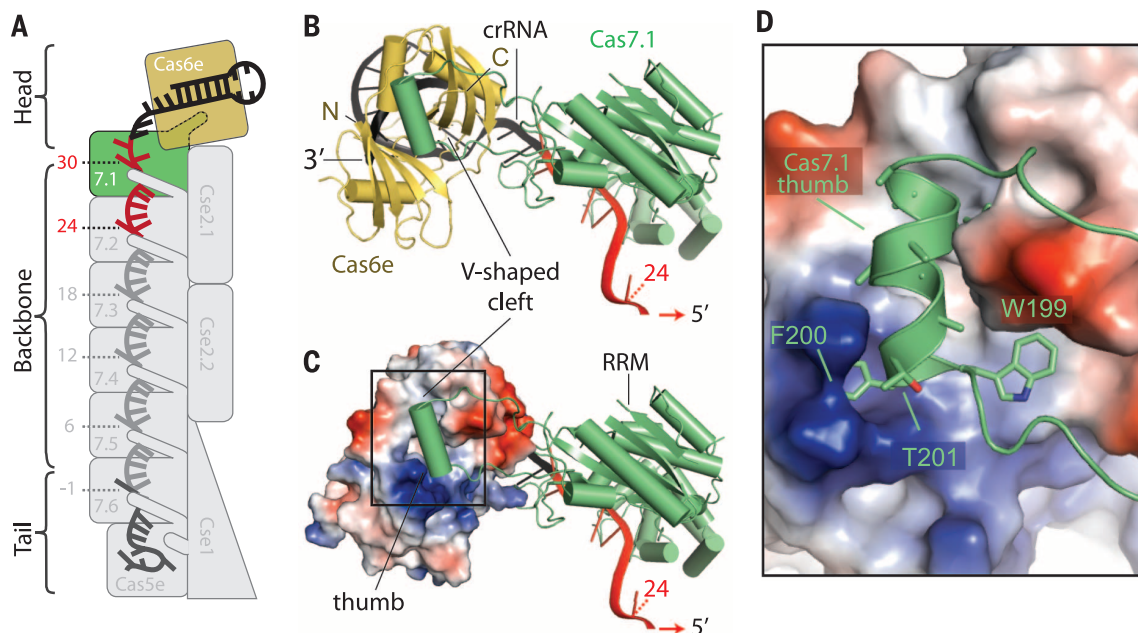
units reveal a ~180° rotation of the finger domain that accommodates the Cas5e thumb and creates a 28 Å gap between the finger domains of Cas7.5 and Cas7.6 (Fig. 5C). A recent cryo-EM structure of Cascade bound to dsDNA reveals that the enlarged separation between these two domains accommodates the dsDNA target (17). Modeling our crystal structure into the cryo-EM density reveals a lysine-rich helix (K137, K138, K141, and K144) on Cas7.5 and Cas7.6 that may play a role in stabilizing the dsDNA during target recognition (fig. S10).

The last seven nucleotides on the 5' end of the crRNA form a distinct S-shaped curve that follows along the arch of the Cas5e thumb and swings across the web of Cas7.6, and the final three bases (-8A, -7U, and -6A) fit into base-specific binding pockets positioned along the top of the glycine-rich $\alpha 1$ helix on Cas5e (Fig. 5 and fig. S9). Nucleotides -5A, -4A, and -3C stack into a well-ordered triplet, whereas the cytosine at position -2 hangs vertically behind this triplet and hydrogen bonds with the phosphate of nucleotide -4A. Mutations at the -2 position interfere with Cascade assembly (9), and the structure reveals that the cytosine at this position participates in maintaining the S-shaped curve in the 5'-handle (Fig. 5B).

Cas5 structures from distantly related CRISPR systems also contain a glycine-rich $\alpha 1$ helix and a positively charged binding pocket that may play a similar role in recognition of nucleotides in the 5' handle of the crRNA (fig. S9) (28–30). Cas5 proteins from type IC systems have an additional C-terminal extension that contains an endonuclease active site (30). In these systems, Cas5d is the CRISPR-specific endoribonuclease responsible for crRNA processing, and structural alignments with Cas5e suggest that Cas5d endonucleases may recognize the 5' handle of the crRNA rather than the 3' stem-loop (fig. S11). These observations

Fig. 3. Connecting the head to the Cas7 backbone.

(A) Schematic of Cascade highlighting the connection between Cas6e and Cas7.1. (B and C) A short helix located on the thumb of Cas7.1 fits in a groove between the N- and C-terminal RRM on Cas6e. The Cas6e helix-binding groove is located opposite the crRNA-binding surface. (D) Conserved hydrophobic residues (F200, T201, and W199) are positioned in binding pockets in the Cas6e V-shaped cleft.



may explain why Cas5d proteins no longer cleave crRNA substrates when portions of the 5' handle are mutated or removed (28, 30).

The Cas5e thumb arches over the top of the fist, creating a cylindrical pore that permits access to the nucleotides in the 5' handle (Fig. 5, B to D). This pore is a docking module for a short α helix on Cse1. This helix is on a loop, previously called loop 1 (L1) (residues 130 to 143), that is

disordered in the crystal structure of the Cse1 protein from *Thermus thermophilus* (31, 32). In the Cascade structure, the L1 helix inserts into the Cas5e helix-binding pore and makes base-specific interactions with the AAC triplet (Fig. 5, A and D) (31).

Cse1 is a large two-domain protein that adopts a distinct globular fold that contains a metal ion coordinated by four cysteines (C140, C143, C250,

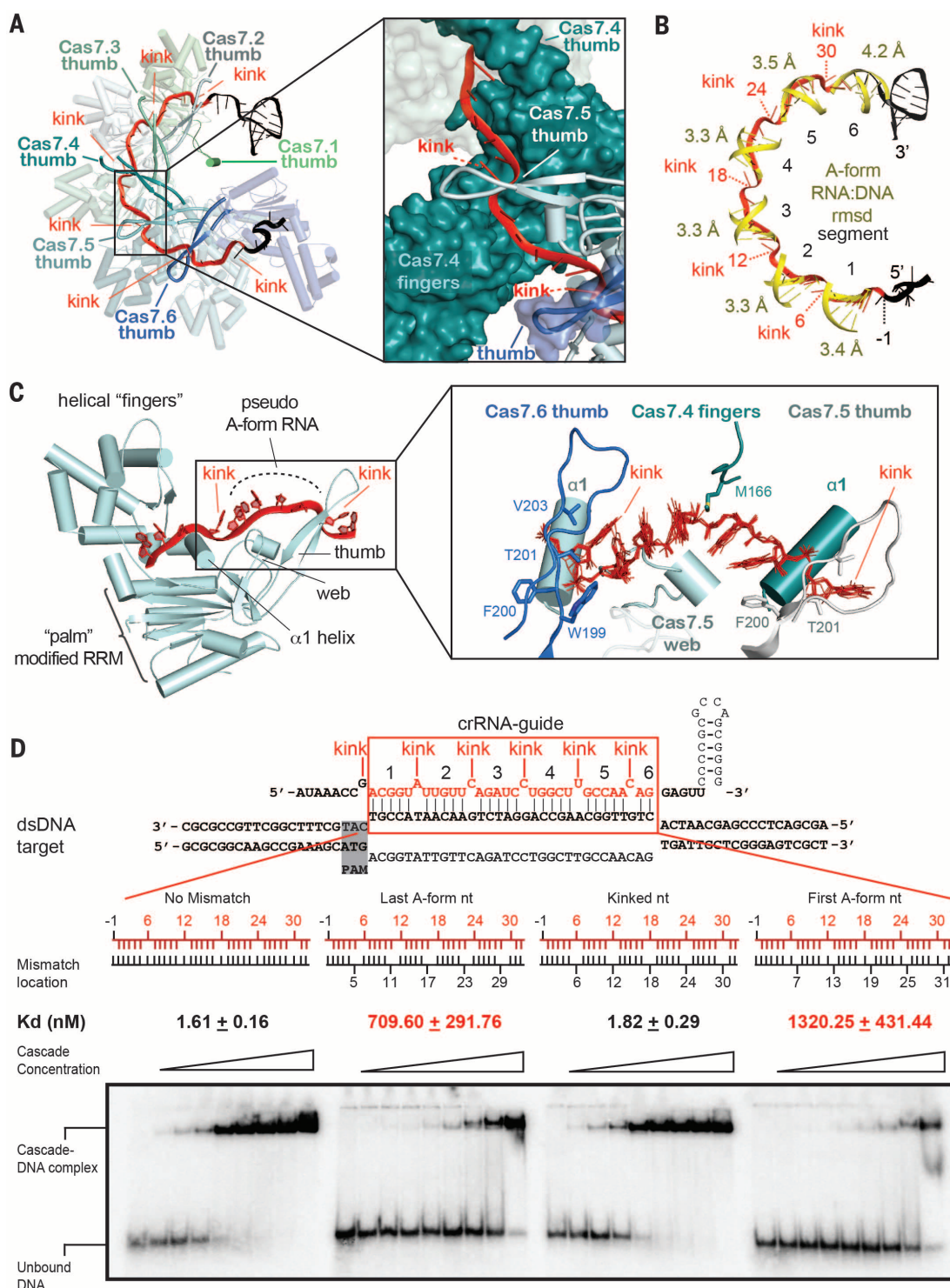
and C253), as well as a C-terminal four-helix bundle (fig. S12). The metal-ion binding motif creates a knob on the end of a loop that may be involved in positioning the L1 helix for docking. In addition to the docking interaction by L1, the globular domain of Cse1 also makes contacts with the modified RRM of Cas5e, and the four-helix bundle on Cse1 extends off the top of the globular domain, making contacts with the C-terminal

Fig. 4. Assembly of the Cas7 backbone creates an interwoven structure that presents segments of the crRNA for target binding.

(A) The Cas7 subunits bind the crRNA in a right-handed helical arrangement in which the thumbs of Cas7.2 to Cas7.6 fold over the top of the crRNA, kinking every sixth nucleotide. (B) Cas7 binding subdivides the crRNA into six segments that are preordered in an A-form-like conformation. Idealized RNA:DNA hybrids are superimposed on each preordered segment of the crRNA, and the RMSD for each section is indicated.

(C) Each Cas7 subunit is shaped like a right hand with fingers (helical domain), palm (modified RRM), webbing, and a thumb. The inset is a zoomed-in view of segments 1 to 5 superimposed on one another. Key residues on the thumbs that flank each segment are indicated.

(D) Electrophoretic mobility shift assays of dsDNA substrates that contain mismatches with the crRNA at 6-nt intervals. Equilibrium dissociation constants are an average from three independent experiments.



domain of Cse2.2 (fig. S8). This interaction completes the structural bridge that connects the four-helix bundle of the Cse1 tail to the Cas6e head.

Discussion

The x-ray crystal structure of Cascade explains how the 12 subunits of this complex assemble into an RNA-guided surveillance machine that targets dsDNA. CRISPR RNA processing by Cas6e is essential for RNA-guided protection from invading DNA (7). Cas6e recognizes the CRISPR RNA repeat sequence through interactions with the RNA stem-loop and specific interactions with bases on the 5' and 3' sides of the stem-loop (Fig. 2 and fig. S4) (18). After cleavage, Cas6e remains tightly associated with the 3' stem-loop of the mature crRNA, and this subcomplex may serve as a platform for the ordered assembly of the re-

maining 10 protein subunits that compose the backbone, tail, and belly of Cascade (movie S1).

Unlike Cas6e and Cas5e, which make sequence-specific interactions with portions of the CRISPR repeat sequence, the Cas7 proteins polymerize along the crRNA via non-sequence-specific interactions (Fig. 4). The structure of Cascade reveals a common thumblike feature on Cas7 and Cas5e proteins that is critical to the oligomeric assembly of the helical backbone. The thumb of each Cas7 protein folds over the top of the crRNA and fits into a positively charged crease on the palm of the adjacent Cas7 protein (Fig. 4). This assembly creates an interwoven architecture that simultaneously protects the crRNA from degradation by cellular nucleases while presenting a series of 5-nt segments for complementary base pairing to a target. EM struc-

tures of crRNA-guided surveillance complexes from type I, type IIIA, and type IIIB systems reveal a similar helical backbone structure, suggesting that this architecture may be a conserved feature of type I and type III CRISPR systems (11, 12, 24, 25, 30, 33–35). Crystal structures of Csa2 (type IA) (24) and Csm3 (type IIIA) (23) reveal modified RRMs with large disordered loops at the same location as the *E. coli* Cas7 thumb, and a mutation in the predicted thumb of Csa2 has been shown to disrupt crRNA binding (fig. S5) (24).

Preordering of the crRNA guide plays an important role in target recognition by reducing the entropic penalty associated with helix formation and provides a thermodynamic advantage for target binding (26). Argonaute proteins enhance target detection using a similar strategy, and a

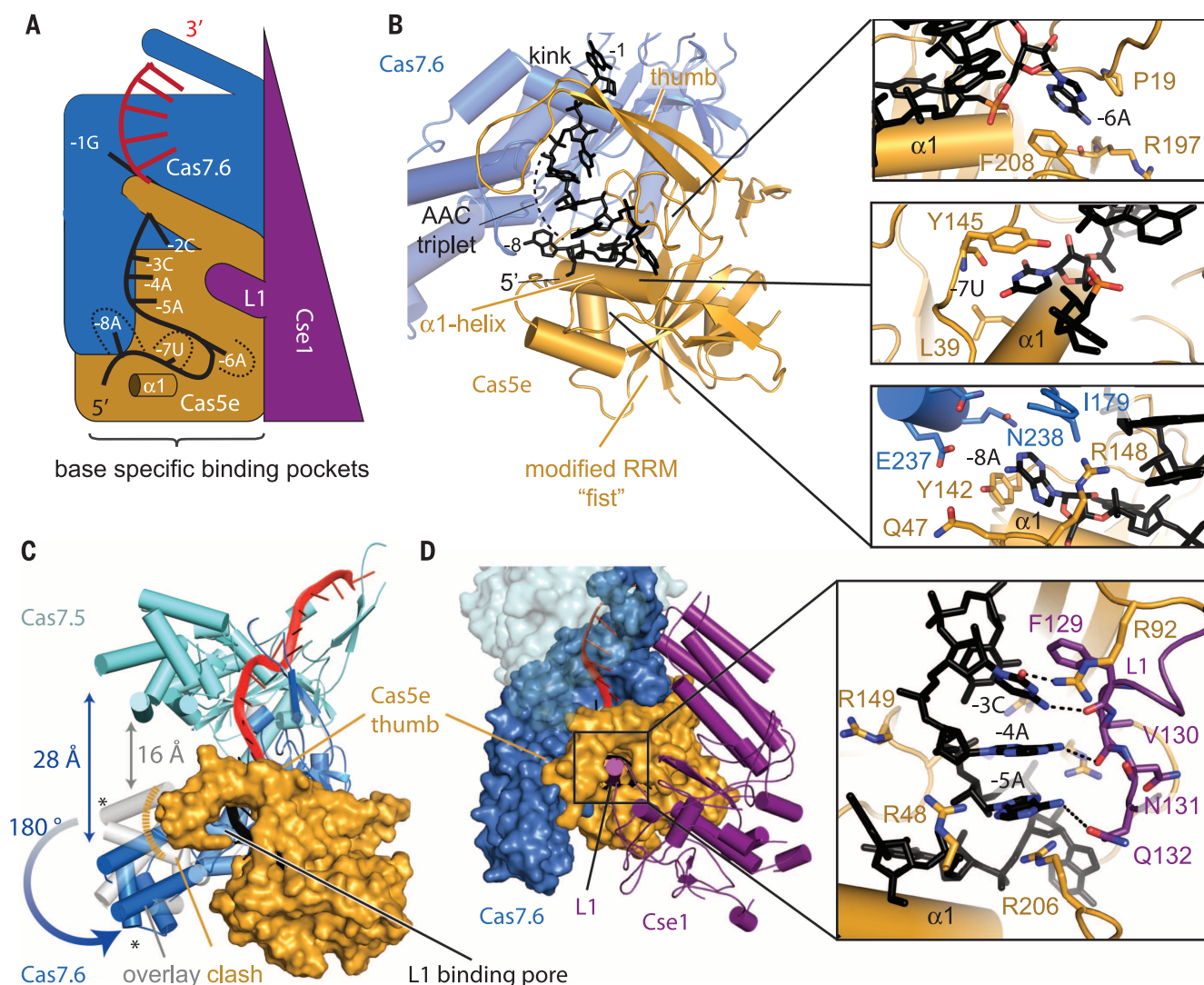


Fig. 5. Mechanism of tail assembly. (A) Schematic view of the 5' tail. Base-specific binding pockets and the $\alpha 1$ helix of Cas5e are highlighted. (B) Cas5e (orange) is composed of a modified RRM and a thumb that interacts with Cas7.6 and the crRNA. The AAC triplet of the 5' handle is indicated, and insets highlight the three base-specific binding pockets. (C) The finger domain of Cas7.6 (dark blue) is rotated 180° relative to the finger domain of the other Cas7 proteins

(white). This rotation increases the distance between the finger domains from 16 to 28 Å. The thumb of Cas5e would clash with the canonical orientation of the Cas7 finger domain (white), suggesting that the rotation of the Cas7.6 finger domain is influenced by Cas5e binding. (D) The L1 helix of Cse1 fits snugly into a pore created by the thumb of Cas5e. The inset shows the base-specific interactions made between L1 residues and the AAC triplet of the 5' handle.

structural comparison of Cascade to eukaryotic Argonautes reveals a similar “kink helix” positioned between nucleotides 6 and 7 (fig. S13) (36). However, in Argonautes there is no thumb that covers the kinked base, and it is expected that target hybridization may release the RNA for contiguous duplex formation (37). Recent crystal structures of the Cas9 protein suggest a similar protein-mediated preordering of the RNA guide (38, 39), and a structure of the target-bound complex suggests that the RNA-DNA hybrid forms a contiguous A-form duplex (39).

Target detection by Cascade relies on protein-mediated recognition of a three-nucleotide PAM and crRNA-guided hybridization to the target. PAM recognition has been proposed to destabilize the target DNA duplex and initiate crRNA-guided strand invasion. L1 in Cse1 has been implicated in this process, and the structure explains why mutations in L1 result in Cascade assembly defects (Fig. 5) (31). However, the structure of Cascade without DNA does not explain how Cascade recognizes the PAM. Structures of Cascade, in association with DNA and Cas3, may provide additional insights into the interplay of Cascade and Cas3 in the process of RNA-guided DNA interference.

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ACKNOWLEDGMENTS

We thank J. Richardson and D. Richardson for technical suggestions and discussion and A. McCoy for implementing the EM scale factor refinement in Phaser. X-ray diffraction data was collected with assistance from J. Nix at Advanced Light Source (ALS) beamline 4.2.2 (DE-AC02-05CH11231), R. Sanishvili and C. Ogata at Advanced Photon Source (APS) beamline 23-ID (Y1-GM-1104), the Structural Biology Center at APS 19-ID (DE-AC02-06CH11357), and Stanford Synchrotron Radiation Lightsources (DE-AC02-76SF00515 and P41GM103393). E.R.W. received funding from the People Program (Marie Curie Actions) of the European Union's Seventh Framework

Program (FP7/2007-2013) under REA grant agreement 327606. S.J.J. B. is supported by a Vidi grant from the Netherlands Organization of Scientific Research (864.11.005) and J.v.d.O. by a Vici grant (865.05.001). R.J.R. is supported by a Principal Research Fellowship from the Wellcome Trust (grant 082961/Z/07/Z). T.C.T. and R.J.R. are supported by a grant (GM063210) from the NIH. J.C. is supported by a grant for undergraduate research from the Howard Hughes Medical Institute (52006931). R.N.J. is supported by a National Research Service Award postdoctoral fellowship (F32 GM108436) from the NIH. Research in the Wiedenheft lab is supported by the NIH (P20GM103500 and R01GM108888), the NSF Experimental Program to Stimulate Competitive Research (EPS-110134), the M. J. Murdock Charitable Trust, and the Montana State University Agricultural Experiment Station. Atomic coordinates have been deposited into the Protein Data Bank with accession code 4TVX.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1473/suppl/DC1
Materials and Methods
Figs. S1 to S13
Tables S1 and S2
References (40–52)
Movie S1

21 May 2014; accepted 24 July 2014
Published online 7 August 2014;
10.1126/science.1256328

STRUCTURAL BIOLOGY

Crystal structure of a CRISPR RNA-guided surveillance complex bound to a ssDNA target

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In prokaryotes, RNA derived from type I and type III CRISPR loci direct large ribonucleoprotein complexes to destroy invading bacteriophage and plasmids. In *Escherichia coli*, this 405-kilodalton complex is called Cascade. We report the crystal structure of Cascade bound to a single-stranded DNA (ssDNA) target at a resolution of 3.03 angstroms. The structure reveals that the CRISPR RNA and target strands do not form a double helix but instead adopt an underwound ribbon-like structure. This noncanonical structure is facilitated by rotation of every sixth nucleotide out of the RNA-DNA hybrid and is stabilized by the highly interlocked organization of protein subunits. These studies provide insight into both the assembly and the activity of this complex and suggest a mechanism to enforce fidelity of target binding.

Prokaryotes use an RNA-based adaptive immune system called the CRISPR (clustered regularly interspaced short palindromic repeats)–Cas (CRISPR-associated) system to prevent invasion by bacteriophage and plasmids (1, 2). Found in the host genome, CRISPR loci comprise an array of identical repeats interspersed by variable foreign DNA. CRISPR loci are transcribed and then cleaved within the repeat regions to generate small CRISPR RNAs (crRNAs) (3–5). In type I and type III CRISPR–Cas systems (6), crRNA and Cas proteins assemble into large multisubunit complexes (3, 7–12). Despite a common seahorse architecture (8, 13–17), these com-

plexes use different mechanisms to destroy their targets: Type I complexes are surveillance complexes that bind DNA complementary to the crRNA guide and then recruit a trans-acting helicase-nuclease, Cas3, to unwind and degrade the invading DNA (18–20), whereas type III-A complexes are effector complexes that destroy targets directly via their intrinsic nuclease activity (7, 10).

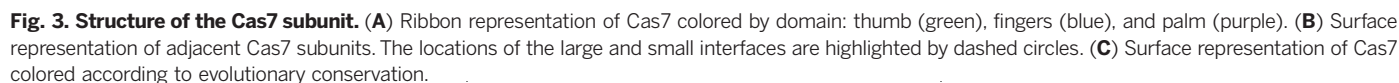
The type I-E surveillance complex in *E. coli* is known as Cascade (CRISPR-associated complex for antiviral defense), a 405-kD complex consisting of 11 subunits of five Cas proteins (CseI₁, Cse2₂, Cas7₆, Cas5₁, and Cas6₆) and a 61-nucleotide (nt) crRNA. The crRNA consists of a 32-nt guide sequence flanked by 5' and 3' handles, the sequences of which are derived from the repeats (Fig. 1A). Cascade recognizes DNA as foreign if it contains a region complementary to the crRNA guide (proto-spacer) that is adjacent to a small 3–base pair (bp)

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Two cryo-EM reconstructions of target-bound Cascade are available, one bound to a 32-nt ssRNA

Fig. 2. Structure of the crRNA and ssDNA target. (A) Overall structure of the two nucleic acids, highlighting the ribbon-like structure of the guide-target hybrid. The crRNA (green) and ssDNA target (orange) are displayed in a spheres representation. (B) Ribbon representation of the crRNA and ssDNA revealing the periodic kinking (marked by asterisks) in the backbone of both strands. (C) Structure of a 5-bp segment and disrupted base pairs. Disrupted RNA and DNA nucleotides are colored red and blue, respectively. In all panels, the crRNA and ssDNA target are colored green and orange, respectively.



target (15) and one bound to a 72-bp dsDNA target with a functional PAM sequence (31). The structural organization of Cascade in the two reconstructions is similar except for the position of the Cse1 base, which has been implicated in PAM binding (21). The crystal structure of Cascade fits best in the EM density of dsDNA-bound Cascade, requiring only small changes in the position of Cas6e and the 3' handle for an optimal fit (fig. S2). Lattice contacts appear to hold the base of Cse1 in the dsDNA-bound conformation. Thus, the structure of ssDNA-bound Cascade observed in the crystal is comparable to that bound to dsDNA, the *in vivo* target of Cascade (3, 31).

The guide-target hybrid adopts a ribbon conformation

The two strands of the guide-target hybrid do not twist around one another in a helix, but instead adopt an underwound ribbon-like structure remi-

niscant of a ladder (Fig. 2A). The 5' and 3' ends of the curved target strand are ~102 Å apart, roughly the length of straight B-form dsDNA with an identical sequence (~107 Å). Underwinding is facilitated by kinks that occur every sixth base pair in the backbone of both strands of the hybrid (Fig. 2B). At each kink, complementary nucleotides are rotated ~90°, in opposing directions, from the axis of the duplex (Fig. 2C). Thus, consistent with cryo-EM studies, the guide-target ribbon contains five 5-bp segments separated by 1-bp gaps (15). The remaining 2 nt of the guide region also form Watson-Crick base pairs with the target strand and are followed by a shear base pair between the nucleotide 5' of the protospacer (A5) and the first nucleotide (G41) in the 5' handle of the crRNA (fig. S3A). The importance of this shear base pair is currently unclear, as there is no reported conservation at this position of target sequence.

The structure of each repeating unit, a 5-bp segment plus the two displaced nucleotides, is essentially identical, superposing to an average root mean square deviation (RMSD) of ~1.20 Å over 209 atoms (fig. S3B). The local conformation of the 5-bp segments is highly distorted from canonical A-form, owing to the nonuniform roll and twist angles between base pairs. However, this distortion is necessary for the hybrid to remain continuous throughout the guide region, as the geometry of an A-form duplex is incompatible with the spacing between kink sites (fig. S3C).

The observed structure of the guide-target hybrid predicts that mutation of the disrupted nucleotides would not affect binding by Cascade. Consistent with this, mutations at position 6 of the protospacer (Fig. 1A) have no effect on target binding, while mutations at positions 1 to 5 or 7 to 8 (Fig. 1A) greatly reduce affinity (32). In addition, recent high-throughput genetic experiments revealed that mutations at the sites of the disrupted bases are readily tolerated *in vivo* (33).

The Cas7 filament and its interaction with the head of Cascade

The structure of Cas7 resembles a right hand, consisting of fingers (residues 59 to 180), palm (residues 1 to 58, 181 to 189, and 224 to 263), and thumb (residues 190 to 223) domains (Fig. 3A). The palm contains the modified RNA-recognition motif (RRM) characteristic of many Cas protein families (34). The domain architecture and key surface features appear conserved among Cas7 family proteins (fig. S4), which suggests that the structure of the Cas7 filament and consequently the ribbon-like structure of the guide-target hybrid (see below) are likely conserved in other type I and type III CRISPR-Cas systems. Within Cascade, the six Cas7 subunits form a right-handed filament, with a pitch of ~135 Å, around the guide-target hybrid (Fig. 1B). The filament is arranged such that the thumb of one Cas7 subunit, composed of an extended β hairpin, extends toward the fingers of the adjacent subunit (Fig. 3B). The conformations of Cas7.2 to Cas7.6 are essentially identical, except that the fingers of Cas7.6 are disordered in the structure (fig. S5A). Three points of contact stabilize the Cas7 filament, one between Cas7 and the guide region of the crRNA (see below) and two involving conserved protein-protein contacts (Fig. 3, B and C) between Cas7 subunits. The larger of these contacts buries ~1500 Å² of solvent-accessible surface area per subunit and is formed by packing the base of the thumb and the back of the palm of one subunit against the front of the palm of the neighboring subunit (Fig. 3B). The second, smaller interface buries ~400 Å² of solvent-accessible surface area per subunit and is formed between the tip of the thumb of one subunit and the fingers of the neighboring subunit (Fig. 3B).

At the head of Cascade, the Cas7 filament and the 3' end of the crRNA are capped by Cas6e (fig. S5B). This region appears to be flexible relative to the rest of Cascade, as the corresponding electron density is quite weak. One face of Cas6e engages the 3' hairpin of the crRNA, as observed

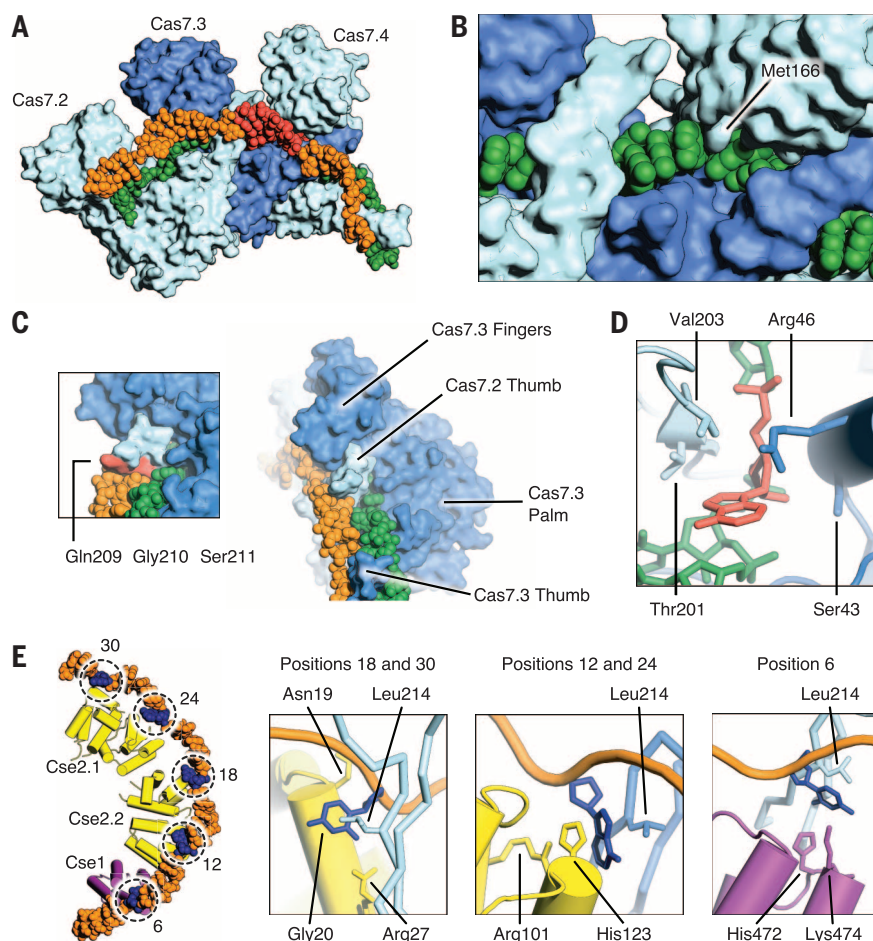


Fig. 4. Stabilization of the guide-target hybrid by Cas7, Cse1, and Cse2. (A) Surface representation showing the interaction of Cas7 subunits with the guide-target hybrid. The 5-bp segment bound between the fingers of Cas7.4 and the palm of Cas7.4 is colored red. (B) Close-up view of the bound crRNA. The DNA target has been removed for clarity. Intercalation by Met¹⁶⁶ from Cas7 is highlighted. (C) Surface representation showing that the thumb of Cas7 pushes through the guide-target hybrid at the 1-bp gaps. Boxed is the interaction of thumb residues with the minor groove face of the hybrid. (D) Binding site for the disrupted DNA nucleotides (dark blue) are indicated. (E) Left: Overview of the interactions between the ssDNA target and Cse2.1, Cse2.2, and Cse1. The proteins are represented as ribbons, the DNA as a surface. The positions of the disrupted DNA nucleotides (dark blue) are indicated. Right: Close-up views of the DNA nucleotide binding pockets. In all panels, the proteins and nucleic acids are colored as in Fig. 1.

with *Thermus thermophilus* Cas6e (28, 30), while the opposite face binds the thumb of Cas7.1 (fig. S5C). Although a portion of the Cas7.1 thumb is disordered in the structure, it is clear that it has a conformation distinct to the other Cas7 subunits. Specifically, the Cas7 thumb rotates $\sim 90^\circ$ toward Cas6e, adopting an open loop structure (fig. S5D) that binds primarily with a conserved face of the C-terminal RRM of Cas6e (fig. S5C).

Subunit interactions with the guide-target hybrid

A highly interdigitated network of protein-nucleic acid interactions stabilizes the ribbon conformation of the guide-target hybrid (Fig. 4A). Extensive contacts between the guide region of the crRNA and the Cas7 filament bury a large portion of the crRNA backbone, leaving the bases solvent-exposed (Fig. 4B). The absence of direct contacts between protein side chains and bases of the crRNA explains the lack of sequence specificity by Cascade for the guide sequence. The target strand is bound primarily through Watson-Crick hydrogen bonding with the guide but also interacts with the Cas7, Cse1, and Cse2 subunits (Fig. 1B). Few interactions are seen with the DNA backbone, rendering the majority of the backbone solvent-exposed (Fig. 1B).

Each 5-bp segment of the hybrid is situated between the palm of one Cas7 subunit and the fingers of the adjacent subunit (Fig. 4A). Several highly conserved polar and positively charged residues (Arg²⁰, Lys²⁷, Ser⁴⁰, Gln⁴², Lys⁴⁵, and Lys⁴⁹) from the palm of one Cas7 contact the RNA backbone, while the fingers from the adjacent Cas7 subunit (residues 109 to 111 and 163 to 169) contact both strands of the hybrid across the minor groove (Fig. 4C). In particular, the side chain of a highly conserved methionine (Met¹⁶⁶) partially intercalates with the bases at the third and fourth position of each RNA segment (Fig. 4B). As a result, the bases at these positions are separated, which helps distort each segment away from A-form geometry, facilitating the underwound structure of the crRNA guide.

The thumb of Cas7 both prohibits base pairing at the kink sites of the hybrid and stabilizes the conformation of the 5-bp segments. As the thumb of one Cas7 subunit extends toward the fingers of the adjacent subunit it passes directly between the strands of the guide-target hybrid, displacing the nucleotides and disrupting their base pairing (Fig. 4C). As a result, adjacent Cas7 subunits completely encircle the guide region of the crRNA (Fig. 4, A to C). Residues from the middle of the Cas7 thumb (Trp¹⁹⁹, Phe²⁰⁰, Thr²⁰¹, Ala²⁰², Leu²¹⁴, and His²¹³) (fig. S6) also stabilize the position of the nucleotides that flank the ends of the 5-bp segments through van der Waals contacts. Residues at the tip of the thumb (Gln²⁰⁹, Gly²¹⁰, and Ser²¹¹) contact the bases of the target strand on the minor groove face of the duplex (Fig. 4C).

Distinct binding pockets accommodate the displaced RNA and DNA nucleotides, respectively. Each binding pocket is solvent-exposed and provides minimal contacts with the Watson-Crick edges of the bases, consistent with the lack of

sequence specificity at these positions. Each displaced RNA nucleotide adopts the syn conformation, is similarly positioned above the backbone of the downstream RNA, and is contacted by residues from both the Cas7 palm (Ser⁴³ and Arg⁴⁶) and thumb (Thr²⁰¹ and Val²⁰³) (Fig. 4D). Each displaced DNA nucleotide adopts the anti conformation, is sandwiched on one side by Leu²¹⁴ from the nearest Cas7 thumb, and, depending on its position along the hybrid, by residues from Cse1, Cse2.1, or Cse2.2 (Fig. 4E). Cse1 contacts the first displaced DNA nucleotide (position 6) (Fig. 1A) with residues Lys⁴⁷⁴ and His⁴⁷² from the four-helix bundle (Fig. 4E). This interaction is consistent with cryo-EM studies showing ordering of this region of Cse1 (residues 406 to 414) on target binding (15). The second and fourth displaced DNA nucleotides (positions 12 and 24) stack with His¹²³ and contact Arg¹⁰¹ from either Cse2.2 or Cse2.1, respectively (Fig. 4E). The third and fifth displaced nucleotides (positions 18 and 30) make contact with Asn¹⁹, Gly²⁰, and Arg²⁷ from either Cse2.2 or Cse2.1, respectively (Fig. 4E). We also observe a salt bridge between Arg²⁶ from Cse2.1 and Cse2.2 and backbone phosphates at positions 17 and 29. Consistent with these observations, the highly conserved Arg²⁶, Arg²⁷, and Arg¹⁰¹ from Cse2 have been implicated as important for DNA binding by electrophoretic mobility shift assays (25).

Interactions capping the tail of Cascade

Cas5 caps the tail of the Cas7 filament at the 5' end of the crRNA (Fig. 5A). The structure of Cascade reveals that Cas5 is structurally related to Cas7, as it consists of a palm (residues 1 to 78 and 115 to 224) and a thumb (residues 79 to 114) do-

main, but lacks a fingers domain (Fig. 5B). The Cas5 palm contains an RRM that superposes with the palm of Cas7 (RMSD of ~ 3.3 Å between 123 C α atoms). The thumb domains of Cas5 and Cas7 adopt a similar orientation with respect to their palm domains and appear to have similar functions within Cascade. The thumb of Cas5 displaces nucleotide G8 in the 5' handle of the crRNA in a manner reminiscent of how the Cas7 thumb displaces RNA nucleotides in the guide. The thumb of Cas5 also provides similar residues (Leu⁸⁹ and Thr⁸⁷) to the binding pocket of the G8 nucleotide, as the Cas7 thumb does for the displaced RNA nucleotides (Val²⁰³ and Thr²⁰¹). Additionally, two residues from the thumb of Cas5 (Tyr⁸⁵ and Gln¹⁰⁵) stabilize the first duplex segment (positions 1 to 5) through van der Waals contacts with the exposed face of the base at position 1 of the hybrid (fig. S6).

The first seven nucleotides of the 5' handle form a hook-like structure (Fig. 5C) that sits between Cas5 and Cas7.6 (Fig. 5D), forming extensive contacts between the RNA backbone and highly conserved charged and polar residues from both Cas5 (Arg²⁵, Ser³⁴, Arg⁴⁸, Arg⁴⁹, Arg¹⁴⁸, and Arg¹⁴⁹) and Cas7.6 (Lys⁴⁵, Arg⁴⁹, and Arg⁴⁶) (Fig. 5E). The first three nucleotides (A1, U2, and A3) are splayed and their bases sandwiched in three distinct pockets formed by conserved residues from Cas5 (fig. S7). Sequence-specific contacts are observed between nucleotide U2 and the backbone of Tyr¹⁴⁵ (fig. S7). The bases of nucleotides A4, A5, and C6 form a triplet stack that sits orthogonal to nucleotide A3 (fig. S7). The backbone of this stack packs against the palm domains of both Cas5 and Cas7.6, leaving the bases solvent-exposed. The side chain of Arg²⁰⁶

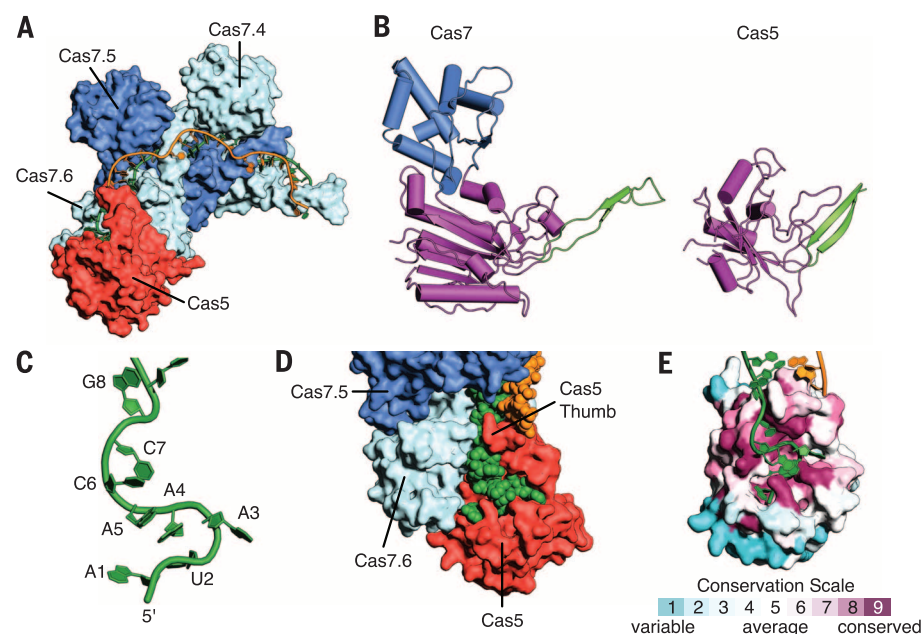


Fig. 5. Structure of Cas5 and the 5' handle of the crRNA. (A) Surface representation of Cas5 capping the end of the Cas7 filament. (B) Ribbon representation of Cas7 and Cas5 colored by domain: thumb (green), fingers (blue), and palm (purple). (C) Structure of the 5' hook of the crRNA. (D) Close-up view of the interaction between Cas5, Cas7.6, and the 5' hook. (E) Surface representation of Cas5 colored according to evolutionary conservation, highlighting the conservation of the residues that bind the 5' hook.

from the palm of Cas5 also stacks with the base of nucleotide A4 (fig. S7). Finally, the position of C7 is stabilized through sequence-specific hydrogen bonds with Arg¹⁰⁸ from the Cas5 thumb (fig. S7). Thus, only nucleotides U2 and C7 form sequence-specific interactions with Cas5 in ssDNA-bound Cascade, consistent with sequence alignments showing these positions are the invariant in the 5' handle (35).

Modeling interactions of Cascade, dsDNA target, and Cas3

Cryo-EM studies (31) revealed that Cascade binds the PAM-proximal end of a dsDNA target between Cas7.5, Cas7.6, and the L1 loop of Cse1, which has been linked to PAM binding (27). The fingers of Cas7.6 and the L1 loop of Cse1 are disordered in the crystal structure of ssDNA-bound Cascade, which suggests that these regions are mobile in the absence of dsDNA. Using the cryo-EM density as a guide, we modeled the position of this dsDNA and the fingers of Cas7.6 onto the crystal structure. The resulting model suggests interactions between the DNA backbone and a cluster of basic residues from the fingers of both Cas7.5 (Lys¹³⁷, Lys¹³⁸, and Lys¹⁴¹) and Cas7.6 (His⁶⁷ and Lys¹⁰⁵) (Fig. 6A). The orientation of the modeled duplex and the path of the target strand agree with atomic force mi-

croscopy studies suggesting that Cascade bends dsDNA upon binding (18).

R-loop formation by Cascade displaces the non-target strand of the protospacer. Footprinting experiments have shown that the 3' end but not the 5' end of the displaced strand is protected by Cascade binding (14, 21). The structure of ssDNA-bound Cascade reveals a prominent basic groove that could serve as a binding site for the 3' end of the displaced strand (Fig. 6B). Spanning from Cas6e to the four-helix bundle of Cse1, the groove is lined with conserved basic residues from Cas7 (Lys³⁴, Lys²⁹⁹, and Lys³⁰¹) and Cse2 (Arg⁵³, Arg¹⁴², Arg¹⁴³, and Arg¹¹⁰) (fig. S8A). Because the PAM is bound at the L1 loop of Cse1 (31), the displaced strand must first loop around the four-helix bundle of Cse1 to gain access to the groove, in agreement with footprinting data showing that the 5' end of the displaced strand is exposed. After R-loop formation, Cascade recruits the Cas3 helicase-nuclease (18–20). Negative stain reconstruction of a complex between dsDNA-bound Cascade and Cas3 revealed Cas3 binds Cascade between the four-helix bundle and the base of Cse1 (31). Mapping this interaction onto the crystal structure reveals the residues that likely mediate Cas3 binding (fig. S8B); these include Glu¹⁹², Glu²⁸⁰, Asn³⁷⁶, Thr³⁸³, and two conserved loops located at the base of Cse1 (residues 288 to 294 and residues 318 to 323).

Cryo-EM studies have shown that target binding triggers structural rearrangements of the Cse1 and Cse2 subunits (15). To further understand these changes, we fit each subunit of the crystal structure of ssDNA-bound Cascade into the ~9 Å cryo-EM reconstruction of apo Cascade as a rigid body, except for Cse1, which required individual placement of its two domains (fig. S9). As previously established, superposition of the apo Cascade model with the structure of ssDNA-bound Cascade reveals a concerted conformational rearrangement. The two Cse2 subunits each move ~16 Å toward the four-helix bundle of Cse1, which subsequently undergoes a ~30° rotation away from Cse2.2 (Fig. 6C). We also observe a ~15° rotation of the base of Cse1 (31). Together, these rearrangements facilitate the formation of binding pockets for the displaced DNA nucleotides (Fig. 6D). Additionally, in the apo conformation, Cse2.1 blocks access to the distal end of the guide (positions 24 to 30) (Fig. 6D). Therefore, repositioning Cse2.1 facilitates base pairing in this region, consistent with previous binding data showing that short oligonucleotides complementary to the 3' region of the crRNA guide have lower affinity than short oligonucleotides that bind to the 5' region (15).

Implications for target binding and recruitment of Cas3

Analysis of the crystal structure, in the context of the reported literature, provides a model for how Cascade recognizes foreign DNA and recruits Cas3. During target recognition, Cascade searches dsDNA for potential PAM sites, possibly facilitated by sequence-independent interactions between the DNA backbone and basic residues from the fingers of Cas7.5 and Cas7.6 (Fig. 6A) (31). Once a PAM is found, the adjacent duplex is destabilized and base pairing between the crRNA guide and the target strand proceeds along the guide in 5-bp increments separated by 1-bp gaps (15, 22, 32).

Cascade incrementally binding 5-nt segments of the target strand likely increases the fidelity of target recognition, by a mechanism similar to that used by RecA (36–38). RecA catalyzes strand exchange in homologous recombination. Thus, despite differing cellular roles, Cascade and RecA both function by correctly pairing a single strand of RNA or DNA, respectively, with a complementary strand in a DNA duplex, respectively. Comparison of the structures of the guide-target hybrid in ssDNA-bound Cascade and the heteroduplex bound to the RecA filament (36) reveals globally similar distortions (fig. S10), as previously predicted (2). RecA is thought to bind its target in 3-nt increments to enforce high fidelity during strand exchange; if so, this suggests that Cascade may use a similar conformational proofreading mechanism (36–38). Specifically, conformational proofreading by Cascade involves optimizing the cost-benefit ratio of binding targets by introducing a structural difference between the crRNA guide and the foreign DNA. The energetics of target binding are the net sum of the energy gained by base pairing and the energy lost in conformational distortion (i.e., formation of the guide-target hybrid). Nonspecific targets lacking

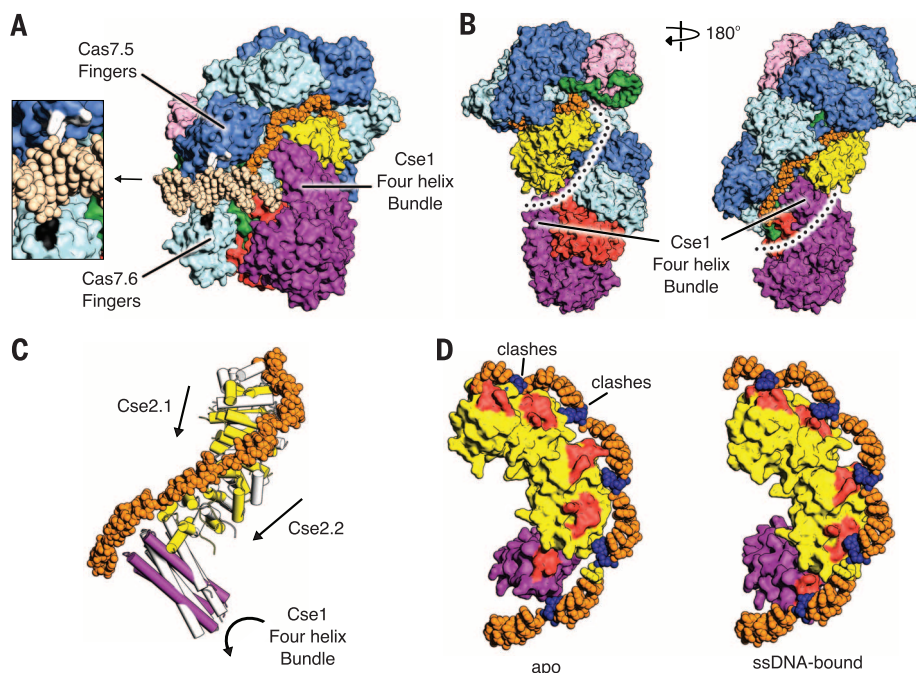


Fig. 6. Modeling interactions with dsDNA and associated conformational changes. (A) Surface representation of Cascade and the modeled dsDNA and Cas7.6 fingers domain. Boxed is a close-up view of the residues predicted to bind the dsDNA: Lys¹³⁷, Lys¹³⁸, and Lys¹⁴¹ from the fingers of Cas7.5 (white) and His⁶⁷ and Lys¹⁰⁵ from the fingers of Cas7.6 (black). (B) Surface representation of Cascade, with the proposed path of the nontarget strand indicated by a dotted line. (C) Ribbon representation of Cse2.1, Cse2.2, and the four-helix bundle of Cse1, highlighting the structural rearrangements that accompany ssDNA binding. The target strand is shown in orange. The subunits in the apo conformation are colored white; those in the ssDNA-bound conformation are colored as in Fig. 1. (D) Surface representations of Cse2.1, Cse2.2, and the four-helix bundle of Cse1 in ssDNA-bound (right) and apo (left) conformations. The binding pockets for displaced DNA nucleotides (dark blue) are colored red. Clashes between Cse2.1 and the distal end of the ssDNA target are indicated.

favorable base pair interactions will not overcome the energetic costs associated with conformational distortion, whereas specific targets can do so. Thereby, enhanced specificity is achieved. Genetic and biochemical assays identified a seed region (positions 1 to 5 and 7 to 8) in crRNA required for high-affinity binding to a target (32). Consistent with this proposed conformational fidelity mechanism, mismatches in the seed region would inhibit formation of the initial 5-nt segments, thereby terminating binding.

Cascade undergoes structural rearrangement of its Cse1 and Cse2 subunits upon target binding. Propagation of base pairing between the crRNA guide and target strand across the target facilitates these conformation changes, which form the binding pockets for the disrupted DNA nucleotides (Fig. 6D). Moreover, movement of Cse2.1 relieves a steric block at the distal end of the guide (positions 24 to 30) (Fig. 6D), enabling base pairing in this region. Recent single-molecule studies monitoring DNA supercoiling revealed that Cascade binding to target DNA is unstable until base pairs form at the distal end of the guide (22). Our structural analysis suggests that base pairing in this region would prevent Cse2.1 from reassuming its apo position, effectively locking Cascade on the DNA. This locking mechanism could also act as an additional proofreading step, as targets that cannot form base pairs across the entire length of the guide will not be stably bound (22).

After target recognition, Cascade recruits the Cas3 helicase-nuclease, likely through interactions with two conserved loops at the base of Cse1 (fig. S8B) (31). Once recruited, Cas3 nicks the displaced nontarget strand ~7 to 11 nt from the 3' end of the PAM (19, 20), consistent with the predicted path of the nontarget strand (Fig. 6B and fig. S8A). Cas3 then loads onto the newly formed ssDNA end (31) and continues to progressively degrade the foreign DNA.

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ACKNOWLEDGMENTS

We thank R. McMacken, B. Learn, J. Berger, D. Leahy, and J. Kavan for helpful discussions; J. Bosch for providing the tungsten clusters used in the soaking experiments; I. Mathews for help with data

collection; and J. Kavan for critical reading of the manuscript. Supported by NIH grant GM097330 (S.B.). Data for this study were measured at beamline X25 of the National Synchrotron Light Source (NSLS) and at beamlines 7-1, 11-1, and 12-2 of the Stanford Synchrotron Radiation Lightsources (SSRL). Funding for X25 comes principally from the Offices of Biological and Environmental Research and of Basic Energy Sciences of the U.S. Department of Energy (DOE) and from National Center for Research Resources grant P41RR012408 and NIH grant P41GM103473. Use of the SSRL is supported by the DOE Office of Science, Office of Basic Energy Sciences, under contract DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research and by NIH grant P41GM103393. The atomic coordinates and structure factors have been deposited into the Protein Data Bank with the accession code 4QYZ.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1479/suppl/DC1

Materials and Methods

Figs. S1 to S10

Tables S1 and S2

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5 June 2014; accepted 4 August 2014

Published online 14 August 2014;

10.1126/science.1256996

REPORTS

QUANTUM GASES

Observation of Fermi surface deformation in a dipolar quantum gas

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In the presence of isotropic interactions, the Fermi surface of an ultracold Fermi gas is spherical. Introducing anisotropic interactions can deform the Fermi surface, but the effect is subtle and challenging to observe experimentally. Here, we report on the observation of a Fermi surface deformation in a degenerate dipolar Fermi gas of erbium atoms. The deformation is caused by the interplay between strong magnetic dipole-dipole interaction and the Pauli exclusion principle. We demonstrate the many-body nature of the effect and its tunability with the Fermi energy. Our observation provides a basis for future studies on anisotropic many-body phenomena in normal and superfluid phases.

The Fermi-liquid theory, formulated by Landau in the late 1950s, is one of the most powerful tools in modern condensed-matter physics (1). It captures the behavior of interacting Fermi systems in the normal phase, such as electrons in metals and liquid ³He (2). Within this theory, the interaction is accounted by dressing the fermions as quasi-particles with an effective mass and an effective interaction. The ground state is the so-called Fermi sea, in which the quasi-particles fill one-by-one all the states up to the Fermi momentum, k_F . The Fermi

surface (FS), which separates occupied from empty states in k -space, is a sphere of radius k_F for isotropically interacting fermions in uniform space. The FS is crucial for understanding system excitations and Cooper pairing in superconductors. When complex interactions act, the FS can get modified. For instance, strongly correlated electron systems violate the Fermi-liquid picture, giving rise to a deformed FS, which spontaneously breaks the rotational invariance of the system (3). Symmetry-breaking FSs have been studied in connection with electronic liquid crystal phases (4) and Pomeranchuk instability (5) in solid-state systems. Particularly relevant is the nematic phase, in which anisotropic behaviors spontaneously emerge and the system acquires an orientational order, while preserving its translational invariance (3).

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A completely distinct approach to studying FSs is provided by ultracold quantum gases. These systems are naturally free from impurities and do not have a crystal structure, realizing a situation close to the ideal uniform case; therefore, the shape of the FS can directly reveal the fundamental interactions among particles. Studies of FSs in strongly interacting Fermi gases have been crucial in understanding the Bose-Einstein condensation (BEC)-to-Bardeen-Cooper-Schrieffer (BCS) crossover, in which the isotropic s -wave (contact) interaction causes a broadening of the always-spherical FS (6). Recently, Fermi gases with anisotropic interactions have attracted much attention in the context of p -wave superfluidity (7, 8) and dipolar physics (9). Many theoretical studies have focused on dipolar Fermi gases, predicting the existence of a deformed FS (10–15).

These studies also include an extension of the Landau Fermi-liquid theory to the case of anisotropic interactions (16). Despite recent experimental advances in polar molecules and magnetic atoms (17–20), the observation of anisotropic FSs has so far been elusive.

Here, we present the direct observation of the deformed FS in dipolar Fermi gases of strongly magnetic erbium (Er) atoms. By virtue of the anisotropic dipole-dipole interaction (DDI) among the particles, the FS is predicted to be deformed into an ellipsoid. To minimize the system's energy, the FS elongates along the direction of the maximum attraction of the DDI, where the atomic dipoles have a “head-to-tail” orientation. To understand the origin of the Fermi surface deformation (FSD), one has to account for both the action of the DDI in k -space and the Pauli

exclusion principle, which imposes antisymmetry on the many-body wave function. In the Hartree-Fock formalism, the FSD comes from the exchange interaction among fermions, known as the Fock term (10, 14, 21). Our observations agree very well with parameter-free calculations based on the Hartree-Fock theory (10, 13, 15). We demonstrate that the degree of deformation, related to the nematic susceptibility in the liquid-crystal vocabulary, can be controlled by varying the Fermi energy of the system and vanishes at high temperatures.

Our system is a single-component quantum degenerate dipolar Fermi gas of Er atoms. Like other lanthanoids, a distinct feature of Er is a large permanent magnetic dipole moment μ of 7 Bohr magneton, which causes a strong DDI between the fermions. Similarly to our previous work (20), we take advantage of elastic dipole-dipole collisions to drive efficient evaporative cooling in spin-polarized fermions. The sample is confined into a three-dimensional optical harmonic trap and typically contains 7×10^4 atoms at a temperature of $0.18(1) T_F$, with $T_F = 1.12(4) \mu\text{K}$ (21). We control the alignment of the magnetic dipole moments by setting the orientation of an external polarizing magnetic field. We label β as the angle between the magnetic field and the z axis (Fig. 1, inset).

To explore the impact of the DDI on the momentum distribution, we performed time-of-flight (TOF) experiments. Since its first use as “smoking-gun” evidence for BEC (22, 23), this technique has proved its power in revealing many-body quantum phenomena in momentum space (6, 24). TOF experiments are based on the study of the expansion dynamics of a gas after it has been released from a trap. For a sufficiently long expansion time, the size of the atomic cloud is dominated by the velocity dispersion and, in the case of ballistic (free) expansions, the TOF images purely reflect the momentum distribution in the trap.

In our experiment, we first prepared the ultracold Fermi gas with a given dipole orientation and then let the sample expand by suddenly switching off the optical dipole trap (ODT). From the TOF images, we derived the cloud aspect ratio (AR), which is defined as the ratio of the vertical to horizontal radius of the cloud in the imaging plane (21). The AR for various values of β are shown in Fig. 1. For vertical orientation ($\beta = 0^\circ$), we observed a clear deviation of the AR from unity with a cloud anisotropy of $\sim 3\%$. TOF images show that the cloud has an ellipsoidal shape, with elongation in the direction of the dipole orientation. When changing β , we observed that the cloud follows the rotation of the dipole orientation, keeping the major axis always parallel to the direction of the maximum attraction of the DDI. In a second set of experiments, we recorded the time evolution of the AR during the expansion for $\beta = 0^\circ$ and $\beta = 90^\circ$ (Fig. 2). For both orientations, the AR differs from unity at long expansion times. Our results are strikingly different from the ones in conventional Fermi gases with isotropic contact interactions, in which the FS is spherical (AR = 1) and the magnetic field orientation has no influence on the cloud shape (6).

Fig. 1. AR of an expanding dipolar Fermi gas as a function of the angle β . In this measurement, the trap frequencies are $(f_x, f_y, f_z) = (579, 91, 611)$ Hz. The data are taken at $t_{\text{TOF}} = 12$ ms. Each individual point is obtained from about 39 independent measurements. The error bars indicate SEM. For comparison, the calculated values are also shown for 0° and 90° (crosses). (Inset) Schematic illustration of the geometry of the system.

Gravity is along the z direction. The atomic cloud is imaged with an angle of 28° with respect to the y axis (21). The magnetic field orientation is rotated within the plane that forms an angle of 14° with the xz plane. Schematic illustrations of the deformed FS are also shown above the panel; k_F is the Fermi momentum for an ideal Fermi gas.

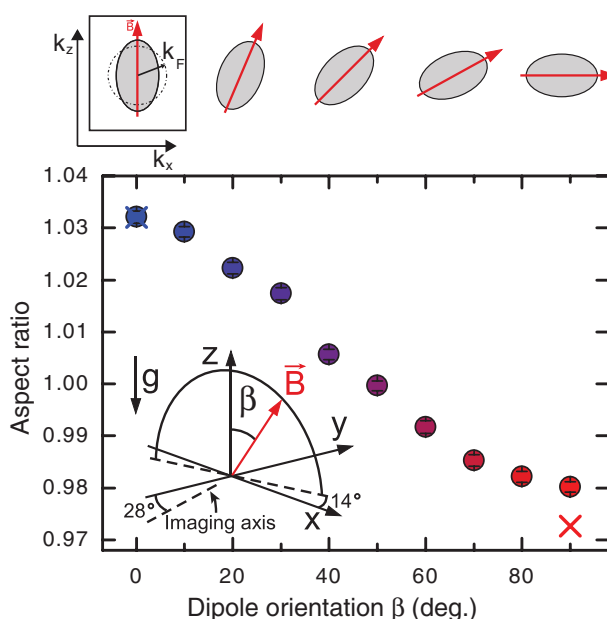
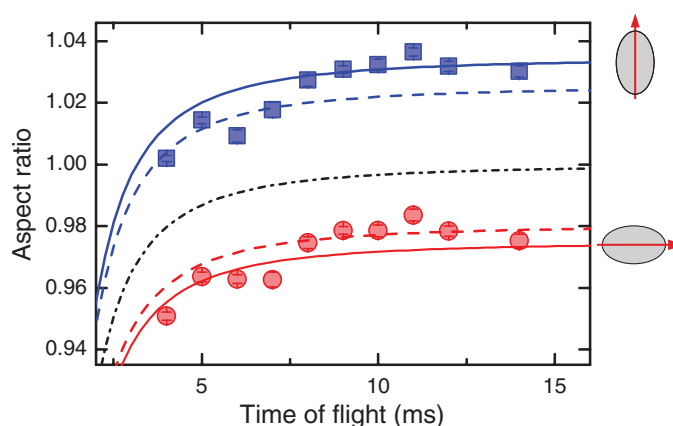


Fig. 2. Time evolution of the AR of the atomic cloud during the expansion. Measurements are performed for two dipole angles, $\beta = 0^\circ$ (squares) and $\beta = 90^\circ$ (circles) under the same conditions as in Fig. 1. The error bars are SEM of about 17 independent measurements. The possible origin of the small fluctuations of the data points at short expansion time is discussed in (21). The theoretical curves show the full numerical calculations (solid lines), which include both the FSD and the NBE effects, and the calculation in the case of ballistic expansions (dashed lines), in the absence of the NBE effect. For comparison, the calculation for a noninteracting Fermi gas is also shown (dot-dashed line).

The theoretical curves show the full numerical calculations (solid lines), which include both the FSD and the NBE effects, and the calculation in the case of ballistic expansions (dashed lines), in the absence of the NBE effect. For comparison, the calculation for a noninteracting Fermi gas is also shown (dot-dashed line).



The one-to-one mapping of the original momentum distribution in the trap and the density distribution of the cloud after long expansion time strictly holds only in the case of pure ballistic expansions. In our experiments, the DDI is acting even during the expansion and could potentially mask the observation of the FSD. We evaluated the effect of the nonballistic expansion (NBE) by performing numerical calculations based on the Hartree-Fock mean-field theory at zero temperature and the Boltzmann-Vlasov equation for expansion dynamics (13, 15, 21). In Fig. 2, the theoretical curves do not have any free parameters and are calculated both in the presence (Fig. 2, solid lines) and absence (Fig. 2, dashed lines) of the NBE effect. The comparison between ballistic and nonballistic expansion reveals that the latter plays a minor role in the final AR, showing that the observed anisotropy dominantly originates from the FSD. The agreement between experiment and theory implies that our model accurately describes the behavior of the system.

Theoretical works have predicted that the degree of deformation depends on the Fermi energy and the dipole moment (10, 12–16). In the

limit of weak DDI, the magnitude of the FSD in a trapped sample is expected to be linearly proportional to the ratio of the DDI to the Fermi energy, $\eta = nd^2/E_F$ (14). Here, $n = 4\pi(2m E_F/\hbar^2)^{3/2}/3$ is the peak number density at zero temperature, $\hbar$ is the Planck constant, m is the mass, $d^2 = \mu_0\mu^2/(4\pi)$ is the coupling constant for the DDI, and μ_0 is the magnetic constant. For a harmonically trapped ideal Fermi gas, the Fermi energy E_F depends on the atom number N and the mean trap frequency $\bar{f} = (f_x f_y f_z)^{1/3}$, $E_F = \hbar\bar{f}(6N)^{1/3}$. Given that $\eta \propto \sqrt{E_F}$, the FSD can be tuned by varying E_F .

To test the theoretical predictions, we first numerically studied the degree of cloud deformation Δ , defined as $\Delta = AR - 1$, as a function of the trap anisotropy, $\sqrt{f_x f_z}/f_y$, and/or $\bar{f}$. To distinguish the effect of the FSD and of the NBE, we keep the two contributions separated in the calculations (Fig. 3, A and B). Our results clearly convey the following information: (i) The FSD gives the major contribution to Δ ; (ii) the FSD is independent of the trap anisotropy but increases with $\bar{f}$; and (iii) the NBE effect reflects the trap anisotropy and vanishes for a spherical trap (13).

In the experiment, we explored the dependence of Δ on the trap geometry for $\beta = 0^\circ$ by keeping the axial frequency (f_y) constant and varying the radial frequencies ($f_x = f_z$ within 5%) (Fig. 3C). This leads to a simultaneous variation of both the trap anisotropy and $\bar{f}$. We observed an increase of Δ with η , which is consistent with the theoretically predicted linear dependence (14).

In analogy with studies in superconducting materials (25), we graphically emphasized the FSD in the measurements at $\eta = 0.009$ by subtracting the TOF absorption image taken at $\beta = 90^\circ$ from the one at $\beta = 0^\circ$ (Fig. 3D). The resulting image exhibits a cloverleaf-like pattern, showing that the momentum spread along the orientation of the dipoles is larger than in the other direction. For comparison, the same procedure is applied for images obtained by a fit to the observed cloud (Fig. 3E). At $\eta = 0.009$, the trap anisotropy is so small that the NBE effect is negligibly small, and the deformation is caused almost only by the FSD.

Last, we investigated the temperature dependence of Δ (Fig. 4). We prepared samples at various temperatures by stopping the evaporative cooling procedure at various points. The final trap geometry is kept constant. When reducing the temperature, we observed the emergence of the FSD, which becomes more and more pronounced at low temperatures and eventually approaches the zero-temperature limit. The qualitative behavior of the observed temperature dependence is consistent with a theoretical result at finite temperatures (14), although further theoretical developments are needed for a more quantitative comparison.

Our observation clearly shows the quantum many-body nature of the FSD and sets the basis for future investigations on more complex dipolar phenomena, including collective excitations (13, 15, 26, 27) and anisotropic superfluid pairing (28, 29). Taking advantage of the wide tunability of cold-atom experiments, dipolar Fermi gases are ideally clean systems for exploring exotic and topological phases in a highly controlled manner (9).

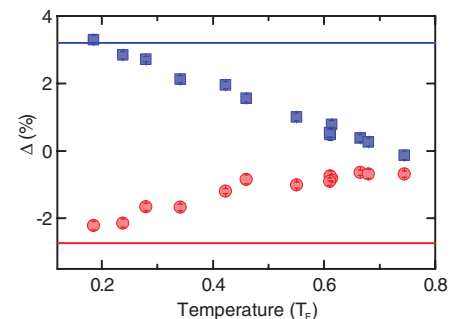


Fig. 4. Δ as a function of the temperature of the cloud. Measurements are performed for two dipole angles, $\beta = 0^\circ$ (squares) and $\beta = 90^\circ$ (circles), under the same conditions as in Fig. 1. The error bars are SEM of about 26 independent measurements. The solid lines show the numerically calculated values at zero temperature for $\beta = 0^\circ$ and $\beta = 90^\circ$.

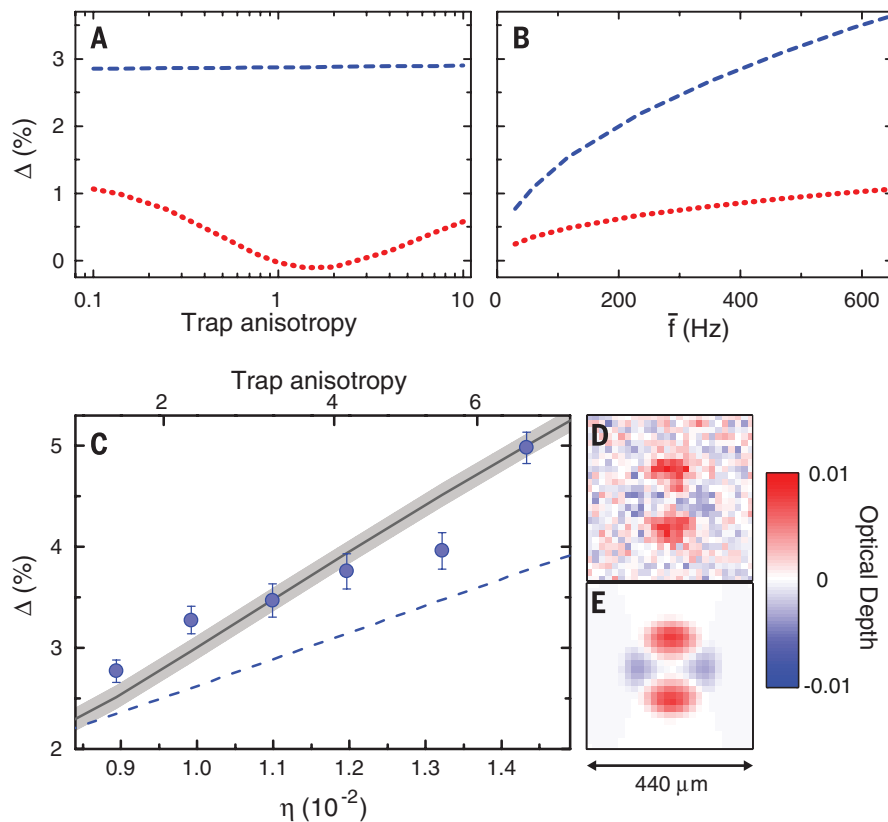


Fig. 3. $\Delta = AR - 1$ for various trap geometries. (A and B) We consider a cigar-shaped trap with $f_x = f_z$ in the calculations and show the behaviors of the FSD (dashed lines) and the NBE (dotted lines) separately as (A) a function of the trap anisotropy $\sqrt{f_x f_z}/f_y$ at $\bar{f} = 400$ Hz and (B) a function of $\bar{f}$ at $\sqrt{f_x f_z}/f_y = 5$. (C) Experimentally observed Δ at $t_{\text{TOF}} = 12$ ms are plotted as a function of η , together with the full calculation (solid line) and the calculation considering only FSD (dashed line). The shaded area shows the uncertainty originating from the uncertainty in determining η in our experiments. The sample contains 6×10^4 atoms at a typical temperature of $T/T_F = 0.15(1)$. The error bars represent SEM of about 15 independent measurements. The variation of the trap anisotropy in the experiment is indicated on the top axis. (D and E) Visualization of the FSD at $\eta = 0.009$ from (D) the experimental TOF image and (E) the fitted image.

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ACKNOWLEDGMENTS

We are grateful to A. Pelster, M. Ueda, M. Baranov, R. Grimm, T. Pfau, B. L. Lev, and E. Fradkin for fruitful discussions. This work is supported by the Austrian Ministry of Science and Research (BMWf) and the Austrian Science Fund (FWF) through a START grant under project Y479-N20 and by the European Research Council under project 259435. K.A. is supported within the Lise-Meitner program of the FWF.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1
References (30–41)

25 April 2014; accepted 15 August 2014
10.1126/science.1255259

ORGANIC ELECTRONICS

Room-temperature coupling between electrical current and nuclear spins in OLEDs

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The effects of external magnetic fields on the electrical conductivity of organic semiconductors have been attributed to hyperfine coupling of the spins of the charge carriers and hydrogen nuclei. We studied this coupling directly by implementation of pulsed electrically detected nuclear magnetic resonance spectroscopy in organic light-emitting diodes (OLEDs). The data revealed a fingerprint of the isotope (protium or deuterium) involved in the coherent spin precession observed in spin-echo envelope modulation. Furthermore, resonant control of the electric current by nuclear spin orientation was achieved with radiofrequency pulses in a double-resonance scheme, implying current control on energy scales one-millionth the magnitude of the thermal energy.

Exceptionally large magnetoresistance effects can be observed at relatively low magnetic fields of a few millitesla in organic semiconductors (*1*). Electron spin resonance (ESR) techniques have provided insight into the microscopic origins of spin-dependent transport in these materials and have pointed to hyperfine interactions as the dominant mechanism. Monitoring the device current during coherent spin excitation (*2–7*) has revealed signatures of hyperfine coupling that manifest themselves as a resonance line-broadening mechanism. In addition, in nutation experiments under strong microwave excitation, such coupling becomes apparent through the beating of both individual charge-carrier spins at the first harmonic pre-

cession frequency, a signature of spin-1 precession (*3, 5*). Ultimate verification of the influence of nuclear magnetic moments on electronic transport in these highly disordered material systems can only be made by direct electrical detection of nuclear magnetic resonance (NMR). However, at room temperature and the relevant energy scale of NMR, the magnitude of nuclear-level Zeeman splitting is on the order of 100 neV—a million times smaller than the thermal energy kT . Nevertheless, we succeeded in measuring the influence of individual ensembles of nuclear spin states on device conductivity directly through current-detected electron spin-echo envelope modulation and NMR-induced nuclear spin manipulation.

We used poly[2-methoxy-5-(2'-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) organic light-emitting diodes (OLEDs) as prototypical device structures for pulsed magnetic resonance (*2, 3*). Electrons and holes were injected electrically to form weakly bound charge carrier pairs within the polymer film (*8*). These pairs exist in either singlet or triplet configuration and can

recombine via an excitonic state or dissociate into free charges. In addition, pairs may undergo intersystem singlet-triplet transitions through either incoherent spin-lattice relaxation, or coherent spin manipulation with microwave pulses under ESR conditions. Because of spin statistics, in thermal equilibrium there is an excess population of electron-hole pairs in the triplet state (*9*). In our pulsed electrically detected magnetic resonance experiments, current changes under constant bias (corresponding to a current of ~100 μ A dc) were detected as a function of time after a resonant microwave pulse to reveal transient changes in the pair populations through the underlying spin-dependent transport mechanism. Hahn echo and stimulated echo sequences could then be implemented by a small modification of the pulse sequence and subsequent temporal integration of the differential current (*4, 10, 11*). This so-called polaron-pair model of spin-dependent transport is not undisputed (*12*), and other carrier-pair mechanisms (such as bipolaron pair formation) have been discussed as the origin for this spin-dependent process (*13, 14*). Although our discussion is based on the polaron-pair model, the same arguments apply consistently to any Pauli blockade-based spin-dependent transport process. The interpretation of our results is independent of the microscopic nature of this process—e.g., with regard to the electrical polarity of the involved charge carriers, or signs and magnitudes of electronic rates and rate coefficients.

To explore NMR control of the OLED current, we first had to reliably detect the influence of hyperfine coupling on the carrier-pair spin state. To this end, we applied an electrically detected (*11, 15*) electron spin-echo envelope modulation (ESEEM) technique (*16–18*). Nuclear coupling was observed indirectly through coherent manipulation of the electronic spins precessing in the nuclear hyperfine fields and manifested as a modulation of the current spin-echo amplitude superimposed on the exponentially decaying echo signal. This approach is particularly suitable for studying systems with comparatively weak hyperfine interaction strength and low nuclear precession frequencies (i.e., below 5 MHz in an X-band

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ESR setup operating at a microwave frequency of 9 to 10 GHz, at a resonant field $B_0 \approx 345$ mT, which corresponds to a g-factor of 2.003; strong excitation fields at low excitation frequencies are difficult to establish owing to technical limitations in many electron-nuclear double resonance setups. We applied this technique to OLED devices with MEH-PPV composed of either hydrogenated or deuterated 2-ethylhexyloxy side groups (3), as there is a pronounced difference in the nuclear Larmor frequencies of protium and deuterium (14.5 versus 2.2 MHz, respectively, at 345 mT). To reveal spin beating between electronic and nuclear spins in ESEEM, it is important to work at low B_1 fields (weak microwave intensities) so as to avoid purely electronic spin-beating within the spin-1 charge carrier pair (3, 5).

Figure 1 shows results from current-detected ESEEM spectroscopy of the OLEDs. The temporal transient of the spin-echo decay was measured as the time-integrated change in current (i.e., total change in charge ΔQ) (4, 10, 15). The echo decays exponentially as expected, but also exhibits a superimposed amplitude modulation (Fig. 1, D and J). This oscillation originates from the dipolar coupling of the carrier-pair spin state and the surrounding nuclear spins. The modulation frequency corresponds to the nuclear Larmor frequency. The charge-carrier pairs are weakly coupled, and under sufficiently weak microwave excitation, only the spin- $\frac{1}{2}$ transition is excited (3, 5). Consequently, only nuclear modulations of the electron spin arise (17), rather than, e.g., electronic spin-spin interactions. The pronounced difference in echo modulation between hydrogenated and deuterated devices is clearly visible without any data processing. The B_0 dependence of the resonance—i.e., the Zeeman splitting (Fig. 1, E and K)—exhibits an unconventional line shape that originates from the pulse sequence projection method used to detect the echoes (11) (Fig. 1B). However, the overall resonance line width is typical for MEH-PPV (2).

Because the information on nuclear Larmor precession is encoded as a modulation signal in the raw data, Fourier transformation was used to extract the individual frequency contributions (Fig. 1, F to H and L to N). Two different characteristics are visible in the Fourier spectra: (i) a flat, B_0 -independent feature close to 15 MHz that persists far off resonance where the echo amplitude itself is zero; and (ii) a strong signal at 14.5 MHz for hydrogenated and at 2.2 MHz for deuterated devices that follows the same B_0 -resonance dependence as the echo amplitude (Fig. 1, E and K). The first feature corresponds to the second harmonic of the Nyquist critical frequency and is thus an artifact of finite sampling. Because data acquisition and processing are carried out in the same way for both devices, this feature is identical in both spectra. The second characteristic, the so-called proton or deuteron matrix line, originates from the nuclear spin precession of the respective isotope. A splitting of the matrix line is not observed. This finding is consistent with weak hyperfine coupling of charge carriers to remote nuclear spins,

as would be expected in a disordered system (16, 18). Weak coupling arises as the strength of the dipolar hyperfine interaction scales with r^{-3} , where r is the distance between nuclear and electronic spins. From the absence of nuclear line

splitting, we can deduce a minimum distance of 0.3 nm between the charge carriers and nuclei (18). Such a separation is consistent with the nature of π -electrons in the polymer (Fig. 1, C and I), where the carriers are localized on the

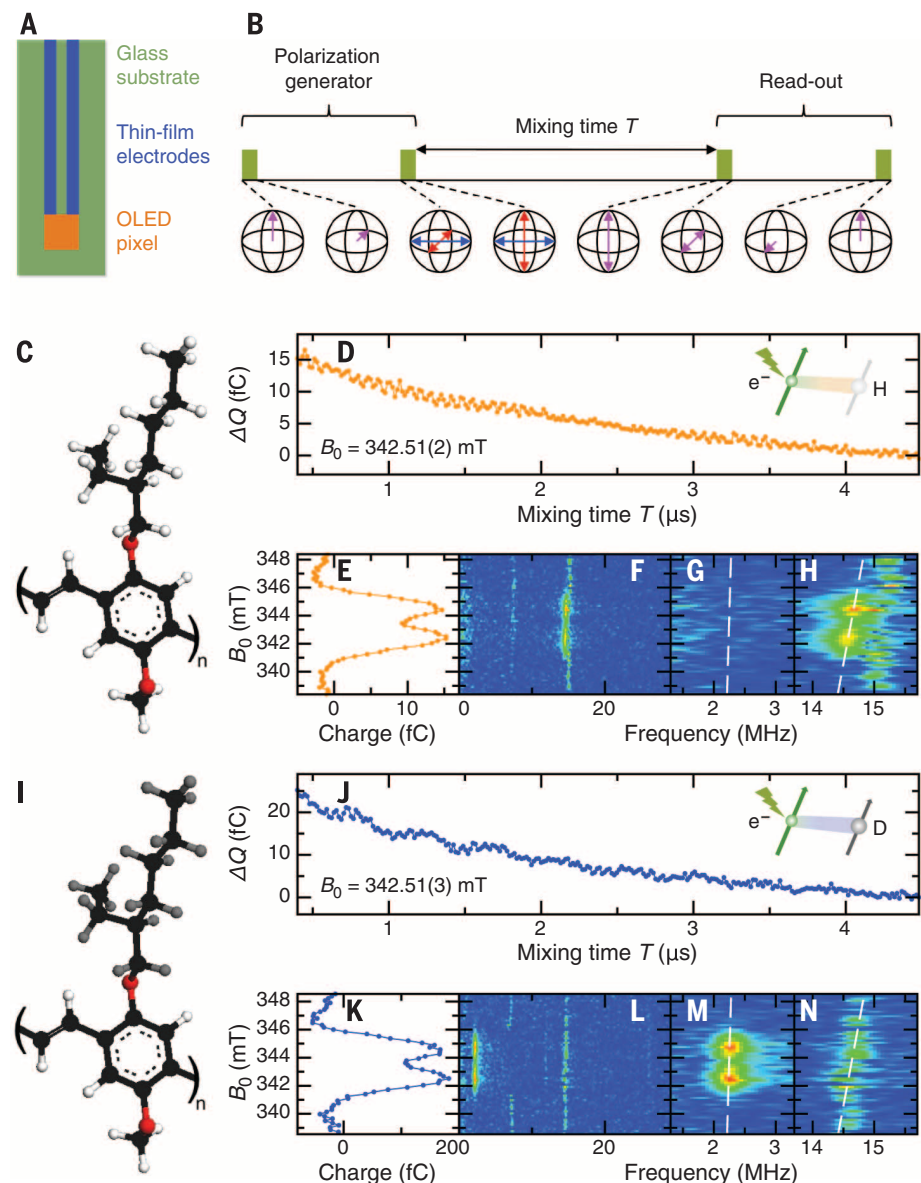


Fig. 1. Isotopic fingerprinting of coherent electronic spin precession in the current of an OLED at room temperature. (A) An ITO/PEDOT:PSS/MEH-PPV/Ca/Al OLED pixel was fabricated on an RF/microwave-compatible substrate with thin-film ITO and aluminum contact leads [ITO: indium tin oxide; PEDOT:PSS: poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate)]. (B) Scheme for the electrical detection of ESEEM in a stimulated echo sequence. After electronic coherence is generated using a $\pi/2$ - τ - $\pi/2$ ESR pulse sequence, electron and nuclear spins are allowed to mix for a time T before the read-out sequence $\pi/2$ - τ - $\pi/2$ is applied. During T , the electron spins beat with the nuclear spins, imposing a periodic modulation on the spin-echo envelope, which is clearly seen. (C and I) Chemical structure of the hydrogenated and deuterated MEH-PPV polymers. Black, white, and gray spheres represent carbon, protium, and deuterium. (D and J) ESEEM signal as a function of mixing time, T . The nuclear resonance signal is contained in the oscillations superimposed on the exponential decay of the current-detected spin echo. The experiments were performed at a microwave frequency of 9.63 GHz. (E and K) B_0 dependence of the signal. (F and L) The ESEEM signal after background correction and Fourier transformation for different values of B_0 across the resonance. (G, H, M, and N) Magnified views around 2 and 15 MHz, the deuteron and proton matrix lines, respectively. The dashed lines indicate the anticipated frequencies of the proton and deuteron resonances.

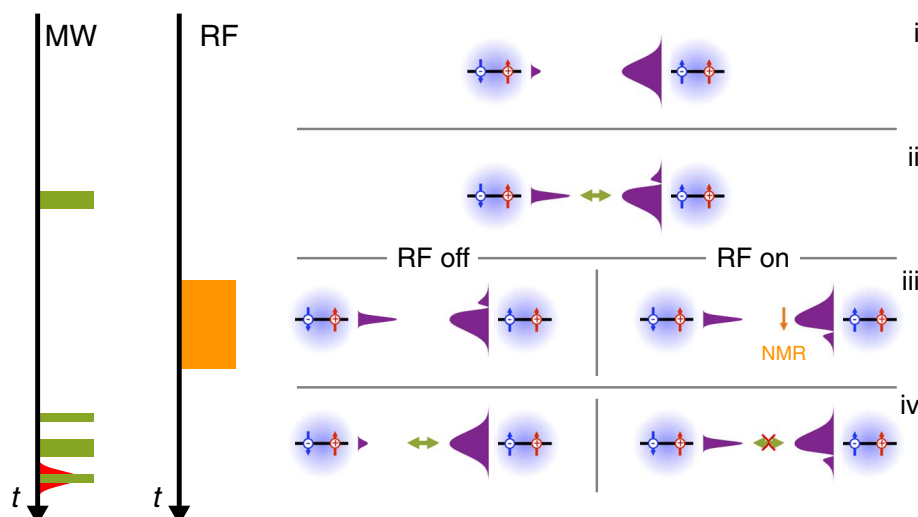


Fig. 2. Microwave (MW) and radiofrequency (RF) pulse sequence and corresponding carrier-pair population distributions for OLED ENDOR spectroscopy. The energetic spread of the populations arises predominantly from hyperfine fields. For simplification, only two of the four singlet and triplet carrier pair states are shown, and the Zeeman splittings between these states are neglected. Transitions between singlet and triplet configurations occur under ESR, leading to a spectral hole in the triplet distribution. Under NMR, the hyperfine field distribution is inverted, blocking resonant refilling of the hole.

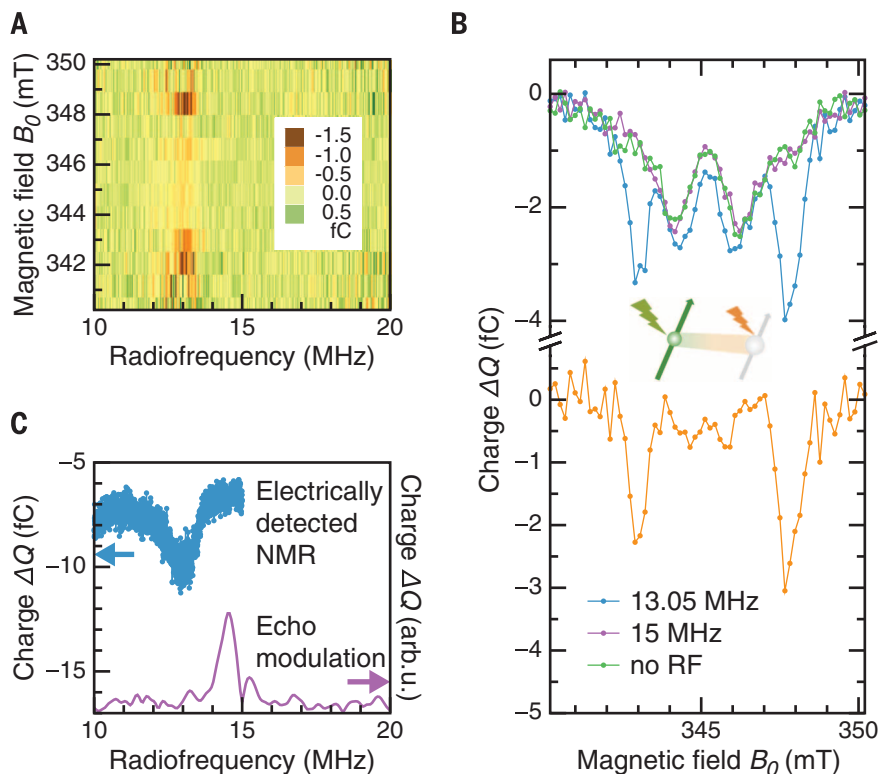


Fig. 3. Direct control of OLED current by nuclear spin transitions. (A) Background-corrected Hahn-echo amplitude as a function of RF excitation and B_0 in an ENDOR experiment. Resonance features only appear close to 13 MHz. The experiments were carried out at a microwave frequency of 9.68 GHz. (B) Hahn-echo amplitude as a function of B_0 for two different ν_{RF} values (13.05 MHz, blue; and 15 MHz, purple). The spectrum with RF irradiation turned off (green) was used to background-correct the 13.05-MHz data (blue), yielding the pure electrically detected NMR spectrum (orange). (C) ENDOR spin-echo amplitude as a function of ν_{RF} at $B_0 = 344.1013$ mT (blue) compared to the ESEEM spectrum from Fig. 1F (purple).

conjugated backbone and the hydrogenic nuclei are located predominantly in the side groups. The deuterated device does contain a small fraction of protium, but the corresponding matrix line is too weak to be separated clearly from the sampling artifact (Fig. 1N).

Rather than simply correlating a qualitative observable such as magnetoresistance with deuteration (3, 6), these ESEEM experiments constitute direct nuclear magnetic fingerprinting of spin-dependent currents in an organic semiconductor. Given the discrete coupling of electronic spins and device current to nuclear moments, as evidenced by the proton and deuteron matrix lines, it should be possible to coherently transfer spin information between electrons and nuclei as in other solid-state qubit systems such as nitrogen vacancy centers in diamond (19) and ^{31}P donor electrons in silicon (20, 21).

To resonantly control the nuclear spin state, we applied high-power radiofrequency (RF) pulses in an electron-nuclear double resonance (ENDOR) configuration that corresponds to a modified Davies-ENDOR sequence (17, 21–23). Figure 2 shows the pulse sequence itself with sketches of the relevant carrier-pair spin states. The sequence is rationalized as follows: (i) Before the pulse sequence is applied, the system is in steady state with an excess population of triplet pairs due to spin statistics. The triplet (spin-1) pair distribution is broadened (5) by magnetic field interactions (such as Zeeman splitting), whereas the singlet (spin-0) is not. (ii) A microwave π -pulse is applied, which rotates one of the pair partners, turning triplet states into singlets. Only those states in resonance with the pulse are transferred to singlets. This step corresponds to spectral hole burning, because a narrow subensemble of the entire population is depleted. (iii) An RF π -pulse is applied. If this pulse is resonant with the hydrogenic nuclear spin transition (i.e., the pulse energy $E = h\nu_{\text{RF}}$ corresponds to the Zeeman splitting of the nuclear moments), the nuclear spins are inverted. Consequently, the spectral hole in the triplet pair distribution is inverted (right-hand side). If the RF pulse is nonresonant (or omitted altogether), the nuclear spins remain unaffected (left-hand side). (iv) A modified Hahn-echo pulse sequence $\pi/2$ - τ - π - τ - $\pi/2$ -echo (4, 10, 11) is applied in the microwave channel. This sequence effectively corresponds to a π -pulse and thus again induces transitions between singlet and triplet manifolds. If the RF pulse in step (iii) is nonresonant, singlets are transferred back to triplets, filling the spectral hole in the triplet distribution. The final configuration will then resemble the initial steady-state situation. If, however, the RF excitation is resonant with the nuclear spin transition, the spectral hole in the triplet distribution, detected in the device current, must shift its resonance frequency because the hyperfine interaction strength is modified selectively (the spectral hole is shifted in energy). The resonant transition between singlets and triplets under microwave irradiation (measured by electrically detected magnetic resonance) is then blocked. When the spin-echo amplitude is

subsequently recorded as a function of RF frequency ν_{RF} , the characteristic hydrogen NMR transition frequency should be visible owing to this population quenching, as the relative singlet-triplet pair population controls conductivity (2, 14). It is effectively irrelevant whether a spectral hole or a peak (a population excess) is formed on one of the spin-pair distributions at a specific nuclear resonance energy. The key aspect is that the ESR pulse leads to a departure from equilibrium configuration.

On the peak of the electron spin resonance, when B_0 coincides with the center of the current-detected resonance spectrum (Fig. 1, E and K), a resonant RF excitation will map the inversion of the spectral hole in the triplet population distribution back onto itself. In this case, there is no net change to the nuclear spin population (i.e., the effective hyperfine field), and the current-detected spin-echo amplitude shows no modification. Therefore, NMR control of the current by ENDOR must be conducted slightly off resonance—i.e., at a frequency offset from that observed in current-detected spin precession (ESEEM, Fig. 1), which in turn corresponds to the maximum of the hyperfine field distribution. This argumentation is somewhat simplified and takes neither spin-lattice relaxation (T_1) (24) nor electronic processes such as carrier hopping into account (2, 4). The nuclear RF excitation pulses lasted for 10 μs (which corresponds to an RF magnetic field strength at the position of the sample of ~ 1.2 mT at 240-W RF power). Spin-lattice relaxation together with spectral diffusion will gradually fill up the spectral hole in the triplet distribution while the RF field is applied, thus reducing the overall signal strength. For charge carrier pairs in MEH-PPV, $T_1 \approx 40$ μs at room temperature (9), which means that the spectral hole is still pronounced even though the experimental fidelity is limited. In addition, the RF pulse duration places an upper limit on the excitation bandwidth (i.e., the Fourier transform of the pulse), which implies that only a small subset of nuclear spins can be excited resonantly, rather than the entire nuclear spin ensemble.

Figure 3 demonstrates direct control of the OLED current through the nuclear spin of the proton [ENDOR experiments on deuterated devices could not be performed because of the weaker nuclear magnetic moment and several technical limitations relating to the lower resonance frequency (11, 17)]. First, we measured the echo amplitude as a function of B_0 (centered around the current-detected electron-spin resonance) and the nuclear excitation frequency, ν_{RF} (Fig. 3A). The spin-echo-detected B_0 -resonance line shape of the electron, which is present both on and off the nuclear resonance (compare to Fig. 1), obscures the ENDOR signal itself. This background was removed by subtracting the off-resonant B_0 -dependent signal from the echo amplitude in the absence of RF excitation. The most pronounced features in the two-dimensional ENDOR spectra appear at a nuclear excitation frequency of ~ 13 MHz and are symmetrical around this resonance with respect to B_0 . With these

features identified, we performed current-detected spin-echo sweeps of B_0 at different fixed values of ν_{RF} (Fig. 3B, upper panel). When ν_{RF} was detuned from resonance (i.e., to 15 MHz, purple curve) or the RF pulse was omitted altogether (green curve), only the echo-detected line shape was visible, analogous to the results in Fig. 1. However, when ν_{RF} was tuned to the resonance determined from Fig. 3A (13.05 MHz), two additional sharp features appeared on either side of the spin-echo-detected resonance spectrum (blue curve), whereas the central spectral feature remained unaffected. The lower panel of Fig. 3B shows the difference between the blue (nuclear resonance) and green (no RF field) lines, revealing the direct effect of nuclear resonant excitation on device current, the effective ENDOR spectrum. The spectrum is consistent with the understanding of the modified Davies-ENDOR experiment described above: No substantial effect is anticipated close to the center of the charge-carrier resonance (see Fig. 2), where the spectral hole in the distribution of hyperfine fields is mapped back onto itself under inversion, resulting in the observed double-dip feature of the spectrum.

The blue curve in Fig. 3C shows a sweep of ν_{RF} across the hydrogen nuclear resonance with B_0 fixed to the higher-field echo-detected ENDOR peak at 348.5 mT (17). For comparison, the nuclear resonance spectrum extracted from the Fourier transform of the current-detected spin-echo envelope modulation of an OLED at the same B_0 field (purple curve, data from Fig. 1) is also shown. Both ESEEM and ENDOR spectra are of comparable width but are shifted with respect to each other by 1.5 MHz. The width of the ENDOR spectrum constitutes a measure of inhomogeneous (disorder) broadening of the hyperfine fields. The shift occurs because the proton matrix line cannot be seen in ENDOR experiments owing to the decreased sensitivity to nuclei with weak (i.e., dipolar) hyperfine-field interaction strengths (16). This insensitivity of the method arises from the finite excitation bandwidth of the RF pulses (17), which does not present a problem in envelope-modulation detection of intrinsic NMR in the absence of RF manipulation, as in ESEEM. In addition, the ESEEM signal is slightly shifted with respect to the nuclear Zeeman frequency. This shift determines the hyperfine-coupling constant of individual nuclei with the charge carriers (17), which in this case accounts for ~ 4 MHz, or an effective magnetic field strength of 0.1 mT, taking into account that the resonance line splits in two. This value is considerably lower than the hyperfine fields that were previously extracted from ESR line-shape analysis (5, 6), as the latter is determined by the net fields of the entire nuclear spin ensemble acting on the charge carriers. In contrast, here we measure the individual contribution of discrete nuclei, which are selected by the spectral hole-burning procedure.

The room-temperature control of OLED conductivity by direct manipulation of nuclear spin states occurs on an energy scale of ~ 100 neV (the Zeeman splitting of the nuclear spins at $B_0 \approx$

345 mT), six orders of magnitude below kT , and therefore constitutes an example of highly efficient switching. Although this effect, originating from strong hyperfine interactions, suggests that protons in organic semiconductors could be used for electrically addressable quantum spin information storage, it is limited with regard to carrier recombination. Coherent writing and read-out of information in nuclear spins in organic semiconductors are not immediately possible because the electronic spins recombine too quickly. However, carrier-pair recombination can be controlled effectively by electric fields (24), which could provide a route to enhancing coherent spin-storage times.

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ACKNOWLEDGMENTS

This work was funded by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Material Sciences and Engineering under Award DESC0000909. We also acknowledge the Utah NSF–Materials Research Science and Engineering Centers program (DMR 1121252) supporting the acquisition and development of the experimental facilities. P.L.B. is a University of Queensland Vice-Chancellor's Senior Research Fellow. We thank J. Yu for preparing the deuterated MEH-PPV.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1487/suppl/DC1
Materials and Methods
Figs. S1 to S4
References (25, 26)

5 May 2014; accepted 31 July 2014
10.1126/science.1255624

NUCLEAR CHEMISTRY

Synthesis and detection of a seaborgium carbonyl complex

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Experimental investigations of transactinoid elements provide benchmark results for chemical theory and probe the predictive power of trends in the periodic table. So far, in gas-phase chemical reactions, simple inorganic compounds with the transactinoid in its highest oxidation state have been synthesized. Single-atom production rates, short half-lives, and harsh experimental conditions limited the number of experimentally accessible compounds. We applied a gas-phase carbonylation technique previously tested on short-lived molybdenum (Mo) and tungsten (W) isotopes to the preparation of a carbonyl complex of seaborgium, the 106th element. The volatile seaborgium complex showed the same volatility and reactivity with a silicon dioxide surface as those of the hexacarbonyl complexes of the lighter homologs Mo and W. Comparison of the product's adsorption enthalpy with theoretical predictions and data for the lighter congeners supported a $\text{Sg}(\text{CO})_6$ formulation.

The heaviest elements, with atomic number $Z \geq 104$, are accessible solely in nuclear fusion reactions of intense heavy-ion beams with suitable targets. Production rates of these radioactive elements range from single atoms per minute down to single atoms per week or even month, with concomitant production of orders-of-magnitude larger amounts of unwanted by-products, some of which strongly interfere with the unambiguous detection of a single transactinoid atom. Harsh ionizing conditions created by the heavy-ion beam and the short half-lives of transactinoid nuclides severely constrain techniques that can be applied to their chemical investigations, which have reached flerovium (Fl, $Z = 114$) (1–4). Chemical studies of the heaviest elements are best suited to shed light on the influence of relativistic effects induced in the electron structure by the high nuclear charge (5).

Gas-phase chemical studies performed in the past 50 years elucidated the chemistry of halide

and oxyhalide compounds of rutherfordium (Rf, $Z = 104$), dubnium (Db, $Z = 105$), seaborgium (Sg, $Z = 106$), and bohrium (Bh, $Z = 107$), as well as the oxyhydroxide of Sg and the tetroxide of hassium (Hs, $Z = 108$). In all of these complexes, the metal is present in its highest formal oxidation state; all outer-valence electrons participate in chemical bonding. Only copernicium (Cn, $Z = 112$) and Fl have been studied in their elemental state. Recently, the combination of physical separation of single ions in a magnetic dipole field, combined with chemical techniques (6), was established. This technique eliminates the beam-induced plasma, which in more classical techniques prevented the formation of fragile compounds directly behind the target. Thus, this development paved the way for studies of distinct compound

classes. Here, we report on the chemical investigation of a seaborgium carbonyl complex, most likely $\text{Sg}(\text{CO})_6$.

The first synthesis of a metal carbonyl complex was reported over 100 years ago (7). Carbonyl complexes have since become of great interest in fundamental and applied research. They are known for many lighter transition metals (8). In carbonyl complexes, electron density is donated from the highest occupied molecular orbital (HOMO) of the CO ligand to empty atomic orbitals in σ -symmetry of the metal, such as the $p_{1/2}$. In return, electron density is back-donated from the metal d-orbitals in π -symmetry to the lowest unoccupied molecular orbital (LUMO) of the CO ligand (9). Group six hexacarbonyl complexes are prominent examples for complexes of octahedral symmetry. The hexacarbonyl complexes of chromium (Cr), molybdenum (Mo), and tungsten (W) are well known and are volatile solids at room temperature.

The hexacarbonyl complex of the pseudohomolog of group 6, uranium (U), has only been observed in matrix isolation experiments at temperatures below 30 K (10) and could not be synthesized in larger quantities (11). This is in agreement with theoretical calculations, which predict the instability of $\text{U}(\text{CO})_6$ (12). In contrast, the transactinoid element Sg is expected to form stable hexacarbonyl complexes (12, 13) like its lighter homologs. Relativistic effects, however, lead to enhanced contraction of the $p_{1/2}$ orbital in Sg, weakening the σ -donation bond. Consequently, because of the relativistic contraction of s and $p_{1/2}$ orbitals, the d-orbitals are better shielded from the atomic nucleus and expand. The overlap with the LUMO of the CO ligand becomes stronger. This may lead to a stronger contribution of π -back-donation relative to σ -donation in comparison with the corresponding ratio in the complexes of the lighter members of group 6. Overall, the first bond dissociation energy should remain similar to that of $\text{W}(\text{CO})_6$ (13). Also, the calculated bond lengths are similar (13, 14). Theory predicts a lower ionization potential and a higher polarizability of $\text{Sg}(\text{CO})_6$ compared with $\text{W}(\text{CO})_6$ (14). Both properties influence the interaction with a surface in a way

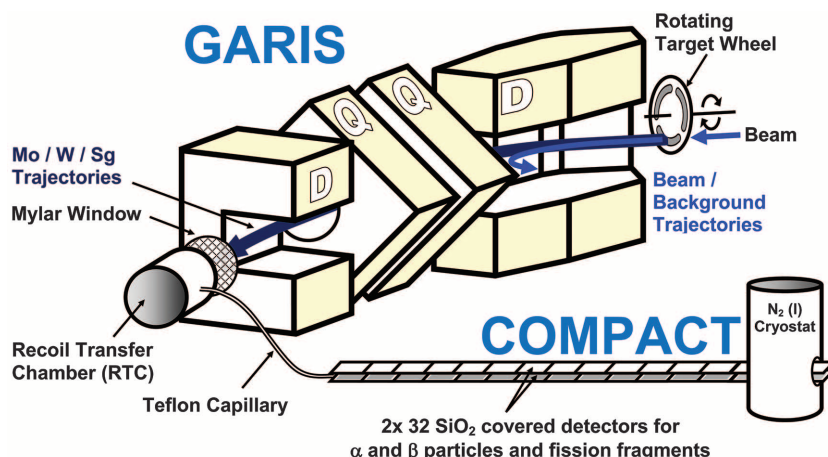


Fig. 1. Schematic drawing of the experimental setup.

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that, for example, equal values for the adsorption enthalpies of $\text{Sg}(\text{CO})_6$ and $\text{W}(\text{CO})_6$ on silicon dioxide (SiO_2) are predicted (14). This promises to facilitate identification of $\text{Sg}(\text{CO})_6$ through a direct comparison with the behavior of $\text{W}(\text{CO})_6$, if both elements are studied under identical conditions. The successful synthesis of $\text{Sg}(\text{CO})_6$ would then present a crucial step toward more detailed studies of the metal-carbon bond involving transactinoid elements.

Synthesis of group 6 hexacarbonyl complexes typically requires high-pressure conditions and temperatures between room temperature and 200°C. Higher temperatures would favor decomposition of the complexes. Hence, doubts arose whether the synthesis of these complexes in an accelerator-based experiment under conditions relevant for a transactinoid experiment would be feasible. Recently, combining physical preparation in a magnetic recoil separator (6) with in situ chemical methods allowed the synthesis and study of hexacarbonyl complexes with short-lived Mo and W isotopes, providing a basis for experiments with Sg (15, 16).

Among the known isotopes, ^{265}Sg is sufficiently long-lived and can be produced in the nuclear fusion reaction of a ^{22}Ne beam with ^{248}Cm targets (17). Two long-lived, α -decaying states are known with half-lives of $(8.5^{+2.6}_{-1.6})$ s and $(14.4^{+3.7}_{-2.5})$ s (17).

Our experiments were performed at the Gas-filled Recoil Ion Separator (GARIS) (18) at RIKEN, Japan. The $\text{Mo}(\text{CO})_6$ and $\text{W}(\text{CO})_6$ complexes, lighter homologs of $\text{Sg}(\text{CO})_6$, were synthesized and studied in preparatory experiments (15, 16) and also in the $\text{Sg}(\text{CO})_6$ experimental campaign, allowing direct comparison among all three compounds.

A schematic of the experimental setup is shown in Fig. 1. Mo isotopes were synthesized in the nuclear fusion of ^{24}Mg with Zn nuclei, forming $^{87,88}\text{Mo}$ after direct emission of few neutrons—the general notation for such nuclear reactions being $^{nat}\text{Zn}(^{24}\text{Mg},x\text{n})^{87,88}\text{Mo}$ (nat, natural isotopic abundance). The energy of the ^{24}Mg ions was 85 MeV, and the thickness of the Zn foil was $558 \mu\text{g}/\text{cm}^2$. W isotopes were produced in the nuclear reaction $^{144}\text{Sm}(^{24}\text{Mg},4\text{-Sn})^{163,164}\text{W}$. Enriched $^{144}\text{Sm}_2\text{O}_3$ (isotopic abundance 93.8%) was evaporated on a 3- μm -thick Ti foil to a Sm layer of thickness $251 \mu\text{g}/\text{cm}^2$ and irradiated with ^{24}Mg ions of 144 MeV energy. For the synthesis of Sg, $264 \mu\text{g}/\text{cm}^2$ of ^{248}Cm , deposited in oxidic form on a 2- μm thick Ti foil, were irradiated with $1.5 \cdot 10^{19}$ ^{22}Ne beam particles of 122 MeV energy.

The ion beam from the RIKEN Linear Accelerator (RILAC) accelerator passed through the rotating target assembly. Nuclear fusion products were separated from the beam within the first dipole magnet (Fig. 1, “D”) of GARIS, focused in a doublet of quadrupole magnets (Fig. 1, “Q”) and guided by another dipole magnet to the GARIS focal plane. Mo, W, and Sg ions passed 3.7, 4.4, and 0.7 μm of Mylar, respectively, which diminished their kinetic energy. The ions were thermalized behind the window in a Recoil Transfer Chamber (RTC) (19). The RTC was flushed with a He/CO mixture at 500 to 750 mbar. Volatile species, which formed in the RTC, were transported by the gas

stream through a 10-m Teflon (DuPont, Wilmington, DE) capillary (inner diameter, 2 mm) to the Cryo-Online Multidetector for Physics And Chemistry of the Transactinoides (COMPACT) (4). COMPACT is a gas-thermochromatography detector with the chromatography column consisting of 32 pairs of Si semiconductor detectors suitable for registering time- and energy-resolved fission fragments and α - and β -particles emitted in the nuclear decay—for example, of transactinoides such as ^{265}Sg and its daughter products or $^{163,164}\text{W}$ and $^{87,88}\text{Mo}$, respectively. The detectors form a channel with a rectangular cross section. The surface of the detectors was covered with a 50-nm-thick SiO_2 layer. The total efficiency to register an α particle emitted from a species residing inside

the chromatography channel is 76%. Along the channel, a negative temperature gradient was applied by using a liquid nitrogen cryostat. To calibrate the detectors, gaseous ^{219}Rn was used, which emanated from a ^{227}Ac source installed in the gas system.

In agreement with observations in previous experiments (15, 16), Mo and W formed volatile carbonyl complexes, which were transported by the gas stream and deposited in COMPACT. The isotopes ^{163}W ($T_{1/2} = 2.75$ s) and ^{164}W ($T_{1/2} = 6.0$ s) were identified in COMPACT by their α -particle energies; a typical spectrum is shown in fig. S1. In the experiments with Mo isotopes, signals from β -decay were recorded. β -particles feature a continuous energy spectrum. Hence, isotopes

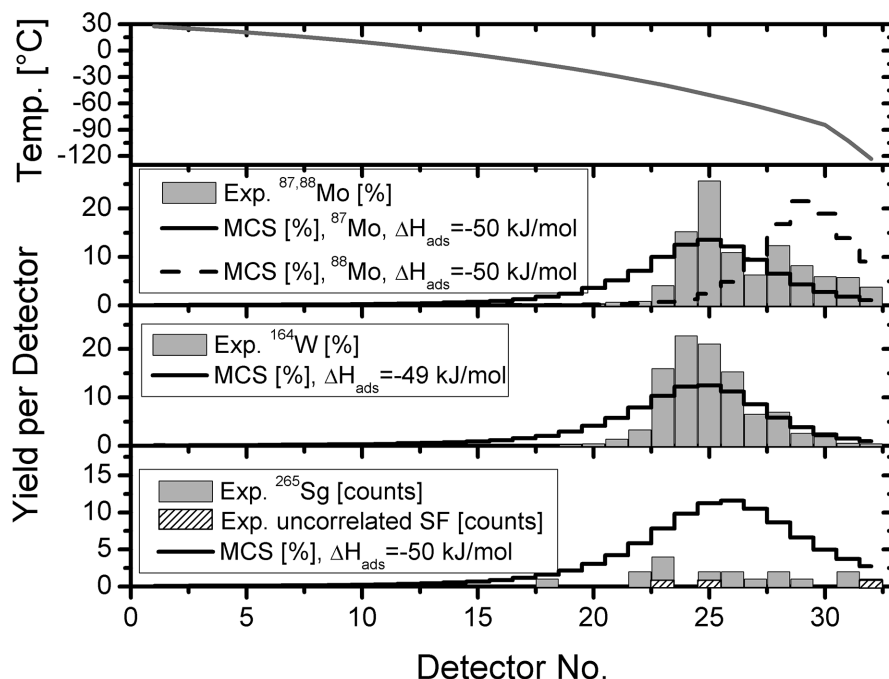


Fig. 2. Deposition patterns of volatile hexacarbonyl complexes in COMPACT. (Top) Temperature gradient along the COMPACT detector array. (Top middle) Bars indicate experimental distribution of ^{87}Mo and ^{88}Mo (gas flow rate, 1 L/min; gas composition, He:CO=1:1); lines indicate result of MCS for ^{87}Mo and ^{88}Mo , assuming $\Delta H_{\text{ads}} = -50$ kJ/mol. (Bottom middle) Bars indicate experimental distribution of ^{164}W (gas flow rate, 1 L/min; gas composition, He:CO=1:1); line indicates result of MCS for $^{164}\text{W}(\text{CO})_6$ assuming $\Delta H_{\text{ads}} = -49$ kJ/mol. (Bottom) Bars indicate distribution of ^{265}Sg (gray) and the uncorrelated spontaneous fission events (hatched). The data were obtained under various conditions (gas flow rates, 1.0 to 2.2 L/min). He:CO was either 1:1 (17 events) or 5:1 (one event). Details are provided in table S2. Line indicates averaged distribution deduced from MCS taking into account the various experimental conditions, assuming $\Delta H_{\text{ads}} = -50$ kJ/mol.

Table 1. Adsorption enthalpies of group 6 carbonyl complexes on SiO_2 surface from this work and previous publications. Data on experiments (second and third columns) are from this work and from (15). Data from theory (fourth column) is from (14).

	$-\Delta H_{\text{ads}}$ [kJ/mol]* (this work)	$-\Delta H_{\text{ads}}$ [kJ/mol]* [from (15)]	$-\Delta H_{\text{ads}}$ [kJ/mol]† [from (14)]
$\text{Cr}(\text{CO})_6$			45.4 ± 2.5
$\text{Mo}(\text{CO})_6$	50 ± 2	42 ± 2.5	48.1 ± 2.5
$\text{W}(\text{CO})_6$	49 ± 2	46.5 ± 2.5	46.5 ± 2.5
$\text{Sg}(\text{CO})_6$	50 ± 4		46.2 ± 2.5

*Error limits include only statistical errors.

†Values predicted with respect to the measured $-\Delta H_{\text{ads}}$ of $\text{W}(\text{CO})_6$ from (15).

emitting the β -particles cannot be identified according to registered energies. Therefore, Mo was accumulated for 15 min in the COMPACT detector. Afterward, the irradiation of the target was stopped, and the decay of the activity deposited on the detectors was monitored. The decay curves of every single detector pair of COMPACT were then recorded, revealing in the detector pairs around detector number 25 a component with a half-life of ~ 13 s to be present, which was assigned to ^{87}Mo . In the detector pairs around detector number 27, a component with a half-life of ~ 8 min was dominant, which agrees with the half-life of ^{88}Mo . Small contributions of a species with a half-life of ~ 2 min have also been observed, which we assigned to ^{89}Mo . Thus, ^{87}Mo and ^{88}Mo as well as small amounts of ^{89}Mo were identified by their half-lives. The identification was confirmed in separate γ -spectroscopic measurements, in which samples collected on a charcoal filter were measured with a germanium γ -ray detector; a typical spectrum is shown in fig. S2.

For the deposition patterns of Mo and of W shown in Fig. 2, on the order of $\sim 10^6$ atoms were collected in the COMPACT detector. The distribution of Mo and W measured at a gas flow rate of 1 L/min of a He:CO=1:1 mixture is shown in Fig. 2. The deposition pattern of Mo shows a double-peak structure. We assign the first one, peaking in detector number 25, to the 14.5-s isotope ^{87}Mo , and the second one, peaking at detector 27, to 8.2-min ^{88}Mo . Because of its longer half-life, the latter is transported to lower temperatures before its decay occurs. The maximum of shorter-lived ^{164}W ($T_{1/2} = 6.0$ s) was registered in detector 24.

In the experiments with Sg, the gas flow rate and gas composition were varied; details are given in table S2. A combined data set is presented in Fig. 2. To identify single Sg atoms, a search for correlated decay chains originating from ^{265}Sg was performed. We searched for 7.4 to 10.0 MeV α particles, followed within 1 hour by another 7.4 to 10.0 MeV α particle or a single fission fragment, which was registered in either the same or an adjacent detector pair to the one in which the first α particle was found. This procedure revealed 15 correlated decay chains. The probability to detect an α - α correlation originating from background activities, unrelated to the decay of ^{265}Sg , was calculated according to (20) to be less than 10^{-5} . In background measurements, no spontaneous fission events were observed. During the experiment, three spontaneous fission events were registered, which were not preceded by an α particle in the relevant energy and time window. These occurred in detectors 23, 25, and 32. The detection positions of the uncorrelated spontaneous fission events agree with those of the observed decay chains. Therefore, and as the probability for these events to originate from background is negligible, we assign them to originate from the decay of ^{265}Sg or its daughter nuclides as well. Thus, in total 18 events were assigned to the decay of Sg or its daughter nuclides. An overview of all these events and the experimental conditions under which they were observed is given in the supplementary materials. Sg deposited in the last third of the detectors, like

its lighter homologs Mo and W (Fig. 2). Evidently, Sg therefore forms a volatile complex, which has properties similar to the hexacarbonyl complexes of its lighter homologs Mo and W. Fully relativistic quantum chemical calculations predict Sg to form a stable, volatile hexacarbonyl complex (13), with interaction properties with a SiO_2 surface closely resembling those of its lighter homologs (14). This is in agreement with our experimental results and suggests the observation of Sg in the form of $\text{Sg}(\text{CO})_6$.

To extract the adsorption enthalpy, ΔH_{ads} , of the hexacarbonyl complexes on the SiO_2 surface, the deposition patterns of Mo, W, and Sg were modeled by means of Monte Carlo Simulations (MCS) of the adsorption chromatographic process (21), where ΔH_{ads} was the only free parameter; the input parameters of the simulations are given in table S1 in the supplementary materials. We estimate the systematic uncertainty of this method in our analyses to ± 6 kJ/mol. In the following, we give only statistical error limits, at the 68% confidence level unless otherwise stated.

The black lines in Fig. 2 show the simulated distribution of $^{87,88}\text{Mo}(\text{CO})_6$, $^{164}\text{W}(\text{CO})_6$, and $^{265\text{a,b}}\text{Sg}(\text{CO})_6$. From comparison with the MCS, the adsorption enthalpies were deduced to be (-50 ± 2) kJ/mol for $\text{Mo}(\text{CO})_6$ and (-49 ± 2) kJ/mol for $\text{W}(\text{CO})_6$. A statistical analysis was applied to extract ΔH_{ads} of the $\text{Sg}(\text{CO})_6$ from the results obtained at different experimental conditions. The probability of detecting a given event in the detector where it was actually registered was determined for a range of ΔH_{ads} values by using MCS of the chromatographic process under the applied experimental conditions when the decay was observed. The total probability to register the experimental distribution was calculated from these values by multiplying all individual probabilities. Thus, a probability distribution of the adsorption enthalpies was deduced, yielding a value for ΔH_{ads} of (-50 ± 4) kJ/mol. Within the statistical error limits, this is equivalent to the values for the $\text{Mo}(\text{CO})_6$ and $\text{W}(\text{CO})_6$ complexes as deduced in the present experiment. Fully relativistic quantum chemical calculations predict adsorption properties of the $\text{Sg}(\text{CO})_6$ complex on SiO_2 to be very similar to those of $\text{W}(\text{CO})_6$ (14). This corroborates our interpretation that Sg forms a volatile hexacarbonyl complex. An overview of the experimental and theoretically calculated adsorption enthalpies is given in Table 1. We note good agreement, except for the case of $\text{Mo}(\text{CO})_6$, in which the current value is higher than that reported in (15, 16). However, a different technique was used in (15, 16) compared with the present work, and systematic uncertainties are different for the work reported here and that reported in (15, 16). To avoid this situation for the present experiment, we have measured all three elements with the same technique. Our experimental adsorption enthalpies are in good agreement with those predicted (14) for the group 6 hexacarbonyl complexes.

Thus, owing to our advanced techniques, we have synthesized a compound of the heavy element Sg that belongs to a new compound class, unknown up to now for the transactinoids. We

have confirmed Sg to be a full homolog of Mo and W, and our results are in agreement with theoretical predictions. Our new approach substantially broadens the range of chemical systems available for studies of transactinoids and other elements that are only available in single-atom quantities. Studies of the thermal stability of $\text{Sg}(\text{CO})_6$ appear feasible, as do syntheses of carbonyl complexes of Bh, Hs, and meitnerium (Mt, $Z = 109$) (15, 16). Furthermore, the combination of physical and chemical separation techniques bears potential for background-free nuclear decay spectroscopy because purified transactinoid samples can be prepared.

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ACKNOWLEDGMENTS

This work was performed at the RI Beam Factory operated by RIKEN Nishina Center and the Center for Nuclear Study, University of Tokyo. We thank the ion source and accelerator staff at the RIKEN Nishina Center for accelerator-based research for providing intense and stable ion beams and V. Pershina for interesting discussions. The present work is partially supported by the Reimei Research Program (Japan Atomic Energy Agency); the German Federal Ministry for Education and Research contract 06M77164; the Helmholtz association contract VH-NG-723; the Ministry of Education, Culture, Sports, Science, and Technology, Japan, Grant-in-Aids 19002005 and 23750072; the Swiss National Science Foundation contract 200020_144511; the Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, Heavy Element Chemistry Program of the U.S. Department of Energy at Lawrence Berkeley National Laboratory under contract DE-AC02-05CH11231; and the National Natural Science Foundation of China (grant 11079006).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1491/suppl/DC1
Figs. S1 and S2
Tables S1 and S2
References (22–25)

7 May 2014; accepted 12 August 2014
10.1126/science.1255720

MOOD REGULATION

GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment

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The lateral habenula (LHb), a key regulator of monoaminergic brain regions, is activated by negatively valenced events. Its hyperactivity is associated with depression. Although enhanced excitatory input to the LHb has been linked to depression, little is known about inhibitory transmission. We discovered that γ -aminobutyric acid (GABA) is co-released with its functional opponent, glutamate, from long-range basal ganglia inputs (which signal negative events) to limit LHb activity in rodents. At this synapse, the balance of GABA/glutamate signaling is shifted toward reduced GABA in a model of depression and increased GABA by antidepressant treatment. GABA and glutamate co-release therefore controls LHb activity, and regulation of this form of transmission may be important for determining the effect of negative life events on mood and behavior.

Excitatory transmission in the brain is generally balanced by feed-forward and feedback inhibitory actions of γ -aminobutyric acid-releasing (GABAergic) interneurons (1). The LHb largely lacks GABAergic interneurons (2), and thus other mechanisms must

operate to control hyperactivity. One potential mechanism is for input neurons to release both excitatory (e.g., glutamate) and inhibitory (e.g., GABA) transmitter [(3–8), but see (7, 9, 10)]; balance could be achieved by regulating the relative amounts of transmitters released (11).

Hyperactivity in the LHb may contribute to depression (12–19), possibly by overprocessing (20) negatively valenced events (21). Although enhanced excitatory input to the LHb contributes to depression-like behaviors in rodents (14, 15), little is known about LHb inhibitory transmission. Here, we examine the nature of the inhibitory input to the LHb from the basal ganglia, as well as its regulation in conditions related to depression. This pathway is of particular interest because it is active during negatively valenced events (22), which are thought to play a causal role in depression (23).

To examine the effects of GABAergic input on LHb output, we infected in vivo the basal ganglia output region, the entopeduncular nucleus (EP), of rodents with an adeno-associated virus (AAV) expressing channelrhodopsin-2 (ChR2) (24). Two to three weeks later, brain slices were prepared, and whole-cell responses were obtained from neurons in the LHb (Fig. 1A). Trains of brief light pulses produced a few synaptically driven action potentials, indicating predominant excitation (25);

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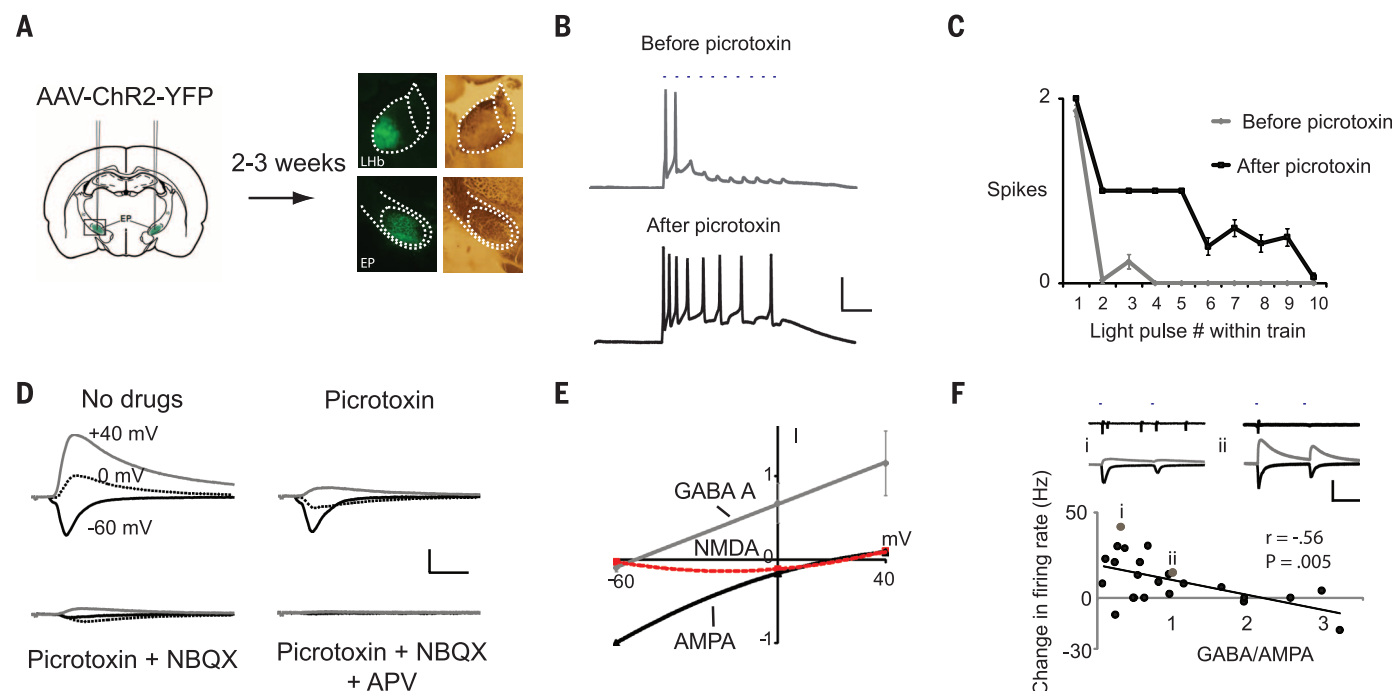


Fig. 1. Mixed excitatory and inhibitory transmission from EP input controls persistent LHb activity. (A) Diagram of experimental protocol. Microscopic images depict example injection of AAV-ChR2-YFP into WT rat; left, yellow fluorescent protein (YFP) fluorescence; right, bright-field illumination. (B) Trains of synaptic responses evoked by light pulses (50 Hz, blue dots) before (top) and after (bottom) bath application of picrotoxin recorded in whole-cell current clamp mode. Scale bars, 25 mV, 50 ms. (C) Summary of average responses for recording in (B). Similar results were obtained in three cells. (D) Whole-cell recordings in voltage-clamp mode of light-evoked EP-LHb transmission at different holding potentials (as indicated) and after application of drugs (as

indicated). Scale bars, 100 pA, 10 ms. (E) Current-voltage plots for different EP-LHb synaptic conductances normalized to AMPA-R-mediated response at -60 mV ($N = 9$ cells). (F) Cell-attached (top) and subsequent whole-cell (middle) recordings at +20 mV (GABA, gray) and -50 mV (AMPA, black) holding potentials from two cells (i and ii) during synaptic responses evoked by pairs of light pulses (as indicated). Below, plot of change in firing rate (measured in cell-attached mode) versus GABA-A-R-mediated/AMPA-R-mediated synaptic response (measured in voltage-clamp mode; $N = 24$ cells); values for cells i and ii indicated by gray symbols. Scale bars, 400 pA, 50 ms. Error bars throughout indicate mean $\pm$ SEM.

bath application of the GABA-A receptor (α -R) antagonist picrotoxin increased the number of action potentials (Fig. 1, B and C), indicating that the EP excitatory drive was balanced by inhibition. In voltage-clamp mode recordings, light-evoked synaptic responses displayed AMPA-R, GABA-A-R, and *N*-methyl-D-aspartate receptor (NMDA-R) components (Fig. 1, D and E). In other brain slice experiments (Fig. 1F), pairs of light pulses elicited EP-LHb transmission and action potentials; the ratio of GABA-A-R to AMPA-R-mediated EP-LHb synaptic transmission was inversely related to the generation of action potentials.

Because the LHb contains few inhibitory neurons (2), we tested whether the GABA-A response was due to long-range monosynaptic EP to LHb transmission. Light-evoked synaptic responses, measured at -15 mV holding potential (to allow simultaneous measurement of AMPA-R and GABA-A-R-mediated currents), displayed inward followed by outward currents (Fig. 2A). An AMPA-R antagonist (NBQX; 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione), blocked the inward current, leaving intact a picrotoxin-sensitive outward current (Fig. 2A and fig. S1), indicating monosynaptic EP to LHb inhibition (see also fig. S2). In some whole-cell recordings of LHb neurons voltage-clamped at -15 mV, we observed isolated spontaneous responses (in the absence

of tetrodotoxin; i.e., action potentials not blocked) that were inward immediately followed by outward currents, indicating co-release of glutamate and GABA from individual axons onto the recorded cell (Fig. 2B and fig. S3). Co-release from some individual vesicles could also be detected. In four of six experiments monitoring spontaneous miniature excitatory postsynaptic currents (in tetrodotoxin), biphasic events occurred more often than could be accounted for by independent inward and outward responses (Fig. 2C and fig. S4) ($P < 0.05$ for four experiments) (26).

To investigate specifically whether EP neurons projecting to the LHb co-release glutamate and GABA, we first took a genetic approach. In *Vglut2*-Cre mice, only glutamatergic neurons expressing the vesicular glutamate transporter *Vglut2* express Cre recombinase (27). We injected their EP with an AAV that expresses ChR2 only in cells expressing Cre (AAV-DIO-ChR2) (28) (figs. S5 and S6) and prepared brain slices 2 to 3 weeks later. As in wild-type (WT) animals, light-evoked synaptic responses, measured at -15 mV holding potential, displayed a NBQX-sensitive inward current followed by a NBQX-insensitive, picrotoxin-sensitive outward current (Fig. 2D), indicating that EP-LHb glutamatergic neurons release both glutamate and GABA. In cell-attached recordings, picrotoxin increased spikes evoked by 50 Hz, but not

2 Hz, stimulation of *Vglut2*-expressing EP axons (Fig. 2E and figs. S7 and S8) (NBQX blocked evoked spikes) (26), indicating that co-release of GABA is important for limiting LHb output during high-frequency EP input; the greater effect at higher frequency is likely due to the slower GABA-A-R kinetics, permitting greater summation of GABA-A-R currents at 50 Hz.

In complementary experiments, we used *GAD67*-Cre mice, in which only GABAergic neurons expressing glutamic acid decarboxylase type 67 express Cre recombinase (29–31). We injected their EP with AAV-DIO-ChR2 (figs. S5 and S6) and prepared brain slices 2 to 3 weeks later. As in WT and *Vglut2*-Cre mice, light-evoked synaptic responses, measured at -15 mV holding potential, displayed a NBQX-sensitive inward current followed by a NBQX-insensitive, picrotoxin-sensitive outward current (Fig. 2F), indicating that EP-LHb GABAergic neurons release both glutamate and GABA. In cell-attached recordings, EP-LHb transmission evoked spikes, indicating that EP GABAergic neurons produce excitatory responses in LHb (Fig. 2G).

Co-release from EP to LHb was further supported by the following observations: (i) the trial-to-trial variability in evoked EP-LHb transmission recorded at -15 mV displayed significantly greater covariation of early inward and late outward currents than would be expected from independent

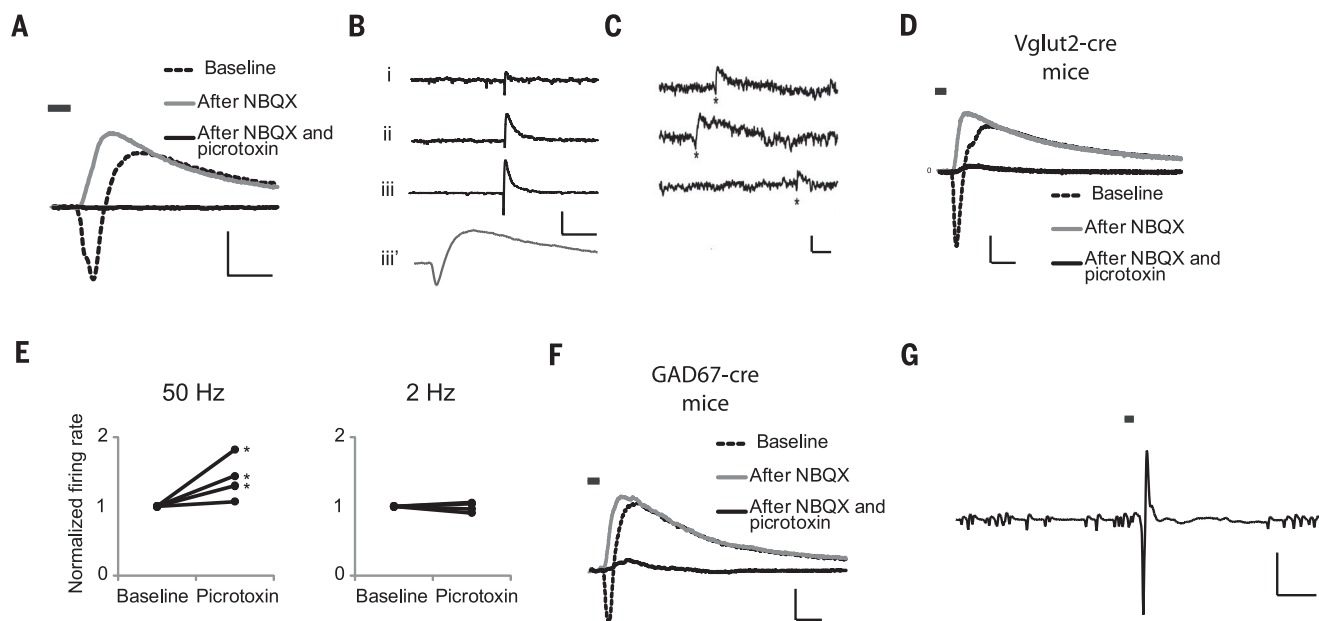


Fig. 2. Co-release of GABA and glutamate from EP inputs to LHb: Electrophysiological, optogenetic, and mouse genetic evidence. (A) Whole-cell recording at -15 mV holding potential of light-evoked EP-LHb transmission after bath application of indicated drugs. Scale bars, 60 pA, 10 ms. (B) Spontaneous responses from whole-cell recordings at -15 mV holding potential from three different LHb neurons (i, ii, and iii) in adult WT mice. Lower trace (iii') is the same as above (iii) at higher temporal resolution. Scale bars, 50 pA, 200 ms (upper), 10 ms (lower). Biphasic events accounted for 2/3 of all large (>15 pA) outward events in these cells (79, 56, and 67%; large, biphasic events were found in 3 of 12 recordings). (C) Spontaneous miniature biphasic events (*) from whole-cell recordings at -40 mV with 0.5 mM chloride internal

solution. Scale bars, 10 pA, 20 ms. (D) Whole-cell recording from *Vglut2*-cre mouse injected with AAV-DIO-ChR2-YFP at -15 mV holding potential of light-evoked EP-LHb transmission with indicated drugs in bath. Both inward and outward currents found in 17 of 17 cells. Scale bars, 100 pA, 10 ms. (E) Plot of effect of light-evoked EP-LHb transmission from *Vglut2*+ axons of adult mice delivered at 50 Hz (left) or 2 Hz (right) on normalized LHb neuronal firing rate before and after bath application of picrotoxin ($*P < 0.05$). (F) Same as (D) for *GAD67*-cre mice. Total of 11 of 13 cells, inward/outward; 2 of 13 cells, outward only. Scale bars, 40 pA, 10 ms. (G) Cell-attached recording (average of 10 trials) from adult *GAD67*-cre mouse LHb neuron; light delivery indicated. Scale bars, 5 mV, 20 ms.

release of glutamate and GABA (fig. S9); (ii) evoked EP-LHb GABA-A-R- and AMPA-R-mediated transmission displayed similar presynaptic modulation by bath application of serotonin (25) (fig. S10); (iii) evoked EP-LHb transmission recorded from different LHb neurons displayed similar GABA-A-R- and AMPA-R-mediated paired-pulse response ratios (fig. S11); and (iv) GABA-A-R- and AMPA-R-mediated transmission displayed similar response latencies and jitter (fig. S12).

We next examined immunohistochemically the synapses made by EP neurons to the LHb. In adult mice, the EP was injected with an AAV expressing green fluorescent protein (AAV-GFP) (fig. S6). Subsequently, 400-nm thin sections of the LHb were prepared, immunolabeled for GFP, glutamic acid decarboxylase (GAD), and Vglut2, and imaged with confocal microscopy. GFP-expressing structures displayed GAD and Vglut2 (Fig. 3A and fig. S13), indicating GABA and glutamate in individual EP-LHb terminals. A control region displayed little GAD and Vglut2 colabeling (Fig. 3B). Quantification of labeling (26) (fig. S14) in structures originating from the EP indicated that ~50% of Vglut2-positive structures

showed GAD and ~80% that were GAD-positive had Vglut2; outside the LHb, Vglut2 showed little overlap with GAD (Fig. 3, C and D, and fig. S15). Thus, in EP-LHb structures, ~50% had both GAD and Vglut2, ~10% had only GAD, and ~40% had only Vglut2. Consistent with co-release, glutamate receptors and GABA receptors displayed overlap in LHb (fig. S16).

To examine anatomically whether GABA and glutamate can be released from individual vesicles at EP-LHb synapses, we used immunogold electron microscopy (EM). Two to three weeks after injecting adult mice with AAV-GFP in the EP, LHb sections were processed for EM and immunolabeled for GFP, Vglut2, and GABA with secondary antibodies linked to gold of three different sizes (26). Structures originating from the EP (Fig. 3E and figs. S17 and S18) showed gold particles of three clearly different sizes (Fig. 3F and fig. S17). The frequency of distances between Vglut2 and GABA gold labels displayed a peak at ~20 to 30 nm (Fig. 3G), consistent with colocalization of Vglut2 and GABA at individual vesicles (32). The distribution of GFP-Vglut2 and GFP-GABA distances was flat, indicating random

distribution of particles. The number of gold particles less than 30 nm apart, compared with the number of gold particles between 30 and 100 nm apart, was significantly higher for Vglut2-GABA (34 of 248) compared with Vglut2-GFP (4 of 92; $P < 0.03$, χ^2 -test) and GABA-GFP (64 of 999; $P < 0.001$, χ^2 -test). Increased enrichment of Vglut2-GABA gold pairs at less than 30 nm was obtained from three separate experiments using different antibodies and size gold (Fig. 3, F and H) (26). Vglut2 and the GABA vesicular transporter (VGAT), which are responsible for glutamatergic and GABAergic transmission, respectively (fig. S19), also displayed enrichment at less than 30 nm (fig. S20).

Activity of the LHb is elevated in animal models of human depression (12, 14, 33). Chronic serotonin-based antidepressant [e.g., citalopram (34)] treatment reduces LHb activity (35) and normalizes behaviors modeling human depression (36). We thus examined EP-LHb transmission as above in mice treated with citalopram or saline for 2 weeks. The ratio of GABA-A-R- to AMPA-R-mediated light-evoked synaptic responses was significantly higher in the citalopram- compared to

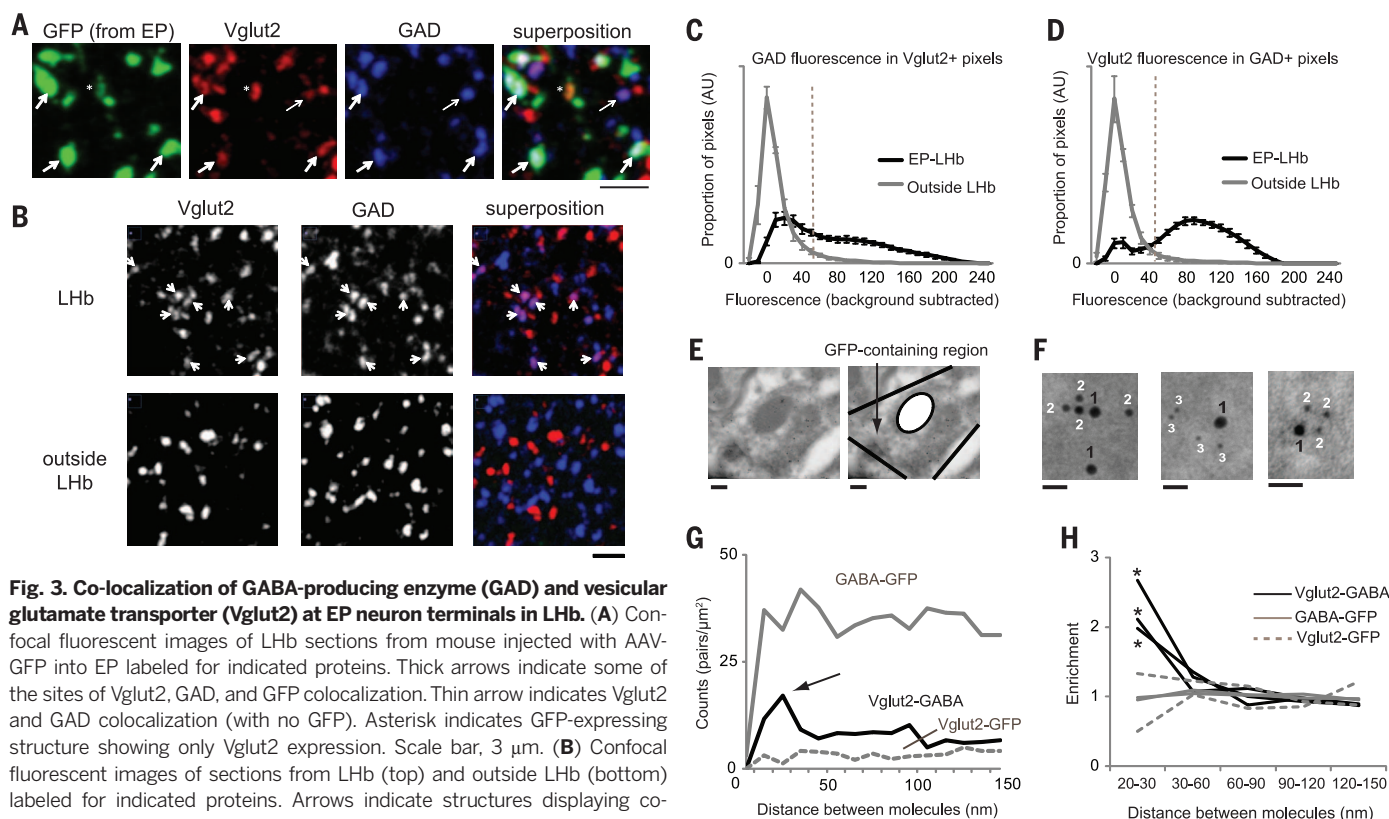


Fig. 3. Co-localization of GABA-producing enzyme (GAD) and vesicular glutamate transporter (Vglut2) at EP neuron terminals in LHb. (A) Confocal fluorescent images of LHb sections from mouse injected with AAV-GFP into EP labeled for indicated proteins. Thick arrows indicate some of the sites of Vglut2, GAD, and GFP colocalization. Thin arrow indicates Vglut2 and GAD colocalization (with no GFP). Asterisk indicates GFP-expressing structure showing only Vglut2 expression. Scale bar, 3 μ m. (B) Confocal fluorescent images of sections from LHb (top) and outside LHb (bottom) labeled for indicated proteins. Arrows indicate structures displaying colabeling for GAD and Vglut2. Scale bar, 3 μ m. (C) Distribution of GAD fluorescence in pixels with Vglut2 intensity above Vglut2 threshold [dashed line in (D); (26)], for images in LHb (for pixels displaying GFP, black line) and outside LHb (gray line). (D) Distribution of Vglut2 fluorescence in pixels with GAD intensity above GAD threshold (dashed line in C), as indicated. [(C) (D)] EP-LHb, $N = 21$ images, 3 mice; outside LHb, $N = 5$ images, 3 mice. (E) Electron micrograph showing a GFP-containing region in the LHb. Gold particles in the mitochondria were not analyzed. Scale bars, 200 nm. See figs. S17 and S18 for larger images of same region. (F) (Left) 18-nm gold labels Vglut2 (1); 12-nm gold labels GABA (2). (Middle) 18-nm gold labels Vglut2 (1); 6-nm gold labels GFP (3). (Right) 12-nm gold labels Vglut2 (1); 6-nm gold labels GABA (2). Scale bars, 30 nm. (G) Density of gold label pairs for distance intervals on x axis for a single experiment. Black line, Vglut2-GABA distance; gray line, GABA-GFP; dotted gray line, Vglut2-GFP. $N = 72$ Vglut2 (18 nm) labels, 486 GABA (12 nm) labels, 416 GFP (6 nm) labels. Arrow indicates peak for Vglut2-GABA counts at 20 to 30 nm. (H) Increased enrichment (26) of Vglut2-GABA (black lines; three experiments) but not GABA-GFP (gray lines; two experiments) or Vglut2-GFP (dotted gray; two experiments) labels at 15- to 30-nm separation. $N = 1624$, 2556, and 1558 total Vglut2, GABA, and GFP labels, respectively. *Values > 4 SD from the mean.

saline-treated group (Fig. 4, A and C, and fig. S21). This ratio was also reduced in cLH rats, a rat model of human depression (37), compared with control animals (Fig. 4, B and D, and fig. S21). No differences in paired-pulse ratio, a measure of presynaptic release probability, were observed (figs. S21 and S22). These changes were

matched immunohistochemically: In animals treated with citalopram, the ratio of GAD to Vglut2 was larger in EP-LHb boutons (Fig. 4E and fig. S21) due to increased GAD labeling (Fig. 4, G and I, and fig. S21) whereas in cLH animals, this ratio was reduced (Fig. 4F and fig. S21) because of decreased GAD labeling (Fig. 4, H and K, and fig. S21). Little

change was seen in Vglut2 labeling (Fig. 4, G, H, J, and L, and fig. S21). Consistent with these results, chronic antidepressant treatment normalized the GAD to Vglut2 ratio in cLH animals (fig. S23).

The LHb plays important roles in reward computations (21, 22), is activated during negatively

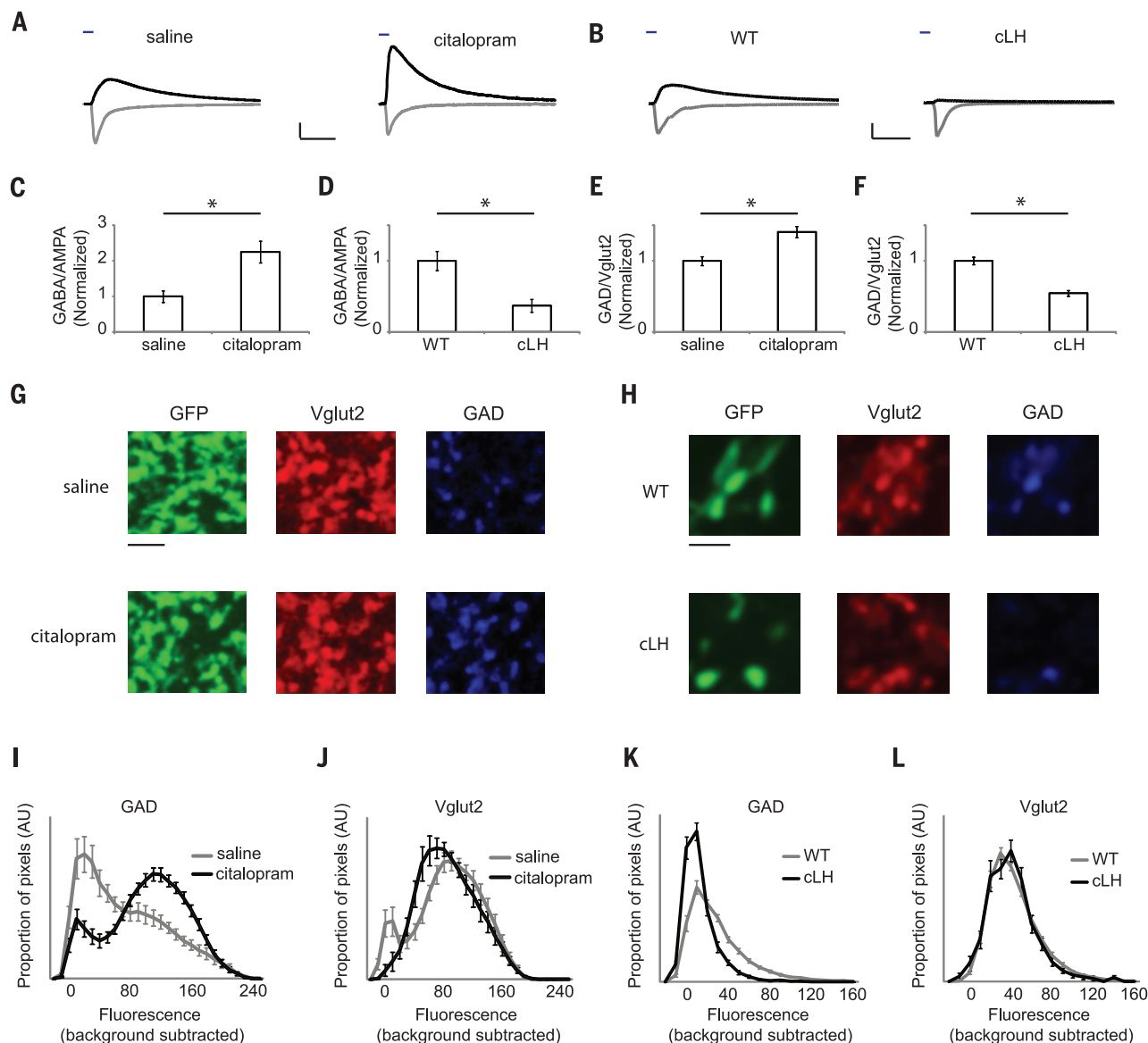


Fig. 4. Altered co-release of GABA in conditions related to depression.

(A) Sample whole-cell recordings of GABA-A-R- (black) and AMPA-R-mediated (gray) light-evoked EP-LHb responses from animals treated with saline or citalopram (10 mg per kg of weight, intraperitoneally). Scale bars, 200 pA, 20 ms. (B) Sample recordings, as in (A), in WT rats and cLH rats. Scale bars, 200 pA, 20 ms. (C) Bar graph of GABA-A-R-mediated/AMPA-R-mediated response at EP-LHb synapses for indicated groups normalized to average of saline ratios ($N = 19$ cells from four saline-treated mice and 24 cells from four citalopram-treated mice). (D) Bar graph of responses, as in (C), for WT and cLH rats normalized to average of WT ratios ($N = 41$ cells from nine WT rats and 23 cells from nine cLH rats). (E) Ratio of average background subtracted pixel intensities for GAD and Vglut2 in terminals originating from EP (26) for indicated groups ($N = 24$ images from three saline-treated mice, 21 images from three citalopram-treated mice). (F) Ratios, as in (E), for WT

and cLH rats ($N = 32$ images from seven WT rats, 21 images from three cLH rats). (G) Example confocal images from a saline and a citalopram-treated mouse. Scale bar, 3 μ m. (H) Example confocal images from a WT rat and a cLH rat. Scale bar, 3 μ m. (I) GAD fluorescence in pixels displaying Vglut2 and GFP labels in tissue from animals treated with citalopram or saline (percentage of Vglut2+, GFP+ pixels showing GAD fluorescence: saline, $54 \pm 5\%$, 21 images; citalopram, $79 \pm 2\%$, 24 images; $P < 10^{-4}$). (J) Vglut2 fluorescence in pixels displaying GAD and GFP labels in tissue from animals treated with citalopram or saline (percentage of GAD+, GFP+ pixels showing Vglut2 fluorescence: saline, $80 \pm 3\%$, 21 images; citalopram, $79 \pm 3\%$, 24 images; $P = 0.8$). (K) GAD fluorescence, as in (I), for images from WT and cLH rats (WT, $50 \pm 3\%$, 32 images; cLH, $19 \pm 3\%$, 21 images; $P < 10^{-7}$). (L) Vglut2 fluorescence, as in (J), for images from WT and cLH rats (WT, $61 \pm 3\%$, 32 images; cLH, $61 \pm 3\%$, 21 images; $P = 0.9$). * $P < 0.002$.

valenced events (21) via its basal ganglia inputs (22), and its hyperexcitation may contribute to human depression (16, 38). We found that co-release of GABA and glutamate occurs in the LHB and that the opponent actions of these neurotransmitters control the level of LHB activity by basal ganglia inputs. Our studies identify an unusual form of inhibition controlling the activity of this key brain region.

There is physiological evidence for co-release of GABA and glutamate (3) in the auditory system (5) and CA3 region of the hippocampus (4, 6–8), where it may contribute to refinement of neuronal connectivity during development (6, 39) and be induced by seizures in the adult hippocampus (11); GABA and glutamate co-release has not been universally accepted (10). Here, we show using a combination of electrophysiological, optogenetic, mouse genetic, and fluorescent and EM immunohistochemical techniques that in the adult rodent brain a substantial fraction of EP inputs to the LHB simultaneously release both glutamate and GABA and that these neurotransmitters have opposite effects on postsynaptic spike output. Co-release of glutamate and GABA can permit very local control, at the level of individual presynaptic terminals, in the balance of excitation and inhibition, a key mechanism controlling neuronal excitability (1).

The LHB receives long-range excitatory and inhibitory inputs from several sources (40–42). It remains to be determined whether other inputs to the LHB co-release glutamate and GABA and the extent to which co-release of glutamate and GABA is used to regulate excitability in the rest of the nervous system (3). Co-expression of Vglut2 and GAD mRNA has been found in EP neurons that project to the motor thalamus, suggesting that co-release of glutamate and GABA may also occur in this pathway (43).

Given the proposed role of increased neuronal activity in the LHB in mood disorders (16), we examined the ratio of GABA to glutamate released at the EP-LHB synapse in conditions related to depression. In animals chronically treated with citalopram, a drug used to treat depression, evidence indicated enhanced GABA at EP-LHB synapses. In line with these data, EP-LHB synapses in cLH rats, which model aspects of human depression, showed evidence of reduced GABA, although additional changes (e.g., in receptor number) cannot be ruled out. Our results identify co-release of GABA and glutamate as a regulator of LHB output and potential determinant of the impact of negatively valenced events on mood and behavior.

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ACKNOWLEDGMENTS

We thank H. Monyer and O. Kiehn for transgenic mice, R. Edwards for Vglut2 antibodies and DNA, K. Deisseroth for Chr2 DNA, J. Lin and R. Tsien for oChIEF DNA, T. Meerloo for electron microscopy assistance, and V. Joseph for histology assistance. We thank N. Spitzer and J. Isaacson and the Malinow laboratory for helpful suggestions. This work was supported by an NIH grant to R.M. awarded to the University of California, San Diego (UCSD) Neuroscience Core (NS047101). C.D.P. was supported by a postdoctoral award by the Instituts de Recherche en Santé du Canada. All primary electrophysiological and immunohistochemical data are archived in the Center for Neural Circuits and Behavior at UCSD.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1494/suppl/DC1
Materials and Methods
Figs. S1 to S23
References (44, 45)

6 January 2014; accepted 5 August 2014
10.1126/science.1250469

CLIMATE CHANGE

Six centuries of variability and extremes in a coupled marine-terrestrial ecosystem

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Reported trends in the mean and variability of coastal upwelling in eastern boundary currents have raised concerns about the future of these highly productive and biodiverse marine ecosystems. However, the instrumental records on which these estimates are based are insufficiently long to determine whether such trends exceed preindustrial limits. In the California Current, a 576-year reconstruction of climate variables associated with winter upwelling indicates that variability increased over the latter 20th century to levels equaled only twice during the past 600 years. This modern trend in variance may be unique, because it appears to be driven by an unprecedented succession of extreme, downwelling-favorable, winter climate conditions that profoundly reduce productivity for marine predators of commercial and conservation interest.

In coastal upwelling zones, alongshore equatorward winds lift deep, cold, nutrient-rich waters into sunlit surface layers, fueling vast phytoplankton blooms that ultimately support large resident and migratory populations of fish, seabirds, and marine mammals (1, 2). These systems are characterized by considerable interannual to multidecadal variability, including,

in some locations, long-term linear trends in upwelling intensity (3). However, observational records rarely span more than 70 years, hindering the ability to identify underlying forcing mechanisms or to assess whether these patterns are exceptional over multicentennial time scales.

In the California Current (CC) upwelling zone along the west coast of North America, ecosystem

function may be critically dependent on cool (winter)-season marine climate (4). Wintertime upwelling intensity, the variability of which is a function of winter sea-level pressure (SLP) over the northeast Pacific [i.e., the North Pacific High (NPH)], synchronizes independent upper-trophic indicators, including rockfish (*Sebastes* spp.) growth and seabird reproductive success (5–7). The same atmospheric conditions that control winter upwelling and associated marine productivity also control onshore precipitation and tree growth by facilitating or blocking storm access to western North America (8). We capitalized on the tight coupling between the oceanic and terrestrial systems to generate a tree-ring reconstruction of CC winter marine climate spanning almost 600 years.

Three physical variables were used to characterize CC winter climate: (i) the Northern Oscillation Index [NOI, an index of the NPH (9)], (ii) the Bakun Upwelling Index (10), and (iii) sea level at San Francisco (SFO). The Upwelling Index and sea level provide a regional CC perspective to complement the broad-scale NOI. All three variables are highly correlated. The offshore high-pressure systems drive northerly upwelling-favorable winds that depress coastal sea levels (Fig. 1, fig. S1, and table S1). The first principal component of the three winter (January to March) instrumental variables (Upwelling Index averaged across 36° and 39°N, SFO sea level, and the NOI) captures 85% of the variability and serves as a multivariate index of marine climate (the California Current Winter Index; CCWI), the target of our reconstruction. The CCWI is negatively correlated with precipitation from central to southern California (Fig. 1 and table S1). Thus, California drought is associated with a strong NPH (positive NOI), robust upwelling, and low sea level (fig. S1).

Biological time series in the CC and adjacent terrestrial ecosystems closely track the CCWI. Among tree-ring chronologies in western North America, significant ($P < 0.01$) correlations with the CCWI are concentrated in California and are exclusively negative (Fig. 1). Because of their length and strong covariation with winter precipitation (8, 11), we used blue oak (*Quercus douglasii*) tree-ring chronologies to develop our reconstruction. Chronologies that contained at

least three trees >400 years old, were sampled after 2002, and correlate to the CCWI at the $P < 0.001$ level were used in the analyses ($n = 16$). In the marine ecosystem, we focused on four biological time series because of their length (>35 years), spatial overlap, and understood responses to winter climate (5, 6). The records include the otolith growth-increment chronology spanning 1948–2006 for splitnose rockfish (*Sebastes diploproa*; valid for 36° to 39°N), mean egg-laying dates for the sea-

birds Cassin's auklet (*Ptychoramphus aleuticus*) and common murre (*Uria aalge*), and the breeding success of the common murre (seabird records span 1972–2006).

The first principal component of the four marine biological time series ($PC_{1\text{bio}}$; 1972–2003) and the average of the 16 blue oak chronologies capture 67% of the total variance among these indicators. PC loadings highlight inverse productivity between the marine and terrestrial ecosystems;

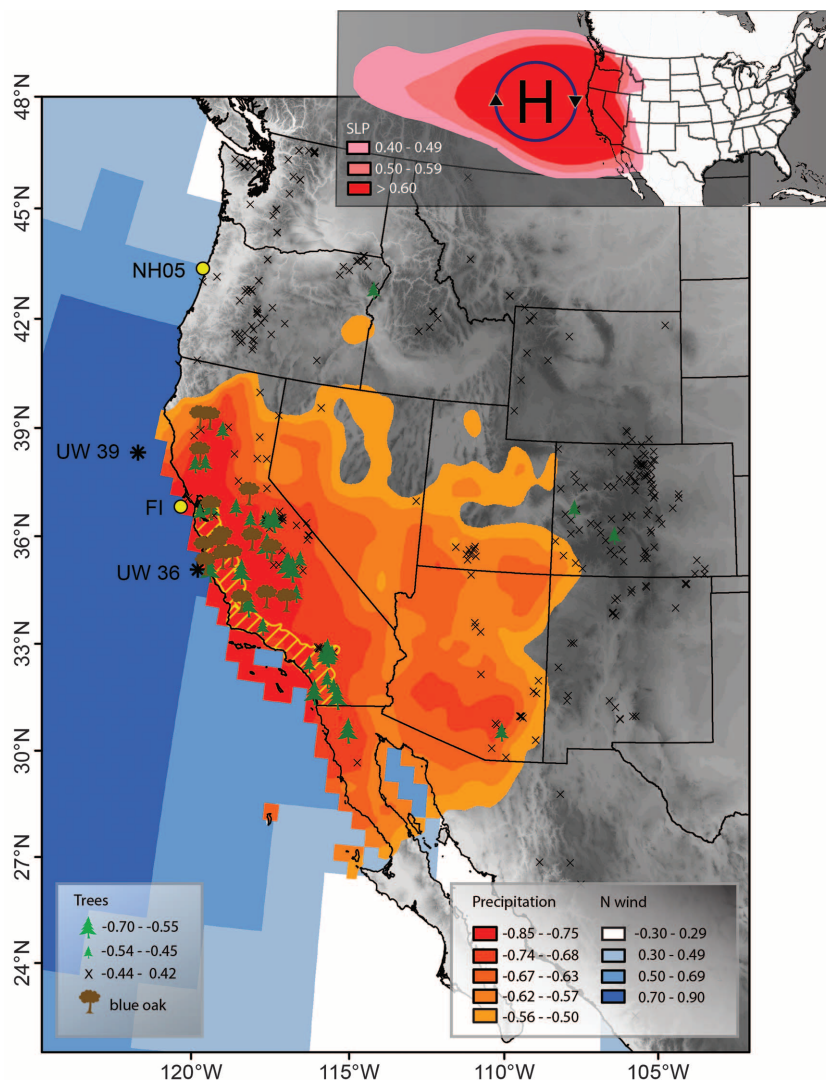


Fig. 1. Climate-driven linkages between marine and terrestrial ecosystems, as captured by the CCWI. (Inset, top right) Correlations (r) between winter SLP and the CCWI in the eastern North Pacific. High pressure (the NPH) blocks the onshore flow of winter storms, decreasing precipitation in the western United States. The clockwise rotation of these systems is associated with northwesterly upwelling-favorable winds. The main figure shows correlations between the CCWI and (i) gridded National Centers for Environmental Prediction/National Center for Atmospheric Research 1948–2010 winter meridional winds, where positive correlations indicate flow from the north (blue scaling); (ii) gridded Climatic Research Unit Time Series 3.1 winter precipitation on land (orange scaling); and (iii) tree-ring chronologies in western North America (green symbols). Also shown are the locations of the blue oak chronologies used in the CCWI reconstruction; upwelling stations at 36° and 39°N latitude (UW39 and UW36); the location of the Farallon Islands (FI) 48 km offshore from San Francisco, California (seabird data); and the locations of the Newport Hydrographic line station 5 (NH05; copepod data). The yellow hatched region represents NOAA NCDC Climate Divisions CA 04 and CA 06, from which precipitation data were obtained for the period 1895–2008 (fig. S1). All correlations in this figure involve an overlap of at least 40 years, but not more than 60 years.

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vigorous fish growth, early seabird egg laying, and high seabird breeding success are all coincident with reduced tree growth (table S1). A large fraction of the interannual variability in $PC1_{bio}$ is explained by the CCWI [coefficient of determination (R^2) = 0.72; Fig. 2 and Table 1]. The stability of the relationship between these climate and biological variables is verified by the early interval of the rockfish growth chronology (R^2 = 0.56 with the CCWI for the 1948–1971 period; Fig. 2, B and C). The CCWI is also closely related to the leading principal component of a broader group (n = 9; 1982–2006) of CC seabird and fish time series (fig. S2) (7), as well as a regional zooplankton index for which positive anomalies indicate abundant cold-water, lipid-rich copepod species associated with high marine productivity (Fig. 2 and Table 1) (12). Such pervasive linkages across the ecosystem further substantiate the biological relevance of this wintertime climate indicator.

Given the tight relationships between marine and terrestrial ecosystems, we used the 16 blue oak chronologies to develop an ensemble-based reconstruction of winter upwelling climate back to 1428 CE (Fig. 3C; median R^2 = 0.58; Fig. 3A and table S4). The stability of this new reconstruction is verified by a median R^2 of 0.51 (P < 0.0001) between independent sea-level instrumental data (1898–1947) and each ensemble member (Fig. 3C and table S4). The reconstruction does not display long-term trends in the mean and is dominated by higher-frequency variability (<30 years; fig. S3), despite employing techniques capable of preserving interannual to centennial fluctuations in tree-ring data. In contrast, long-term trends are evident in variance, which increased sharply from approximately 1950 CE, (Fig. 3, B and D), which is consistent with observations of rising variability over recent decades in a number of CC biological time series (13). However, the multicentennial perspective afforded by winter climate reconstruction demonstrates that both the maximum variance and the rate of late-20th- and early-21st-century variance increase are high, but not unprecedented in the context of the past 600 years (Fig. 3D). Comparable levels in variance occurred during the mid-20th century and the latter 19th century, two peaks in an oscillating pattern that dates back to at least the mid-1600s. Additional instrumental records suggest that the late-20th-century rise in variance was particularly pronounced for precipitation (fig. S6) (14). The CCWI and associated winter variables correlate only weakly with the Pacific Decadal Oscillation (PDO), an important multidecadal mode of variability in the northeast Pacific (15) (table S1 and fig. S7). Establishing correspondence between the PDO and periods of high and low variance in the CCWI is hindered by the low number of PDO regime shifts in the instrumental record and the modest level of agreement among PDO reconstructions (fig. S7) (16).

Although our reconstruction indicates that modern winter climate variability is within historical bounds, the frequency of negative anomalies

per century, as calculated for each ensemble member, is between three and five times greater after 1950 than before 1950 (Fig. 3F and table S5). This is of particular ecological significance, given that the relationship between the CCWI and $PC1_{bio}$

is nonlinear, so that negative CCWI values exert disproportionately severe impacts on marine biological functioning (Fig. 2A and fig. S2). Consequently, although rising wintertime variability over recent decades may not be unprecedented, it has

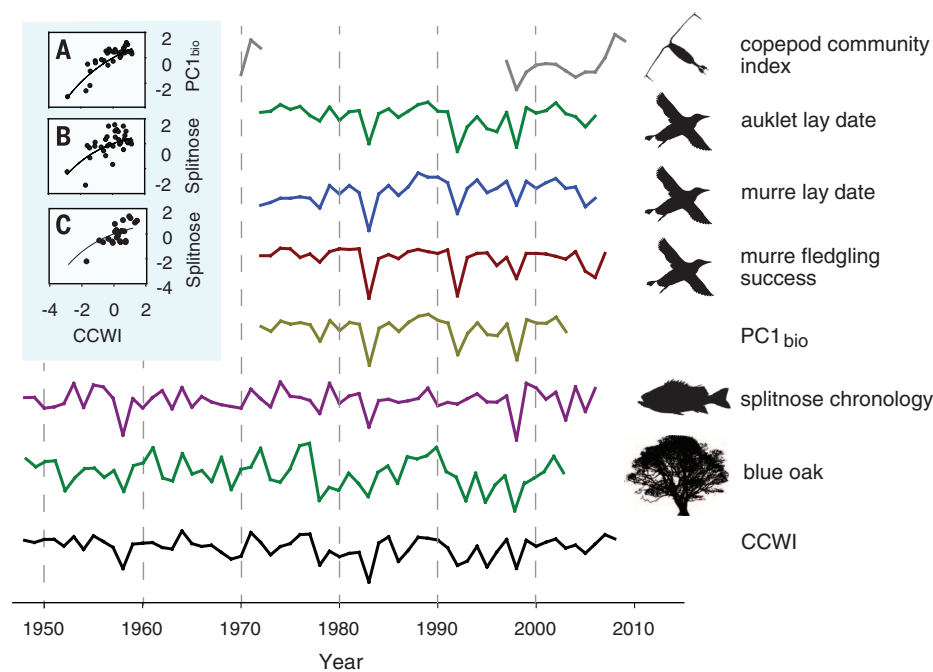


Fig. 2. The CCWI and its coherence with biological time series. Data sets shown are the winter copepod index, murre laying date, auklet laying date, murre fledgling success, a splitnose rockfish otolith chronology, and the mean of the 16 blue oak tree-ring chronologies used in the CCWI reconstruction. The leading principal component ($PC1_{bio}$) for all marine biological time series except copepods is also included. All time series are normalized, and the lay date, copepod, and blue oak time series are inverted to illustrate synchrony. (Inset) (A) Bivariate plot of CCWI and $PC1_{bio}$ (1973–2006; regression with an exponential rise to maximum function; R^2 = 0.72; P < 0.001). (B) Bivariate plot of CCWI and the latter portion of the splitnose chronology (1972–2006; regression with an exponential rise to maximum function; R^2 = 0.38; P < 0.001). (C) Bivariate plot of CCWI and the early portion of the splitnose chronology (1948–1971; regression with exponential rise to maximum function developed for late portion of the chronology; R^2 = 0.55; P < 0.001).

Table 1. Correlations among biological time series and the CCWI. Pearson correlation coefficients (r) and nominal P values (in italics; not corrected for autocorrelation) among marine biological time series, the CCWI, the mean of the 16 blue oak chronologies used in the CCWI reconstruction, and the CCWI tree-ring reconstruction.

Biological variable	CCWI*	CCWI reconstruction†		Blue oak‡		Time span§
Murre lay date	−0.66 <0.001	−0.42	0.018	0.37	0.039	1972–2006
Auklet lay date	−0.78 <0.001	−0.64	<0.001	0.58	<0.001	1972–2006
Murre success	0.57 <0.001	0.38	0.035	−0.34	0.059	1972–2006
Splitnose chronology	0.62 <0.001	0.41	0.001	−0.32	0.015	1948–2006
Winter copepod	−0.78 <0.001	−0.74	0.015	0.74	0.014	1970–1972; 1996–2010
$PC1_{bio}$	0.84 <0.001	0.67	N/A¶	−0.61	N/A¶	1972–2003

*The CCWI is the leading principal component of the winter Northern Oscillation Index, winter sea level at SFO, and winter upwelling index in the central CC. †Reconstruction of the CCWI from tree-ring data (ensemble median). ‡Mean of 16 blue oak chronologies used in the CCWI reconstruction. §Years spanned by the time series. ||The leading principal component of murre laying date, auklet laying date, murre fledgling success, the splitnose rockfish otolith growth-increment chronology, and the mean of 16 blue oak chronologies. ¶Level of significance is not applicable; the blue oak mean is used in calculating $PC1_{bio}$ and the reconstructed CCWI.¶

been driven by a succession of winters with anomalously low SLP over the northeast Pacific, each of which profoundly reduced fish growth and seabird productivity (Figs. 2A and 3F). The frequency of positive, upwelling-favorable, winter climate anomalies does not differ before and after 1950 (table S6), underscoring the role of negative (downwelling-favorable) anomalies in driving the rise of late-20th-century variability.

Winter climate extremes along the western North American coast are teleconnected to the El Niño–Southern Oscillation (ENSO). Forty-two percent of the variance in the CCWI is explained by the mean Jan–Mar Multivariate ENSO Index, and four of the most negative reconstructed CCWI values (1998, 1983, 1941, and 1868) correspond to major El Niño events (17) (Fig. 3). ENSO reconstructions indicate that the 20th century was highly variable in the context of the past centuries (18–20) or even millennia (21), which could explain, at least in part, rising variance in the CCWI. Linkages between anthropogenic climate change and

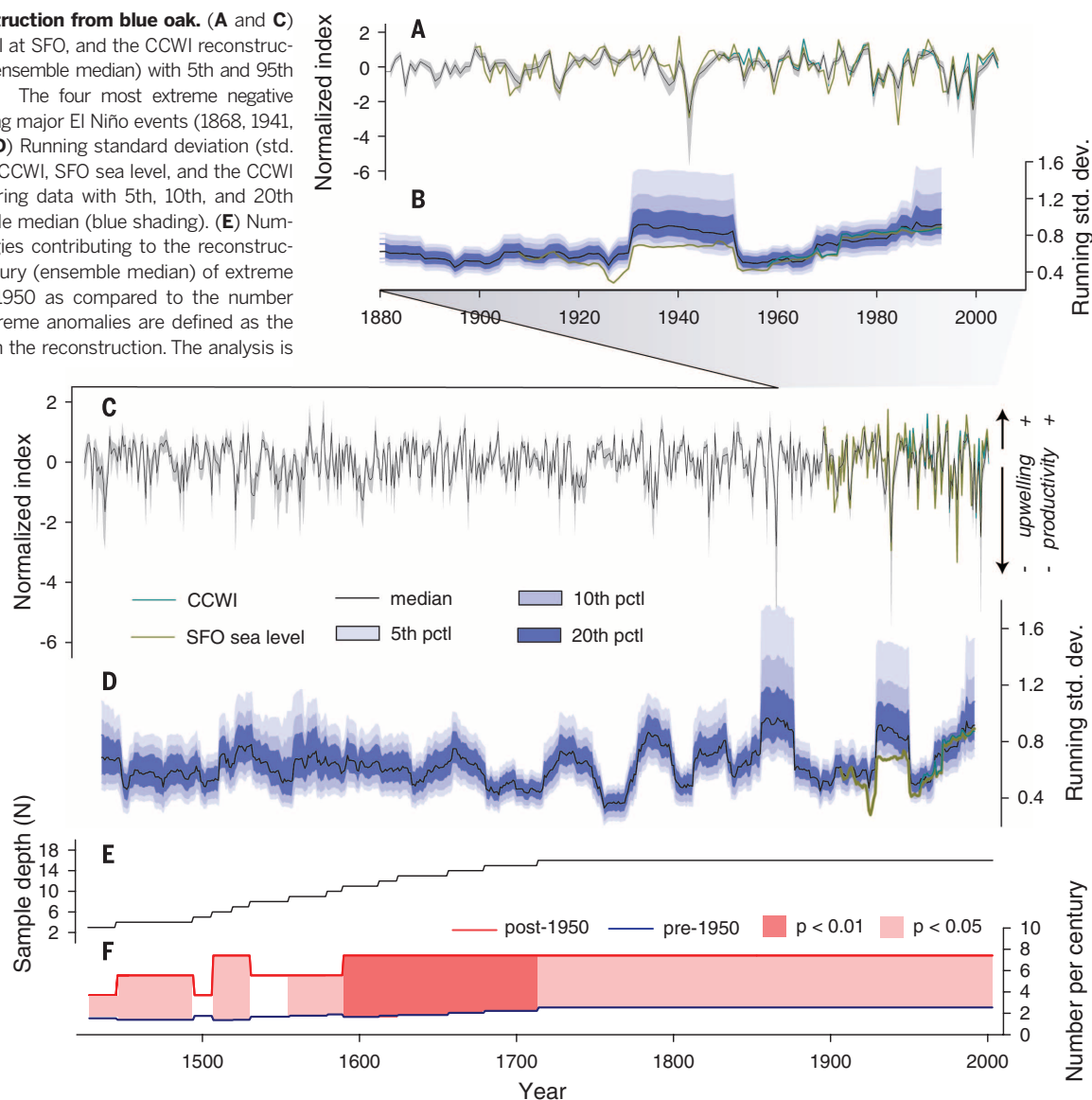
rising ENSO variability remain unclear, and models of future ENSO behavior have not yet reached consensus (22). Thus, CC winter climate trends cannot be easily attributed or forecast. However, the importance of these patterns is clear in the CC and adjacent forests, with implications for biological processes beyond those examined here. For example, rising variance in average cool-season precipitation probably hastened the recent extinction of two checkerspot butterfly (*Euphydryas editha bayensis*) populations in the San Francisco Bay Area (23). Moreover, blocking high pressure (strong NPH) and associated drought, snowpack deficits, low river flows, and elevated wildfire risks in early 2013 and now 2014, illustrate the importance of winter climate to agriculture, ecosystems, water security, and even hydroelectric power generation in central and southern California, a region where freshwater resources are currently overallocated, so that any reductions in rainfall cause conflict over this valuable resource. Although the temporal resolution of this tree-ring

reconstruction is well suited to identifying these high-frequency patterns, other proxies with greater temporal depth, such as sedimentary records, demonstrate that western North American coastal precipitation, and possibly winter upwelling, was highly dynamic over the course of the Holocene (24, 25). Extremely low-frequency, multicentennial fluctuations at time scales exceeding those considered here may an important component of the winter climate system.

Owing to strong linkages with terrestrial productivity, we were able to generate an annually resolved multicentennial reconstruction of CC wintertime climate that reflects marine biological functioning in a coastal ecosystem. Such a highly targeted tree-ring approach may be possible in other coupled systems, especially eastern boundary upwelling zones, assuming that regional climate drivers of productivity have been identified and are linked to atmospheric processes that influence forest growth. Depending on the seasonality of biological response, trees may

Fig. 3. The CCWI reconstruction from blue oak. (A and C)

The CCWI, winter sea level at SFO, and the CCWI reconstruction from tree-ring data (ensemble median) with 5th and 95th percentiles (gray shading). The four most extreme negative estimates all occurred during major El Niño events (1868, 1941, 1983, and 1998). (B and D) Running standard deviation (std. dev.) (21-year window) of CCWI, SFO sea level, and the CCWI reconstruction from tree-ring data with 5th, 10th, and 20th percentiles of the ensemble median (blue shading). (E) Number of blue oak chronologies contributing to the reconstruction. (F) Number per century (ensemble median) of extreme negative anomalies post-1950 as compared to the number per century pre-1950. Extreme anomalies are defined as the 10 most negative values in the reconstruction. The analysis is repeated at each change in reconstruction sample depth to illustrate that pre- and post-1950 differences persist, even where the number of contributing chronologies is low and the time period constituting “pre-1950” is longest (i.e., 1428–1950 at a sample depth of 3 versus 1714–1950 at a sample depth of 16). Pink shading indicates a significant difference pre- and post-1950. See table S5 for values.



also capture signals of warm-season upwelling, which in the CC is uncorrelated to winter upwelling and dominated by decadal-scale variability (3, 5, 26). Overall, multivariable indices such as those we developed and describe here highlight broad physical-ecological connections and may provide new options for monitoring and “hindcasting” ecosystem states. In this example, covariance among fish, seabirds, and trees demonstrates a remarkable degree of connectivity across the coastal interface that not only provides context for interpreting variability in observational records but may also be relevant to management. Identifying biologically important indicators, their current status, and their ranges of historical variability is central to the desired transition from single-species fisheries stock assessments to the next generation of integrative ecosystem-based strategies (27).

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ACKNOWLEDGMENTS

Funding was provided by NSF (grant no. 1130125), the NOAA Fisheries and the Environment (FATE) program, the California Department of Fish and Wildlife Ecosystem Restoration Program (grant no. ERP02-P30), and the Nippon Foundation-University of British Columbia Nereus Program. D.G. was supported by a NOAA Climate and Global Change Postdoctoral Fellowship. Seabird data were obtained from Point Blue Conservation Science/Farallon National Wildlife Refuge. We are grateful to contributors to the International Tree-Ring Databank and thank M. O’Connor for assistance with Fig. 1. Finally, we thank the reviewers for their comments and insights, which greatly improved the paper. All data used in this work, or the sources from which they are available, are included in the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1498/suppl/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 to S6
References (28–30)

11 March 2014; accepted 7 August 2014
10.1126/science.1253209

SOCIAL SCIENCE

Publication bias in the social sciences: Unlocking the file drawer

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We studied publication bias in the social sciences by analyzing a known population of conducted studies—221 in total—in which there is a full accounting of what is published and unpublished. We leveraged Time-sharing Experiments in the Social Sciences (TESS), a National Science Foundation–sponsored program in which researchers propose survey-based experiments to be run on representative samples of American adults. Because TESS proposals undergo rigorous peer review, the studies in the sample all exceed a substantial quality threshold. Strong results are 40 percentage points more likely to be published than are null results and 60 percentage points more likely to be written up. We provide direct evidence of publication bias and identify the stage of research production at which publication bias occurs: Authors do not write up and submit null findings.

Publication bias occurs when “publication of study results is based on the direction or significance of the findings” (1). One pernicious form of publication bias is the greater likelihood of statistically significant results being published than statistically insignificant results, holding fixed research quality. Selective reporting of scientific findings is often referred to as the “file drawer” problem (2). Such a selection process increases the likelihood that published results reflect type I errors rather than true population parameters, biasing effect sizes upwards. Further, it constrains efforts to assess

the state of knowledge in a field or on a particular topic because null results are largely unobservable to the scholarly community.

Publication bias has been documented in various disciplines within the biomedical (3–9) and social sciences (10–17). One common method of detecting publication bias is to replicate a meta-analysis with and without unpublished literature (18). This approach is limited because much of what is unpublished is unobserved. Other methods solely examine the published literature and rely on assumptions about the distribution of unpublished research by, for example, comparing the precision and magnitude of effect sizes among a group of studies. In the presence of publication bias, smaller studies report larger effects in order to exceed arbitrary statistical significance

thresholds (19, 20). However, these visualization-based approaches are sensitive to using different measures of precision (21, 22) and also assume that outcome variables and effect sizes are comparable across studies (23). Last, methods that compare published studies to “gray” literatures (such as dissertations, working papers, conference papers, or human subjects registries) may confound strength of results with research quality (7). These techniques are also unable to determine whether publication bias occurs at the editorial stage or during the writing stage. Editors and reviewers may prefer statistically significant results and reject sound studies that fail to reject the null hypothesis. Anticipating this, authors may not write up and submit papers that have null findings. Or, authors may have their own preferences to not pursue the publication of null results.

A different approach involves examining the publication outcomes of a cohort of studies, either prospectively or retrospectively (24, 25). Analyses of clinical registries and abstracts submitted to medical conferences consistently find little to no editorial bias against studies with null findings (26–31). Instead, failure to publish appears to be most strongly related to authors’ perceptions that negative or null results are uninteresting and not worthy of further analysis or publication (32–35). One analysis of all institutional review board–approved studies at a single university over 2 years found that a majority of conducted research was never submitted for publication or peer review (36).

Surprisingly, similar cohort analyses are much rarer in the social sciences. There are two main reasons for this lacuna. First, there is no process in the social sciences of preregistering studies

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comparable with the clinical trials registry in the biomedical sciences. Second, even if some unpublished studies could be identified, there are likely to be substantial quality differences between published and unpublished studies that make them difficult to compare. As noted, previous research attempted to identify unpublished results by examining conference papers and dissertations (37) and human subjects registries of single institutions (36). However, such techniques may produce unrepresentative samples of unpublished research, and the strength of the results may be confounded with research quality. Conference papers, for example, do not undergo a similar process of peer review as do journal articles in the social sciences and therefore cannot be used as a comparison set. This work is distinctive in the study of publication bias in the social sciences in that we analyzed a known population of conducted studies, and all studies in the population exceed a substantial quality threshold.

We leveraged TESS (Time-sharing Experiments in the Social Sciences), a National Science Foundation-sponsored program established in 2002 in which researchers propose survey-based experiments to be run on nationally representative samples. These experiments typically embed some randomized manipulation (such as visual stimulus or question wording difference) within a survey questionnaire. Researchers apply to TESS, which then submits the proposals to peer review and distributes grants on a competitive

basis (38). Our basic approach is to compare the statistical results of TESS experiments that eventually got published with the results of those that remain unpublished.

This analytic strategy has many advantages. First, we have a known population of conducted studies and therefore have a full accounting of what is published and unpublished. Second, TESS proposals undergo rigorous peer review, meaning that even unpublished studies exceed a substantial quality threshold before they are conducted. Third, nearly all of the survey experiments were conducted by the same high-quality survey research firm (Knowledge Networks, now known as GfK Custom Research), which assembles probability samples of Internet panelists by recruiting participants via random digit dialing and address-based sampling. Thus, there is remarkable similarity across studies with respect to how they were administered, allowing for comparability. Fourth, TESS requires that studies have requisite statistical power, meaning that the failure to obtain statistically significant results is not simply due to insufficient sample size.

One potential concern is that TESS studies may be unrepresentative of social science research, especially scholarship based on nonexperimental data. Although TESS studies are clearly not a random sample of the research conducted in the social sciences, it is unlikely that publication bias is less severe than what is reported here. The baseline probability of publishing experimental findings based on representative sam-

ples is likely higher than that of observational studies using “off-the-shelf” data sets or experiments conducted on convenience samples in which there is lower “sunk cost” involved in obtaining the data. Because the TESS data were collected at considerable expense—in terms of time to obtain the grant—authors should, if anything, be more motivated to attempt to publish null results.

The initial sample consisted of the entire online archive of TESS studies as of 1 January 2014 (39). We analyzed studies conducted between 2002 and 2012. We did not track studies conducted in 2013 because there had not been enough time for the authors to analyze the data and proceed through the publication process. The 249 studies represent a wide range of social science disciplines (Table 1). Our analysis was restricted to 221 studies, 89% of the initial sample. We excluded seven studies published in book chapters and 21 studies for which we were unable to determine the publication status and/or the strength of experimental findings (40). The full sample of studies is presented in Table 2; the bolded entries represent the analyzed subsample of studies.

The outcome of interest is the publication status of each TESS experiment. We took numerous approaches to determine whether the results from each TESS experiment appeared in a peer-reviewed journal, book, or book chapter. We first conducted a thorough online search for published and unpublished manuscripts and read every manuscript in order to verify that it relied on data collected through TESS and that it reported experimental results (40). We then e-mailed the authors of more than 100 studies for which we were unable to find any trace of the study and asked what happened to their studies. We also asked authors who did not provide a publication or working paper to summarize the results of their experiments.

The outcome variable distinguishes between two types of unpublished experiments: those prepared for submission to a conference or journal, and those never written up in the first place. It is also possible that papers with null results may be excluded from the very top journals but still find their way into the published literature. Thus, we disaggregated published experiments according to their placement in top-tier or non-top-tier journals (40) (a list of journal classifications is provided in table S1). The results from the majority of TESS studies in our analysis sample have been written up (80%), whereas less than half (48%) have been published in academic journals.

We also ascertained whether the results of each experiment are described as statistically significant by their authors. We did not analyze the data ourselves to determine whether the findings were statistically significant for two main reasons. First, it is often very difficult to discern the exact analyses the researchers intended. The proposals that authors submit to TESS are not a matter of public record, and many experiments have complex experimental designs with

Table 1. Distribution of studies across years and disciplines. Field coded based on the affiliation of the first author. “Other” category includes Business, Computer Science, Criminology, Education, Environmental Studies, Journalism, Law, and Survey Methodology.

Year	Communication	Economics	Political science	Public health	Psychology	Sociology	Other	Total
2002	0	0	1	0	0	0	0	1
2003	0	1	4	0	6	2	1	14
2004	0	2	9	1	5	0	0	17
2005	2	2	13	0	10	7	1	35
2006	3	1	12	1	9	6	0	32
2007	0	0	5	0	3	2	0	10
2008	2	0	11	1	4	2	1	21
2009	0	0	12	1	8	2	3	26
2010	3	3	22	0	5	6	2	41
2011	2	0	19	1	9	6	2	39
2012	1	1	5	1	1	3	1	13
Total	13	10	113	6	60	36	11	249

Table 2. Cross-tabulation between statistical results of TESS studies and their publication status. Entries are counts of studies by publication status and results. Bolded entries indicate observations included in the final sample for analysis (40). Results are robust to the inclusion of book chapters (table S7).

	Unpublished, not written	Unpublished, written	Published	Book chapter	Missing	Total
Null results	31	7	10	1	0	49
Mixed results	10	32	40	3	1	86
Strong results	4	31	56	1	1	93
Missing	6	1	0	2	12	21
Total	51	71	106	7	14	249

numerous treatment conditions, outcome variables, and moderators. Second, what is most important is whether the authors themselves consider their results to be statistically significant because this influences how they present their results to editors and reviewers, as well as whether they decide to write a paper. Studies were classified into three categories of results: strong (all or most of hypotheses were supported by the statistical tests), null (all or most hypotheses were not supported), and mixed (remainder of studies) (40). Approximately 41% of the studies in our analysis sample reported strong evidence in favor of the stated hypotheses, 37% reported mixed results, and 22% reported null results.

There is a strong relationship between the results of a study and whether it was published, a pattern indicative of publication bias. The main findings are presented in Table 3, which is a cross-tabulation of publication status against strength of results. A Pearson χ^2 test of independence is easily rejected [$\chi^2(6) = 80.3, P < 0.001$], implying that there are clear differences in the statistical results between published and unpublished studies. Although around half of the total studies in our sample were published, only 20% of those with null results appeared in print. In contrast, ~60% of studies with strong results and 50% of those with mixed results were published. Although more than 20% of the studies in our sample had null findings, <10% of published articles based on TESS experiments report such results. Although the direction of these results may not be surprising, the observed magnitude (an ~40 percentage point increase in the probability of publication from moving from null to strong results) is remarkably large.

However, what is perhaps most striking in Table 3 is not that so few null results are published, but that so many of them are never even written up (65%). The failure to write up null results is problematic for two reasons. First, researchers might be wasting effort and resources in conducting studies that have already been executed in which the treatments were not efficacious. Second, and more troubling, if future researchers conduct similar studies and obtain statistically significant results by chance, then the published literature on the topic will erroneously suggest stronger effects. Hence, even if null results are characterized by treatments that “did not work” and strong results are characterized by efficacious treatments, authors’ failures to write up null findings still adversely affects the universe of knowledge. Once we

condition on studies that were written up, there is no statistically significant relationship between strength of results and publication status (table S2).

A series of additional analyses demonstrate the robustness of our results. Estimates from multinomial probit regression models show that studies with null findings are statistically significantly less likely to be written up even after controlling for researcher quality (using the highest-quality researcher’s cumulative *h*-index and number of publications at the time the study was run), discipline of the lead author, and the date the study was conducted (supplementary text and table S3). Further, the relationship between strength of results and publication status does not vary across levels of these covariates (supplementary text and tables S4 and S5). Another potential concern is that our coding of the statistical strength of results is based on author self-reports, introducing the possibility of measurement error and misclassification. A sensitivity analysis shows that our findings are robust to even dramatic and unrealistic rates of misclassification (supplementary text and fig. S1).

Why do some researchers choose not to write up null results? To provide some initial explanations, we classified 26 detailed e-mail responses we received from researchers whose studies yielded null results and did not write a paper (table S6). Fifteen of these authors reported that they abandoned the project because they believed that null results have no publication potential even if they found the results interesting personally (for example, “I think this is an interesting null finding, but given the discipline’s strong preference for $P < 0.05$, I haven’t moved forward with it”). Nine of these authors reacted to null findings by reducing the priority of writing up the TESS study and focusing on other projects (for example, “There was no paper unfortunately. There still may be in future. The findings were pretty inconclusive”). Two authors whose studies “didn’t work out” eventually published papers supporting their initial hypotheses using findings obtained from smaller convenience samples.

How can the social science community combat publication bias of this sort? On the basis of communications with the authors of many experiments that resulted in null findings, we found that some researchers anticipate the rejection of such papers but also that many of them simply lose interest in “unsuccessful” projects. These findings show that a vital part of developing institutional solutions to improve scientific transparency

would be to understand better the motivations of researchers who choose to pursue projects as a function of results.

Few null findings ever make it to the review process. Hence, proposed solutions such as two-stage review (the first stage for the design and the second for the results), pre-analysis plans (41), and requirements to preregister studies (16) should be complemented by incentives not to bury statistically insignificant results in file drawers. Creating high-status publication outlets for these studies could provide such incentives. The movement toward open-access journals may provide space for such articles. Further, the pre-analysis plans and registries themselves will increase researcher access to null results. Alternatively, funding agencies could impose costs on investigators who do not write up the results of funded studies. Last, resources should be deployed for replications of published studies if they are unrepresentative of conducted studies and more likely to report large effects.

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Table 3. Cross-tabulation between statistical results of TESS studies and their publication status (column percentages reported). Pearson χ^2 test of independence: $\chi^2(6) = 80.3, P < 0.001$.

	Null (%)	Mixed (%)	Strong (%)
Not written	64.6	12.2	4.4
Written but not published	14.6	39.0	34.1
Published (non-top-tier)	10.4	37.8	38.4
Published (top-tier)	10.4	11.0	23.1
Total	100.0	100.0	100.0

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ACKNOWLEDGMENTS

Data and replication code are available on GitHub (DOI: 10.5281/zenodo.11300). All authors contributed equally to all aspects of the research. No funding was required for this article. The authors declare no conflicts of interest. We thank seminar participants at the 2014 Annual Meeting of the Midwest Political Science Association, the 2014 Annual Meeting of the Society for

Political Methodology, the 2014 West Coast Experiments Conference, Stanford University, and University of California, San Diego. We thank C. McConnell and S. Liu for valuable research assistance.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1502/suppl/DC1
Materials and Methods
Supplementary Text
Fig. S1
Tables S1 to S7
Reference (42)

1 May 2014; accepted 14 August 2014
Published online 28 August 2014;
10.1126/science.1255484

NEUROMUSCULAR DISEASE

DOK7 gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction

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The neuromuscular junction (NMJ) is the synapse between a motor neuron and skeletal muscle. Defects in NMJ transmission cause muscle weakness, termed myasthenia. The muscle protein Dok-7 is essential for activation of the receptor kinase MuSK, which governs NMJ formation, and *DOK7* mutations underlie familial limb-girdle myasthenia (*DOK7* myasthenia), a neuromuscular disease characterized by small NMJs. Here, we show in a mouse model of *DOK7* myasthenia that therapeutic administration of an adeno-associated virus (AAV) vector encoding the human *DOK7* gene resulted in an enlargement of NMJs and substantial increases in muscle strength and life span. When applied to model mice of another neuromuscular disorder, autosomal dominant Emery-Dreifuss muscular dystrophy, *DOK7* gene therapy likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. These results suggest that therapies aimed at enlarging the NMJ may be useful for a range of neuromuscular disorders.

The neurotransmitter acetylcholine (ACh) is released from the presynaptic motor nerve terminal and binds to ACh receptors (AChRs) on the postsynaptic muscle membrane of the neuromuscular junction (NMJ), which forms in the central region of each myotube (1, 2). To achieve efficient neuromuscular transmission, AChRs must be densely clustered on the postsynaptic membrane (1, 2). Impaired AChR clustering is associated with disorders of neuromuscular

transmission, including subtypes of congenital myasthenic syndromes and myasthenia gravis (2–4). The muscle-specific receptor tyrosine kinase MuSK is required for the formation and maintenance of NMJs (1, 2).

The cytoplasmic protein Dok-7 (downstream of tyrosine kinases 7) is an essential activator of the receptor kinase MuSK, and mice lacking Dok-7 form no NMJs (5–8). Recessive loss- or reduction-of-function mutations in the human *DOK7* gene underlie a limb-girdle type of congenital myasthenic syndrome, *DOK7* myasthenia, a disorder characterized by NMJs that are about half the normal size (7, 9, 10). In contrast to many NMJ channelopathies (11), *DOK7* myasthenia is not associated with abnormalities in the function and local density of AChRs or the quantal release per unit size of the endplates (the region of synaptic specialization on the myotube). These observations suggest that *DOK7* myasthenia should be classified as a synaptopathy rather than a channelopathy (2, 7). Interestingly, there is ac-

cumulating evidence that NMJ structural defects may be a common feature of other neuromuscular disorders (12–18), including muscular dystrophy (MD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and age-related muscle weakness or sarcopenia. Indeed, studies of patients with autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) and a mouse model of this disease have produced data suggestive of inefficient neuromuscular transmission (12). Because the size of NMJs is an important determinant of NMJ function (2), these observations raise the possibility that enlargement of the synaptic area may mitigate muscle weakness associated with defective NMJ structure.

We previously generated Dok-7 transgenic (Tg) mice that overexpress Dok-7 uniformly throughout the skeletal muscle under the control of the human skeletal α -actin (HSA) promoter (6). Using these mice, we found that forced expression of Dok-7 in vivo enhanced the activation of muscle-specific kinase MuSK and subsequent NMJ formation at the correct, central region of muscle fibers in embryos (6). Consistent with this, Dok-7 Tg mice showed greatly enlarged NMJs at 12 weeks of age (fig. S1A). Because exogenous Dok-7 was expressed only in the skeletal muscle (6), these data indicate that forced expression of Dok-7 in muscle triggers not only intramuscular signaling but also retrograde signaling that enlarges motor axon terminals. Interestingly, although these mice have enlarged NMJs, they did not exhibit obvious defects in motor activity, as determined by wire-hang and rotarod tests (fig. S1, B and C). Together, these findings suggest that Dok-7-mediated enhancement of NMJ formation merits investigation as a possible therapeutic approach for neuromuscular disorders associated with an NMJ synaptopathy.

To facilitate Dok-7-mediated NMJ formation in the muscle, we generated AAV-D7, a recombinant adeno-associated virus (AAV) serotype 9 (AAV9) vector carrying the human *DOK7* gene tagged with enhanced green fluorescent protein (EGFP) under the control of the cytomegalovirus (CMV) promoter. This promoter shows higher activity in skeletal muscle than the HSA promoter (19). The AAV vector is a powerful tool for delivering therapeutic genes to skeletal muscle and other tissues (20, 21). We first treated C2C12 myotubes

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with AAV-D7 and observed an increase in the number of AChR clusters (fig. S2), as expected from previous work (5, 7, 9). We next treated 8-week-old wild-type (WT) mice with 4.0×10^{11} viral genomes (vg) of AAV-D7, delivered by a single intravenous injection, and compared them with control, untreated mice. One week after the injection, NMJs were clearly enlarged in the central region of the diaphragm muscle of AAV-D7-treated mice, with exogenous expression of Dok-7 throughout the myotubes (fig. S3 and Fig. 1, A and B). MuSK activation was augmented in the muscle as judged by elevated phosphorylation of MuSK and AChR, the latter known to be dependent on MuSK activation (Fig. 1, C and D). These results demonstrate that forced expression of Dok-7 in adult mice promotes MuSK-mediated formation of NMJs, leading to their enlargement within a week of AAV-D7 treatment. WT mice treated with AAV-D7 did not show any abnormalities in motor activity, as determined by grip strength, wire-hang, and rotarod tests (fig. S4), or in histology of the skeletal muscle, heart, and liver, which are the major target tissues of this AAV9 vector in mice (fig. S5) (22). We confirmed exogenous expression of Dok-7 in the heart (fig. S6) and in skeletal muscle (fig. S3 and Fig. 1A) after AAV-D7 treatment.

To investigate whether forced expression of Dok-7 and subsequent enlargement of NMJs in vivo mitigates disease progression after onset of *DOK7* myasthenia, we generated Dok-7 knock-in (KI) mice homozygous for the frameshift mutation (c.I124_I127dupTGCC), which corresponds to the most prevalent mutation in patients (fig. S7) (7, 10, 23–25). Dok-7^{KI} and littermate WT mice displayed no obvious abnormal phenotype. By contrast, Dok-7^{KI/KI} mice (Dok-7 KI mice) exhibited characteristic features of severe muscle weakness: These mice died between postnatal day 13 (P13) and P20, exhibited about 25% of the body weight of WT mice at P12, and developed apparent disturbance in gait by P9 (fig. S8). Unlike Dok-7^{KI} and littermate WT mice, Dok-7 KI mice were too weak for the measurement of muscle strength. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding (figs. S9 and S10), a pathological feature seen in patients with *DOK7* myasthenia (26). The c.I124_I127dupTGCC mutation is a reduction-of-function mutation in terms of MuSK activation in C2C12 myotubes (7, 9). Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, as judged by attenuated phosphorylation of AChR and MuSK (fig. S11) (see below). Thus, the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice (hereafter referred to as “*DOK7* myasthenia mice”) exhibit a more severe phenotype.

To determine whether *DOK7* gene therapy provides beneficial effects to *DOK7* myasthenia mice, we administered 2.0×10^{11} vg of AAV-D7 by intraperitoneal injection to the animals at P9 to P12. At P9, these mice required at least 10 s to right themselves after being placed on their side, confirming disease onset. A single-dose treatment with AAV-D7 led to marked recovery of the *DOK7*

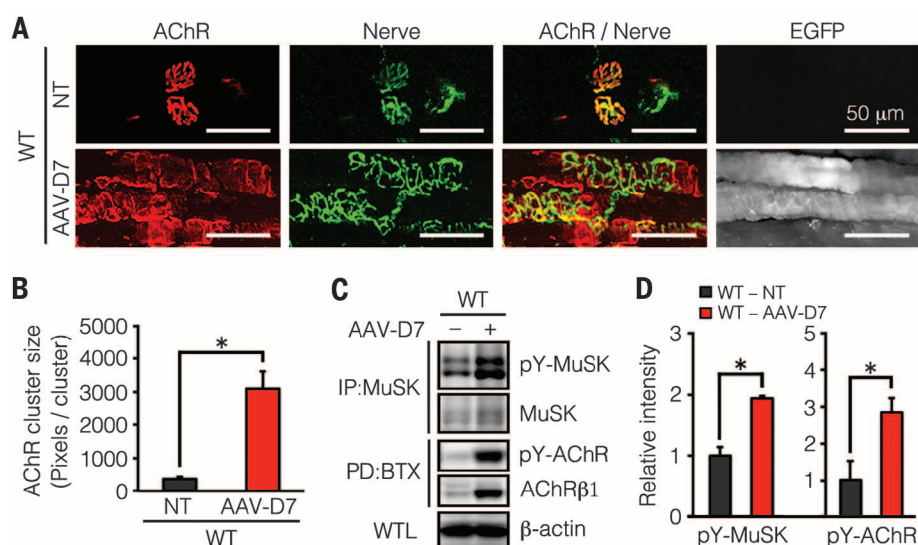


Fig. 1. AAV-D7 treatment promotes MuSK-mediated NMJ formation. WT mice were treated or untreated with 4.0×10^{11} vg of AAV-D7 at P56 and subjected to the following assays at P63. **(A)** Whole-mount staining of NMJs on the diaphragm muscle. Axons and nerve terminals (green) were stained with antibodies to neurofilament and synaptophysin, and AChRs (red) were labeled with α -bungarotoxin (BTX). Expression of Dok-7 tagged with EGFP (gray) was monitored by EGFP. NT, not treated. **(B)** Quantified data for the size of each AChR cluster in the diaphragm muscle ($n = 30$ microscopic fields in 6 mice; male = 3, female = 3). **(C)** Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β -actin in the hind-limb muscle. MuSK immunoprecipitates (IP) from whole-tissue lysates (WTL) of the hind-limb muscle were subjected to immunoblotting for phosphotyrosine (pY) and MuSK. AChRs pulled down with BTX-Sepharose (PD) from WTL were subjected to immunoblotting for pY or the β 1 subunit of AChR. **(D)** Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle ($n = 3$ mice; male = 1 to 2, female = 1 to 2). Values in (B) and (D) are means $\pm$ SD. * $P < 0.05$ by Student's t test.

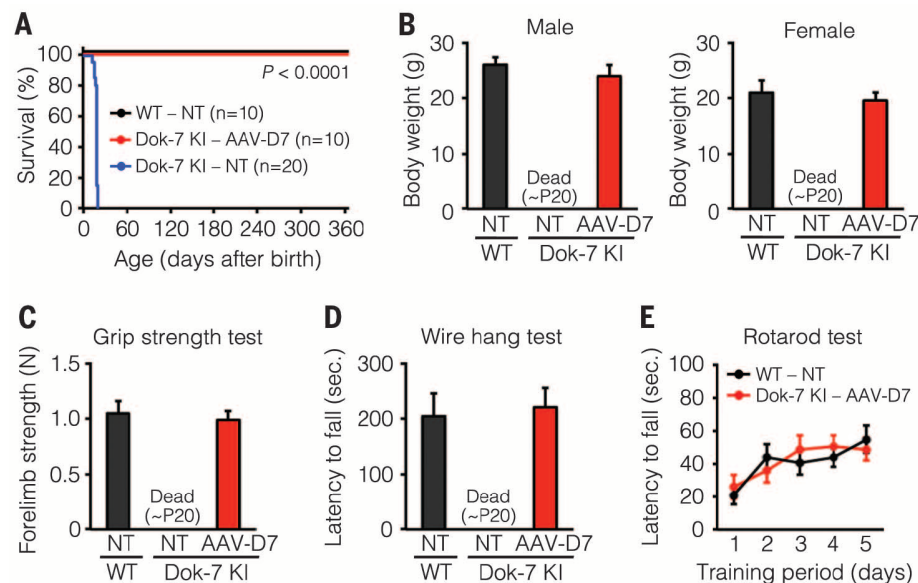


Fig. 2. Dok-7 therapy restores motor activity and survival of *DOK7* myasthenia mice. Mice were treated or untreated with 2.0×10^{11} vg of AAV-D7 at P9 to P12. **(A)** Kaplan-Meier survival curves of WT littermates and Dok-7 KI mice (*DOK7* myasthenia mice) ($n = 10$ to 20 mice; male = 4 to 10, female = 6 to 10). NT, not treated. **(B)** Body weight at P56 ($n = 4$ to 6 mice; male = 5, female = 4 to 6). **(C to E)** Motor activity at P56 determined by (C) grip strength, (D) wire-hang, and (E) rotarod tests ($n = 10$ mice; male = 4 to 5, female = 5 to 6). P value (A) was calculated by log-rank test (Dok-7 KI – NT versus Dok-7 KI – AAV-D7). Values in (B) to (E) are means $\pm$ SD.

myasthenia mice. All AAV-D7-treated mice survived for at least 1 year with no apparent abnormality, whereas all untreated and AAV-EGFP-treated

control mice died by P20 (Fig. 2A and fig. S12). Indeed, body weight and motor activity—as determined by grip strength, wire-hang, and rotarod

tests—of AAV-D7-treated mice approximated those of age-matched WT controls by P56 (Fig. 2, B to E). Similarly, forced expression of a *DOK7* transgene

Fig. 3. Dok-7 therapy promotes MuSK-mediated NMJ formation in *DOK7* myasthenia mice.

Mice were treated or untreated with 2.0×10^{11} vg of AAV-D7 at P9 and subjected to the following assays at P14. (A) Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β -actin in the hind-limb muscle. Experiments were performed as in Fig. 1C. (B) Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle ($n = 3$ mice; male = 1 to 2, female = 1 to 2). (C) Whole-mount staining of NMJs on the diaphragm muscle. Axons and nerve terminals (green), AChRs (red), and Dok-7 tagged with EGFP (gray) were visualized as in Fig. 1A. (D) Quantified data for the size of each AChR cluster in the diaphragm muscle ($n = 30$ microscopic fields in 6 mice; male = 3, female = 3). Values in (B) and (D) are means $\pm$ SD. * $P < 0.05$ by analysis of variance (ANOVA) and Dunnett's test.

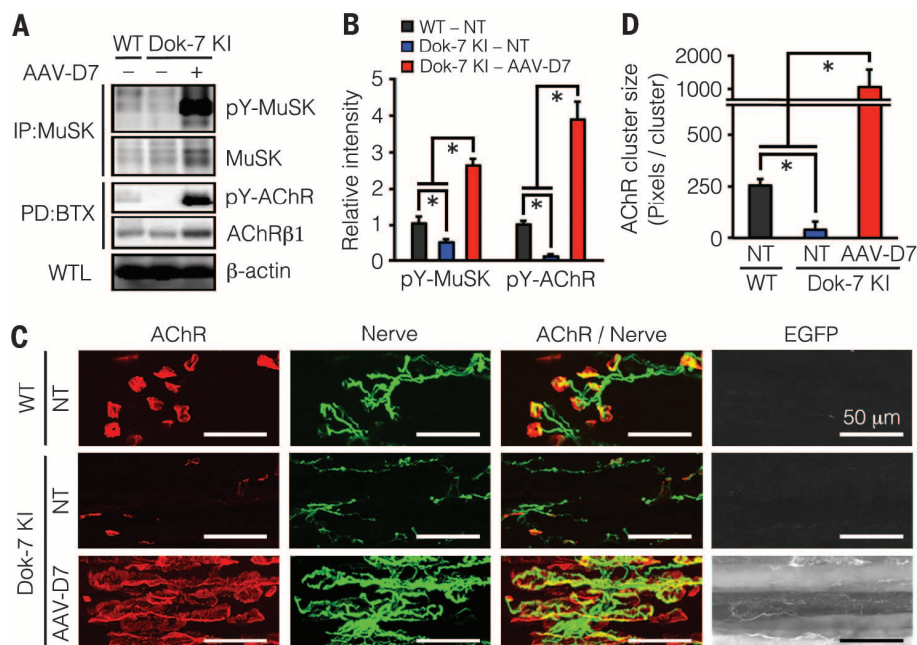
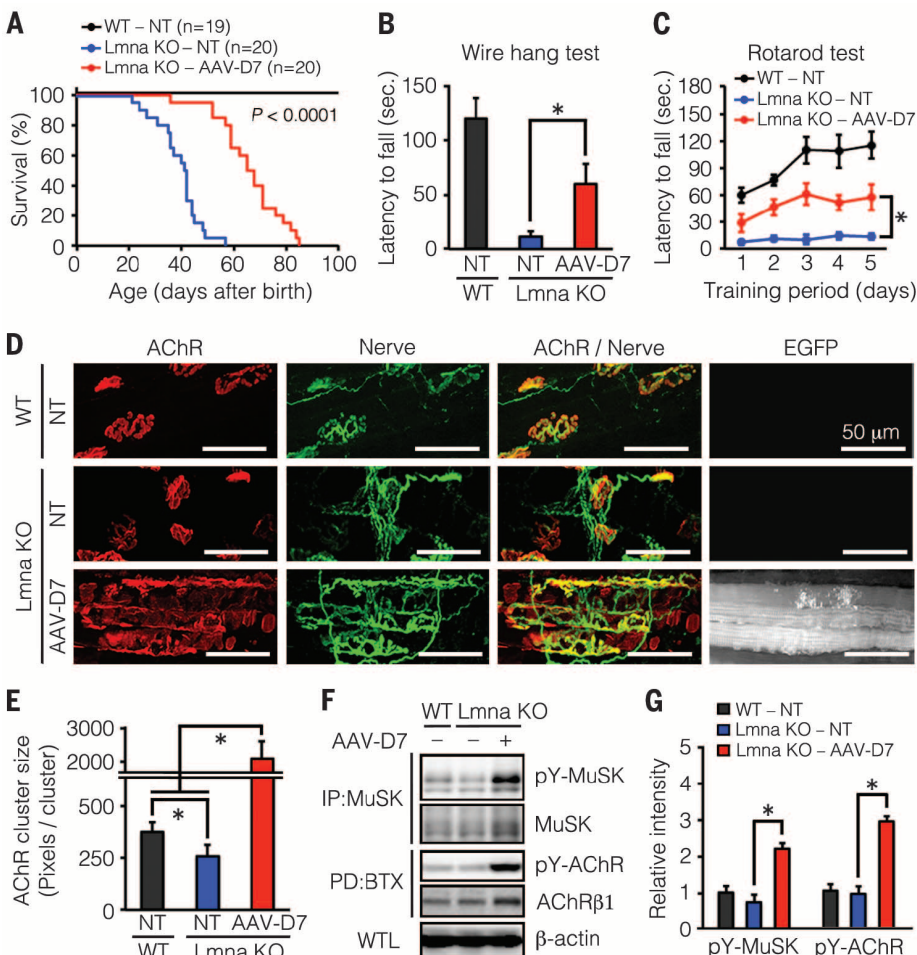


Fig. 4. The effect of Dok-7 therapy in a mouse model of AD-EDMD.

Mice were treated or untreated with 4.0×10^{11} vg of AAV-D7 at P16 and subjected to the following assays. (A) Kaplan-Meier survival curves of WT littermates and *Lmna* KO mice (AD-EDMD mice) ($n = 19$ to 20 mice; male = 9 to 12, female = 8 to 11). NT, not treated. (B and C) Motor activity at P35, determined by (B) wire-hang and (C) rotarod tests ($n = 8$ to 10 mice; male = 4 to 6, female = 4 to 5). (D) Whole-mount staining of NMJs on the diaphragm muscle at P42. Axons and nerve terminals (green), AChRs (red), and Dok-7 tagged with EGFP (gray) were visualized as in Fig. 1A. (E) Quantified data for the size of each AChR cluster in the diaphragm muscle at P42 ($n = 30$ microscopic fields in 6 mice; male = 3 to 4, female = 2 to 3). (F) Immunoblotting for tyrosine phosphorylation of MuSK or AChR and for β -actin in the hind-limb muscle at P42. Experiments were performed as in Fig. 1C. (G) Quantified data for tyrosine phosphorylation of MuSK and AChR in the hind-limb muscle at P42 ($n = 3$ mice; male = 1 to 2, female = 1 to 2). P value (A) was calculated by log-rank test (*Lmna* KO – NT versus *Lmna* KO – AAV-D7). Values in (B), (C), (E), and (G) are means $\pm$ SD. * $P < 0.05$ by analysis of variance (ANOVA) and Dunnett's test.



specifically in skeletal muscle restored survival and motor activity of *DOK7* myasthenia mice as determined by wire-hang and rotarod tests, indicating that muscle-specific expression of Dok-7 is sufficient to rescue these mice (fig. S13, A to C).

We next investigated whether AAV-D7 treatment promotes activation of MuSK and subsequent enlargement of NMJs in *DOK7* myasthenia mice. We found that MuSK and AChR phosphorylation was strongly elevated in *DOK7* myasthenia mice just 5 days after treatment with AAV-D7 (Fig. 3, A and B). Consistent with this, NMJs were greatly enlarged in *DOK7* myasthenia mice within 5 days of treatment (Fig. 3, C and D, and fig. S14) and remained enlarged at 8 weeks of age (fig. S15). Together, these data demonstrate that treatment with AAV-D7 (hereafter referred to as “Dok-7 therapy”) facilitates MuSK-mediated NMJ formation, resulting in stable enlargement of NMJs, restoration of motor activity, and enhanced survival of *DOK7* myasthenia mice.

Because AAV-D7 enlarges NMJs not only in *DOK7* myasthenia mice but also in WT mice, we hypothesized that Dok-7 therapy might be applicable to other types of neuromuscular disorders that are associated with abnormalities of NMJ structure but not caused by *DOK7* mutations. As noted above, AD-EDMD is one such candidate. AD-EDMD is caused by mutations in the *LMNA* gene, which encodes lamin A/C, an important determinant of interphase nuclear architecture (27). Patients with AD-EDMD develop cardiac defects and skeletal muscle weakness (28). Although pacemaker and implantable cardioverter defibrillator (ICD) insertion helps address the cardiac defects (29), there is no effective treatment for skeletal muscle weakness. Histology and gene expression profiles of muscle biopsies from patients with AD-EDMD are suggestive of alterations in NMJ structure (12, 30, 31).

We studied a mouse model of AD-EDMD (hereafter referred to as “AD-EDMD mice”) that is genetically deficient in lamin A/C and that has structurally abnormal and functionally inefficient NMJs (12, 30). At P16, we administered 4.0×10^{11} vg of AAV-D7 into the AD-EDMD mice by a single intraperitoneal injection. Disease onset was confirmed in each AD-EDMD mouse at P16, when the animals showed hind-limb paralysis and required at least 10 s to right themselves after being placed on their sides. We found that Dok-7 therapy prolonged survival of AD-EDMD mice (Fig. 4A). Mice receiving Dok-7 therapy, but not those receiving AAV-EGFP treatment, lived an average of 29 days longer than untreated mice ($P < 0.0001$, log-rank test) (Fig. 4A and fig. S16). In addition, Dok-7 therapy increased latency to fall of AD-EDMD mice, as determined by wire-hang and rotarod tests by 49 s and 44 s, respectively, over untreated mice ($P < 0.05$, Dunnett’s test) (Fig. 4, B and C), indicating enhanced motor activity. Forced expression of Dok-7 specifically in skeletal muscle via a transgene also enhanced AD-EDMD mouse survival and motor activity, as determined by wire-hang and rotarod tests, indicating that muscle-specific expression of Dok-7 is sufficient to benefit these mice (fig. S13, D to F).

Dok-7 therapy enhanced MuSK activation and enlarged NMJs in the skeletal muscle within 26 days of treatment (Fig. 4, D to G), and the NMJs in control, untreated AD-EDMD mice were significantly smaller than those in WT mice (Fig. 4, D and E). Electrocardiographic and histological analyses showed that Dok-7 therapy did not benefit heart function in AD-EDMD mice (fig. S17). It is possible that the beneficial effects of Dok-7 therapy on muscle weakness are partially masked in these mice by heart failure, which, as noted above, would be treatable in patients with AD-EDMD by pacemaker and ICD insertion (29). These findings suggest that Dok-7 therapy might be beneficial for patients with AD-EDMD.

The mechanisms through which Dok-7 therapy alleviates muscle weakness in mouse models of *DOK7* myasthenia and AD-EDMD remain to be determined. We speculate that, in addition to an effect on the postsynaptic region of muscle, the mechanism likely involves retrograde signaling from the muscle to the nerve. This idea is consistent with the observation that enlargement of the nerve terminals at NMJs is seen in the Dok-7 Tg mice that overexpress Dok-7 only in the skeletal muscle, as well as in AAV-D7-treated mice.

Recent studies of mouse models of ALS and SMA revealed that peripheral motor nerve degeneration first manifests as reduction of the nerve terminal area with subsequent denervation at NMJs, and then proceeds proximally, a pattern known as “dying-back” pathology (32, 33). Consistent with this, autopsies of patients with ALS or SMA suggest that motor neuron pathology begins at the distal axon and proceeds proximally (32, 34). Because AAV-D7 has the potential to enlarge the nerve terminals at NMJs, it is tempting to speculate that Dok-7 therapy may counteract the “dying-back” pathology at NMJs and be beneficial in these multifactorial disorders of mostly unknown etiology. Interestingly, in a recent study of the SOD1 (superoxide dismutase 1) G93A Tg mouse model of ALS, it was reported that a modest, muscle-specific increase in MuSK expression via a transgene delayed denervation at NMJs and improved motor activity, but not survival, of the mice (35). However, a previous study had shown that higher-level expression of MuSK in the muscle induces scattered NMJ formation throughout myotubes, leading to severe muscle weakness and ultimately to death (36). Dok-7 gene therapy may be a safer approach because it greatly facilitates correct, centrally localized NMJ enlargement without lethal effects for more than 1 year in *DOK7* myasthenia mice (Figs. 2A and 3C and figs. S14 and S15).

AAV-mediated gene transfer to skeletal muscle can result in long-term expression of the therapeutic gene. For instance, in a patient with hemophilia B, AAV-mediated expression of factor IX was detected even 10 years after a single intramuscular injection (37). In addition, although AAV capsid, rather than transgene, is the antigen that is targeted by the host immune responses in humans, this process can be controlled by a short course of treatment with immunosuppressant without loss of transgene expression (38), raising

prospects for long-term use of these vectors in therapies.

Our findings demonstrate that elevated Dok-7 expression, or any equivalent method that stably and safely enlarges the NMJ, has potential as a therapy for a variety of neuromuscular disorders that feature defects in NMJ structure, including those of unknown etiology. Such NMJ-targeted therapies could be administered alone or in combination with other therapies.

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ACKNOWLEDGMENTS

We are grateful to C. L. Stewart and Y. Hayashi for the *lmna* knock-out (KO) mice (AD-EDMD mice) and to J. M. Wilson for the helper plasmid pRep2Cap9. We thank R. F. Whittier and S. Miyoshi for critical reading of the manuscript. This work was supported by Grants-in-Aid of Scientific Research and of the Translational Research Network Program from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Y.Y. is an inventor on a patent (Japan patent P5339246 and U.S. patent 8222383) that covers the use of human *DOK7* cDNA for commercial purposes. University of Pennsylvania holds a patent (Japan patent P5054975 and U.S. patent 7906111) that covers the use of AAV9 for commercial purposes.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1505/suppl/DC1
Materials and Methods
Figs. S1 to S17
Reference (39–43)

13 January 2014; accepted 22 August 2014
10.1126/science.1250744

CORD BLOOD EXPANSION

Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal

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The small number of hematopoietic stem and progenitor cells in cord blood units limits their widespread use in human transplant protocols. We identified a family of chemically related small molecules that stimulates the expansion *ex vivo* of human cord blood cells capable of reconstituting human hematopoiesis for at least 6 months in immunocompromised mice. The potent activity of these newly identified compounds, UM171 being the prototype, is independent of suppression of the aryl hydrocarbon receptor, which targets cells with more-limited regenerative potential. The properties of UM171 make it a potential candidate for hematopoietic stem cell transplantation and gene therapy.

A llogeneic HSC transplant is the only curative therapy for numerous hematologic malignancies. Unfortunately 30 to 40% of patients will not have a human leukocyte antigen (HLA)-identical donor and will be excluded from therapy (1). Cord blood (CB) transplants offer several advantages, namely,

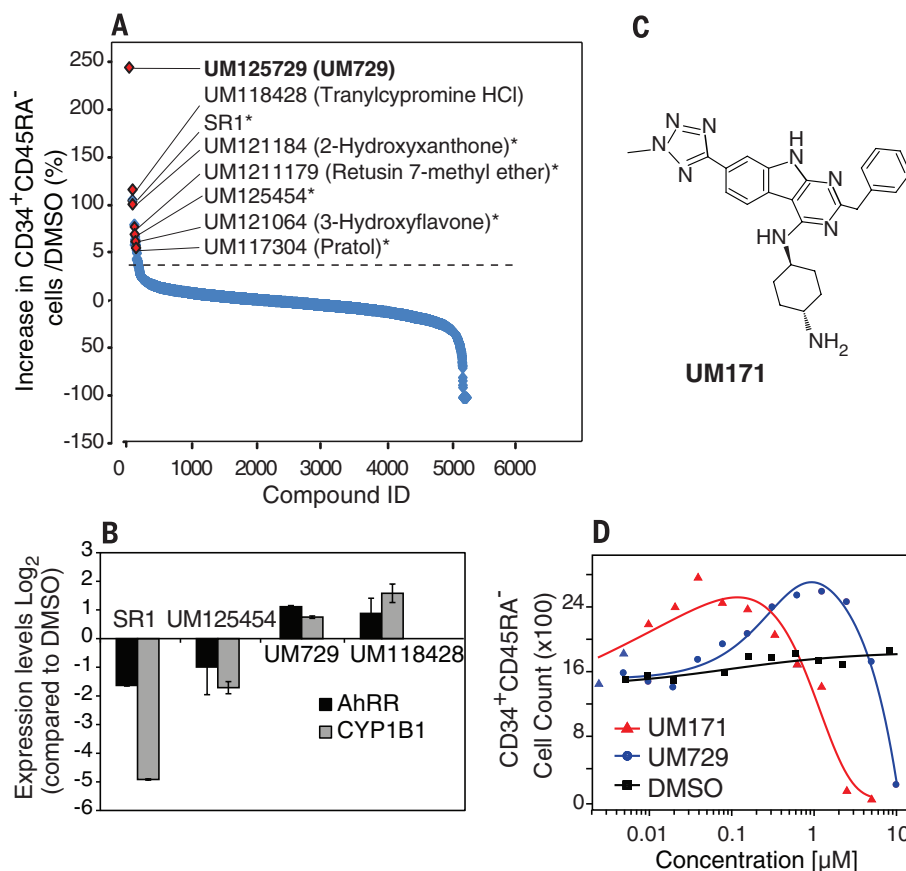
the reduced need for HLA matching [thereby extending transplantation availability to nearly all patients (2)] and the decreased risk of chronic graft-versus-host disease, the most important determinant of long-term quality of life in transplant patients. However, CB transplants suffer from limited progenitor cell dose, leading to

delayed neutrophil engraftment and increased mortality (3, 4).

Recent studies in immunodeficient mice have confirmed the existence of human CB-derived long-term-repopulating hematopoietic stem cells (LT-HSCs) capable of regenerating the life-long production of all mature blood cells (5). These LT-HSCs show a delayed engraftment pattern, in opposition to short-term HSCs (ST-HSCs) that produce short-lived progenitors responsible for the production of mature blood cells and prompt neutrophil recovery (3, 5). Hence, there is great interest in the development of conditions for robustly expanding these progenitor cells while maintaining or expanding LT-HSCs. Unfortunately, most expansion systems available to date achieve progenitor cell

Fig. 1. Identification of previously unknown compounds promoting human CD34⁺ cell expansion.

(A) Results of primary screen; asterisks denote the compounds that suppress the AhR pathway. (B) Changes in expression levels of AhR targets (AhRR and CYP1B1) measured by quantitative reverse transcription polymerase chain reaction after a 12-hour incubation with selected compounds compared with DMSO (using glyceraldehyde-3-phosphate dehydrogenase and hypoxanthine-guanine phosphoribosyltransferase as control, mean $\pm$ SD). (C and D) Chemical structure of UM171, the optimized version of UM729, and their comparative activity on expansion of CD34⁺CD45RA⁺ mPB cells after 7-day cultures. The cytostatic/cytotoxic effects of UM729 and UM171 were observed at values above 1 and 0.125 μ M, respectively.



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expansion at the expense of the LT-HSC loss (6), increasing the risk of late graft failure.

Recent studies showed that aryl hydrocarbon receptor (AhR) antagonists and a notch ligand agonist promote the *in vitro* expansion of human CB cells, with repopulating activity lasting up to 16 weeks in immunodeficient mice (7, 8). We developed an automated and continuous medium delivery system that produces an equivalent expansion of CB cells with similar repopulation properties (9). This fed-batch culture system optimizes the balance of stimulatory and inhibitory factors in a small culture volume. We hypothesized that small molecules with potent LT-HSC-stimulating activities might be identified and potentiated in this fed-batch culture system.

We screened a library of 5280 low-molecular-weight compounds for their ability to expand human CD34⁺CD45RA⁺ mobilized peripheral blood (mPB) cells, which are enriched in LT-HSCs (10) (fig. S1, A and B). Seven hits were identified after excluding the autofluorescent compounds (Fig. 1A and fig. S1C), five of which were known [four (11, 12)] or previously unknown (one, UM125454, fig. S2) suppressors of the AhR pathway (Fig. 1B). The other two compounds, UM729 (fig. S2) and UM118428, did not suppress the AhR path-

way (Fig. 1B). Because of its apparent superior activity in expanding CD34⁺CD45RA⁺ cells, UM729 was selected for further characterization and optimization by structure activity relationship (SAR) studies that determine the link between the chemical structure of the compound and its biological activity in expanding CD34⁺CD45RA⁺ cells. More than 300 newly synthesized analogs of UM729 were examined, of which one (UM171, Fig. 1C) was 10 to 20 times more potent than UM729, with effective concentrations of 17 to 19 nM when tested for its ability to stimulate the expansion of a HSC-enriched population, CD34⁺CD45RA⁺ cells (10) (Fig. 1D and fig. S3, A and B). UM729 did not expand mouse HSCs (fig. S4). UM729 and UM171 treatment enhanced the engraftment potential of CD34⁺ macaque cells by threefold when compared with controls (fig. S5).

Optimization of fed-batch culture duration indicated that the highest expansion of multipotent progenitors and long-term culture-initiating cells (LTC-ICs) was obtained on day 12 (fig. S3, C to E). Likewise, the proportion of apoptotic cells was lower at that time when compared with day 16 (fig. S3F). We also observed that the effect of UM171 requires its constant presence in

the media and that the molecule lacks direct mitogenic activity (fig. S6). Cell division tracking further showed that UM171 does not affect the division rate of phenotypically primitive populations (fig. S7).

We next designed experiments to compare the impacts of UM171 and SR1 on outputs of CD34⁺ CB cells introduced in fed-batch cultures. Control (dimethyl sulfoxide, DMSO) fed-batch cultures contained mostly differentiated cells (Fig. 2A, DMSO) and a reduced frequency of CD34⁺CD45RA⁺ cells (compare red box of the two top right graphs in Fig. 2B). In contrast, this phenotype remained prominent in cultures containing UM171 (Fig. 2A and red box in Fig. 2B). Although CD34⁺ cell frequencies in cultures containing SR1 or UM171 were similar (Fig. 2B, middle graphs), CD34⁺CD45RA⁺ cells were proportionally more abundant when UM171 was present (Fig. 2B, right-hand graph, red box; $P < 0.005$, Mann-Whitney test). Determining the absolute numbers of these primitive phenotypes and functionally defined cells confirmed the greater effect of UM171 when compared with control or SR1 [compare UM171 (red) with fed-batch (black) and SR1 (blue) in Fig. 2C and figs. S8 and S9 for fold expansion and absolute cell numbers, respectively]. Furthermore, the effect of UM171 on

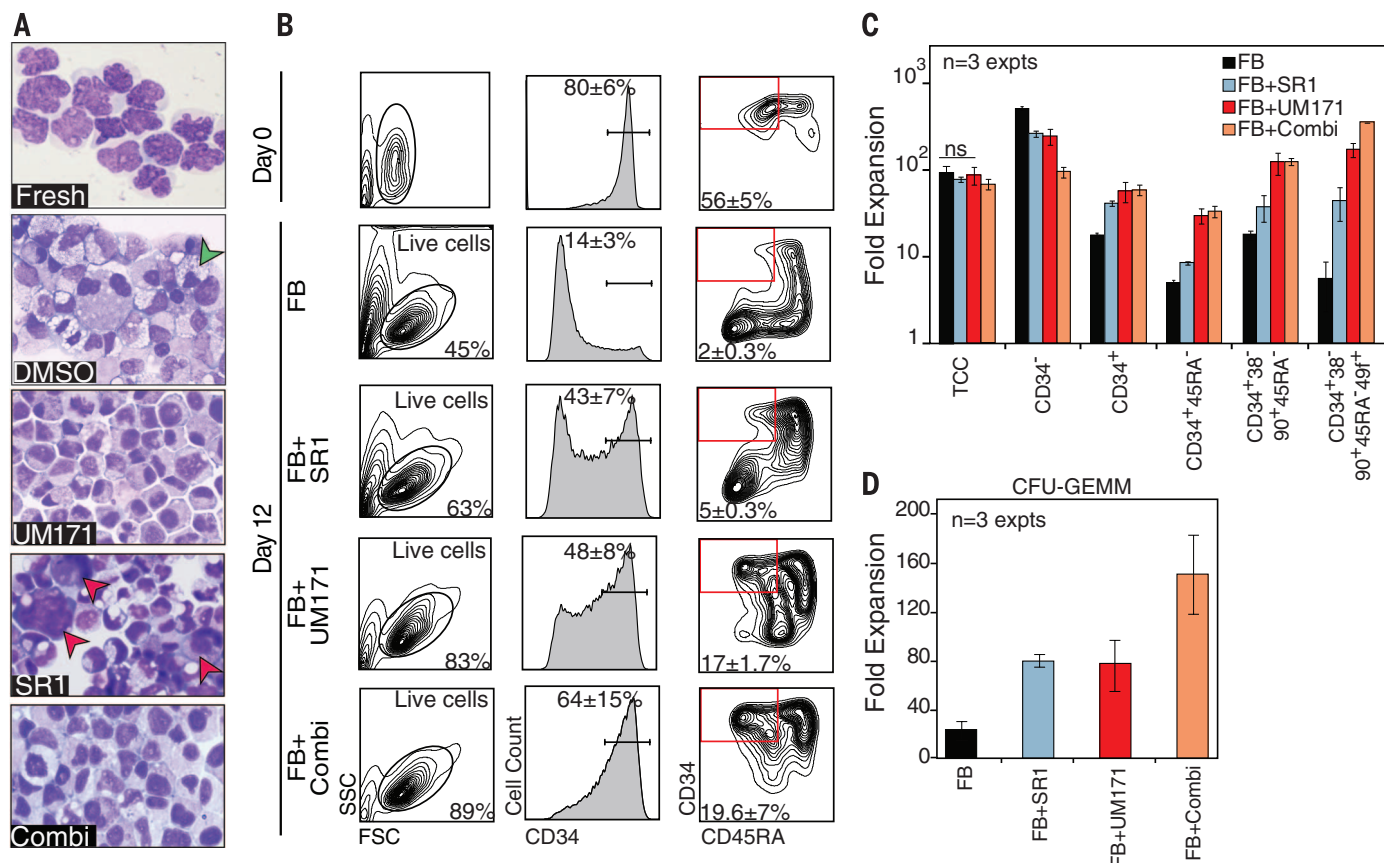


Fig. 2. UM171 attenuates cell differentiation and promotes *ex vivo* expansion of primitive human hematopoietic cells. (A) Wright-stained cytopsin preparation of CD34⁺ CB cells at day 0 and after 12 days in fed-batch cultures supplemented with vehicle (DMSO 0.1%), UM171 (35 nM), SR1 (750 nM), or a combination of SR1 (500 nM) and UM171 (35 nM). Arrowheads show macrophages (green) and megakaryocyte (red). (B) Representative FACS profiles

of CD34⁺ and CD34⁺CD45RA⁺ populations in fresh (day 0) or cultured (day 12) CB cells. (C) The fold expansion of phenotypically defined cell subsets after 12 days in fed-batch cultures supplemented with indicated compounds [mean ± SD unless specified (ns, not significant); all values are significant when compared with control (black bars); $P < 0.05$, Mann-Whitney test]. (D) Fold expansion of CFU-GEMMs after 12 days in cultures.

colony-forming unit of granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) expansion (Fig. 2D) and on mature cell output suppression (e.g., CD34⁺ cells in Fig. 2, B and 2C) was enhanced by the addition of SR1. To-

gether, these observations show that these two compounds cooperate to enhance ex vivo expansion of progenitor cells and that they suppress mature cell output (differentiation). These data also suggest that UM171 targets pheno-

typically more primitive cells than those targeted by SR1.

By using conditions described in fig. S10A, we next determined the frequencies (adjusted to numbers of CD34⁺ cells at day 0, hereafter called d0 equivalent) and the absolute numbers of LT-HSCs in fed-batch cultures supplemented with DMSO (control), UM171, SR1, or the combination of both (Fig. 3A and table S1, respectively). When analyzed at 20 weeks posttransplantation, LT-HSC frequencies in fresh (uncultured) CD34⁺ CB were measured at the expected frequency of ~1 per 880 CD34⁺ starting cells [95% confidence interval (CI) of 470 to 1600; see Fig. 3A in which frequencies (red line) and 95% CIs (gray box) are indicated]. Similar LT-HSC frequencies were obtained from fed-batch cultures (DMSO) whether or not they contained SR1 (Fig. 3A). Frequencies of d0 equivalent LT-HSCs were 13-fold higher in cultures supplemented with UM171 when compared with DMSO or to fresh (uncultured) controls (Fig. 3A). Absolute LT-HSC values determined after 20 weeks posttransplantation in all culture conditions are provided in table S1. Simultaneous addition of SR1 to UM171-treated cultures did not significantly change these numbers, indicating that the cooperativity between these two molecules is restricted to short-lived progenitors and that LT-HSC output is selectively enhanced in the presence of UM171 (Fig. 3A and table S1).

We analyzed the nature of human hematopoietic reconstitution obtained by transplanting fresh or expanded cells in NOD *scid* gamma (NSG) mice. Levels of human cell engraftment, whether total (CD45), myeloid (CD33), or B lymphoid (CD19), were determined for ~300 mice and represented in the form of a heat map in Fig. 3B (raw data in table S2). Analysis of this data set indicates two emerging patterns of human reconstitution, one from predominantly lymphomyeloid LT-HSCs, observed at high cell doses with most conditions, and the other from LT-HSCs that display a lymphoid-deficient differentiation phenotype mostly observed with UM171 treatment, with or without SR1 (Fig. 3B). However, neither

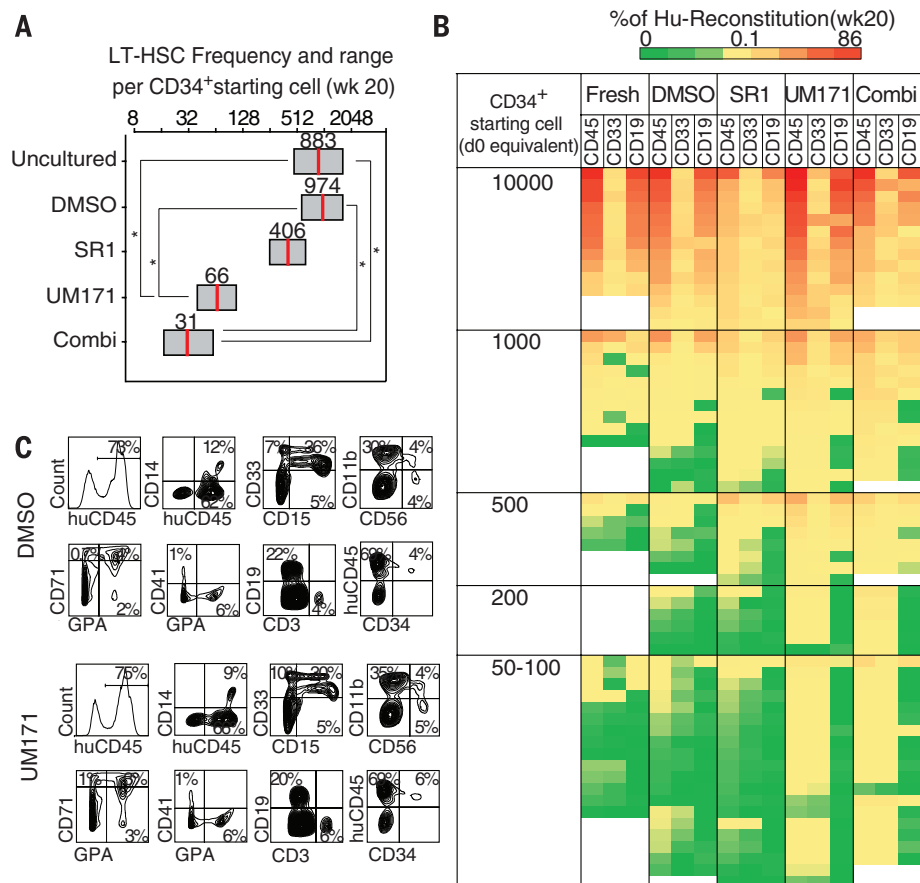


Fig. 3. UM171 promotes expansion of LT-HSCs. (A) LT-HSC frequencies (red lines) and 95% CIs (gray boxes) presented as 1/number of starting cell (day 0) equivalent for each condition; $n = 5$ independent experiments performed with a pool of two to three human CB units per experiment. Significance level $*P < 0.05$ (Mann-Whitney test). (B) Levels of human (Hu) engraftment in NSG mice transplanted with different cell doses (column 1); red, 86%; green, 0%. See table S2 for raw data. (C) Representative FACS profiles showing multilineage repopulation of NSG mice (GPA, glycophorin A or CD235a).

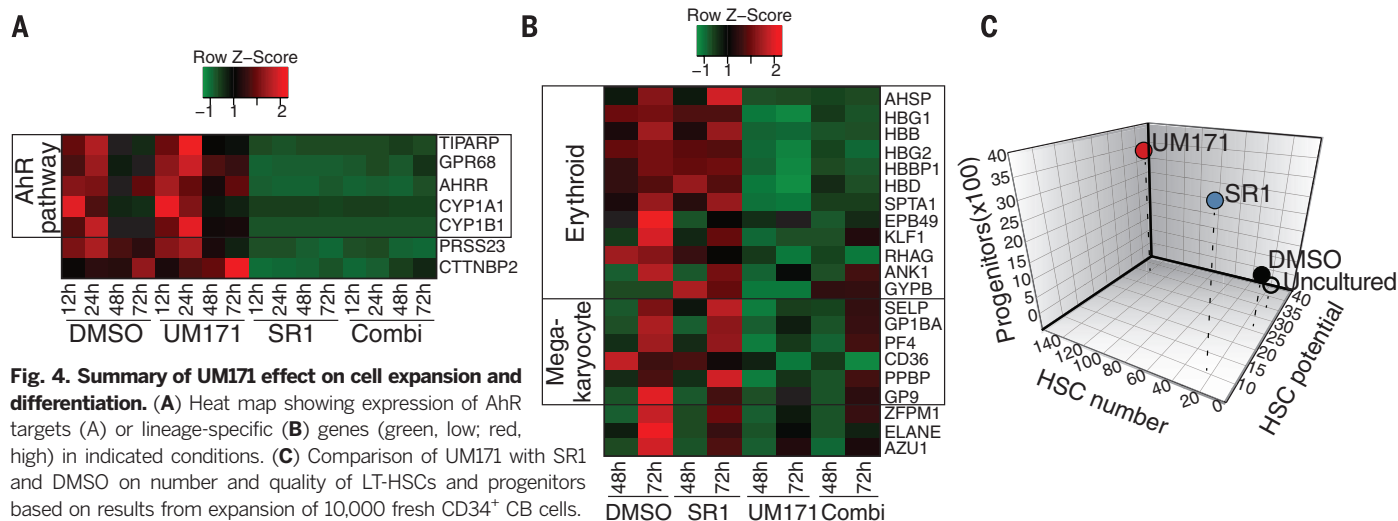


Fig. 4. Summary of UM171 effect on cell expansion and differentiation. (A) Heat map showing expression of AhR targets (A) or lineage-specific (B) genes (green, low; red, high) in indicated conditions. (C) Comparison of UM171 with SR1 and DMSO on number and quality of LT-HSCs and progenitors based on results from expansion of 10,000 fresh CD34⁺ CB cells.

B lymphopoiesis nor the frequency or number of lymphomyeloid LT-HSCs is negatively affected by UM171 (Fig. 3B). SR1 treatment appeared to compromise the *in vivo* proliferative potential, although not the number, of lymphomyeloid LT-HSCs (compare reconstitution levels of SR1 with uncultured or UM171 conditions in Fig. 3B). In support of this, the presence of SR1 in UM171 treated cultures appears to slightly hamper the proliferative potential of the expanded cells (see reduction in red in Combi versus UM171 conditions in Fig. 3B). The impact of UM171 on LT-HSC was preserved at 30 weeks posttransplantation (fig. S10B and table S3), at which time multilineage contribution remained obvious at the high cell dose (Fig. 3C). At this extended time point post-transplant, we also noted a slight augmentation in myeloid cell output, a phenomenon recently described with normal unexpanded cells (5, 13). The molecular and cellular mechanisms underlying this effect of UM171 on expanding LT-HSCs that show a lymphoid-deficient differentiation pattern are of interest given previous studies of a similar self-perpetuating LT-HSC subset in mice (14) whose prominence is increased in the bone marrow as soon as HSCs begin to migrate from the fetal liver to that site (15).

To further evaluate the impact of UM171-treated LT-HSC population(s), we performed transplantation experiments in secondary recipients. For these studies, four to six primary recipients were selected per condition in which human reconstitution ranged between 10 and 70%. Results (table S4) indicate that UM171 *ex vivo* treatment did not appear to affect the capability of LT-HSC to expand in primary recipients and hence similarly reconstituted secondary animals for at least 18 more weeks, thus indicating that cells exposed to the molecule *ex vivo* are still competent in secondary recipients, where they show no advantage when compared to unmanipulated CD34⁺ cells.

We next performed RNA sequencing (RNA-seq) expression profiling experiments to gain insights into the mode of action of UM171. SR1-treated cells were also analyzed for comparison. As expected, SR1 but not UM171 treatment resulted in down-regulation of AhR target genes such as *CYP1B1*, *CYP1A1*, and *AhR* (Fig. 4A and fig. S11A) (7, 16). Unlike SR1, UM171 treatment was accompanied by a marked suppression of transcripts associated with erythroid and megakaryocytic differentiation (Fig. 4B and fig. S11B). Only six to seven genes were commonly up- or down-regulated in cells exposed to UM171 or SR1 (fig. S12A). In line with these results, gene expression signatures were very different between cells exposed to UM171 versus those treated with SR1 (fig. S11C and fig. S12B). Most notably, we found that the transmembrane protein of unknown function, TMEM183A, was the most up-regulated transcript in both conditions (fig. S11, A and B) and that the most highly up-regulated genes in UM171-treated cells encode for surface molecules (fig. S11B, highlighted in red). These genes include *PROCR* (also called EPCR or CD201), which represents a known marker of mouse LT-HSCs (17). Additional RNA-

seq experiments and fluorescence-activated cell-sorting (FACS) analyses confirmed that expression of this receptor is modulated, in a dose-dependent manner, by UM171 treatment (fig. S13).

UM171 enables a robust *ex vivo* expansion of human CB cells with functionally validated long-term *in vivo* repopulating capability (Fig. 4C). On the basis of these findings, we suggest that UM171 acts by enhancing the human LT-HSC self-renewal machinery independently of AhR suppression. Conversely, AhR inhibitors' activity appears restricted to the production of cells with less-durable self-renewal activity (Fig. 4C). By expanding LT-HSCs and downstream cells *in vitro* using UM171, it may become possible for small, well HLA-matched CB units to become a prioritized source of cells for transplantation in future donor selection algorithms.

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ACKNOWLEDGMENTS

We acknowledge the help of M. Cooke and A. Boitano in setting up the assay for the primary screen, the expert help of J. Duchaine at IRIC for assistance with the chemical screen, D. Gagné (also at IRIC) for technical support with flow cytometry, M. Frechette and V. Blouin-Chagnon for assistance with mice experiments, J. Roy and J. Krosi for scientific support and for critical reading of the manuscript, Héma-Québec and the Women's and Children's Hospital of British Columbia for providing cord blood, and Maisonneuve-Rosemont hospital cell therapy laboratory staff for mobilized peripheral blood. Financial support was from a grant to G.S. and collaborators from the Stem Cell Network of Canada and from IRIC Commercialization of Research. D.-C.R. is also supported through Fonds de Recherche du Québec Santé-ThéCell. H.-P.K. is also supported through NIH grant HL84345. D.J.H.F.K. held a Vanier Scholarship, and P.M. a Banting and Best Studentship, both from the Canadian Institutes of Health Research. G.S., Y.G., R.R., S.G., and I.F. are inventors on a patent application filed by the University of Montreal, Canada, that covers pyrimidoindoles and their use in expansion of HSCs and progenitor cells. P.W.Z. and E.C. are inventors on a patent application filed by the University of Toronto, Canada, that covers the fed-batch system. Compounds UM729 and/or UM171 can be obtained from the G.S. laboratory at compound.sauvageaulab@gmail.com under a material transfer agreement with the University of Montreal. We report no conflict of interest.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1509/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S13
Tables S1 to S4
References (18, 19)

21 May 2014; accepted 26 August 2014
10.1126/science.1256337

ENZYME REGULATION

IRBIT is a novel regulator of ribonucleotide reductase in higher eukaryotes

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Ribonucleotide reductase (RNR) supplies the balanced pools of deoxynucleotide triphosphates (dNTPs) necessary for DNA replication and maintenance of genomic integrity. RNR is subject to allosteric regulatory mechanisms in all eukaryotes, as well as to control by small protein inhibitors Sml1p and Spd1p in budding and fission yeast, respectively. Here, we show that the metazoan protein IRBIT forms a deoxyadenosine triphosphate (dATP)-dependent complex with RNR, which stabilizes dATP in the activity site of RNR and thus inhibits the enzyme. Formation of the RNR-IRBIT complex is regulated through phosphorylation of IRBIT, and ablation of IRBIT expression in HeLa cells causes imbalanced dNTP pools and altered cell cycle progression. We demonstrate a mechanism for RNR regulation in higher eukaryotes that acts by enhancing allosteric RNR inhibition by dATP.

Ribonucleotide reductase (RNR) provides building blocks for genomic and mitochondrial DNA replication and repair, and uncontrolled RNR activity has been associated with malignant transformation and tumor cell growth. The key role of RNR in DNA synthesis has made it a target for both anticancer

and antiviral therapy (1). Many chemotherapeutic RNR inhibitors are nucleoside analogs whose association with other nucleotide-binding

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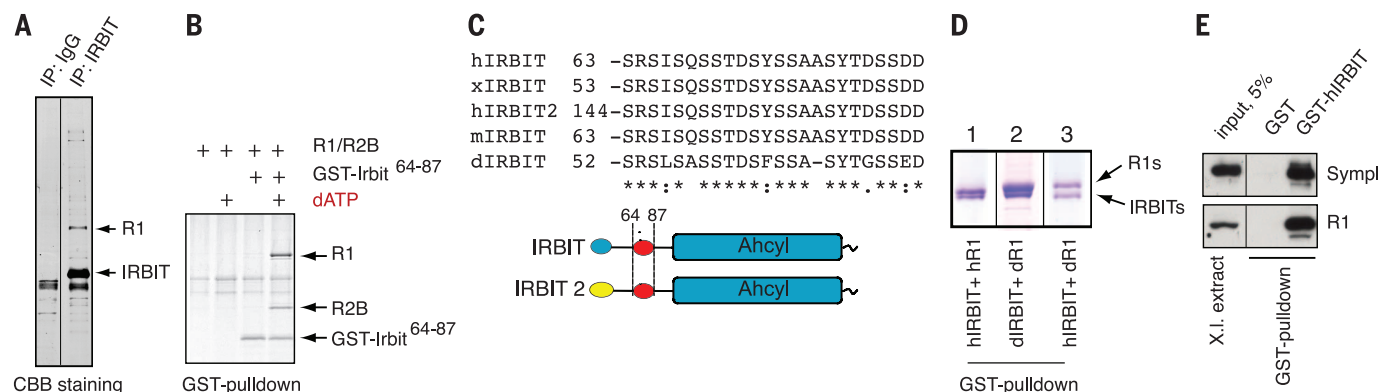


Fig. 1. IRBIT interacts with RNR in a dATP-dependent manner. (A) IRBIT-RNR interaction. IRBIT was immunoprecipitated from asynchronous HeLa cell lysates; bound proteins were resolved on SDS-PAGE and stained with Coomassie brilliant blue (CBB). IRBIT and R1 proteins are indicated by arrows. (B) Minimal domain required for dATP-dependent IRBIT-RNR interaction. GST-IRBIT⁶⁴⁻⁸⁷ was mixed with R1/R2B and 3 μ M dATP. Complexes were retrieved by glutathione-Sepharose and resolved by SDS-PAGE and stained with CBB.

(C) Alignment of IRBIT domain from different metazoans. (D) R1-IRBIT interaction is evolutionarily conserved. GST-IRBIT (hIRBIT) or GST-CG9977 (dIRBIT) were mixed with either human 6His-R1 (hR1) or *Drosophila* 6His-R1 (dR1) along with 3 μ M dATP and analyzed as in (B). (E) hIRBIT binds *Xenopus* R1. GST-hIRBIT was mixed with cytosolic factor-arrested *Xenopus laevis* egg extracts. IRBIT-bound proteins were purified and probed with antibodies against R1 and Symplekin (4).

proteins can contribute to their toxicity, and so the search continues for more specific drugs with less adverse effects (2).

IRBIT is a conserved metazoan protein that has been implicated in diverse functions (3–6). IRBIT consists of a putative enzymatic domain that has similarity to *S*-adenosylhomocysteine hydrolase and an essential N-terminal domain of 104 amino acids (3–5). To identify proteins that bind IRBIT, we performed immunoprecipitation from lysates of HeLa cells, followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and protein staining. Prominent bands were excised and analyzed by mass spectrometry. RNR's large subunit R1 was identified as one of the most abundant proteins in the sample (Fig. 1A and table S1). We expressed RNR (R1/R2B) and IRBIT in insect cells and purified these proteins. It should be noted that only R2B (p53R2), but not R2, formed a stable complex with R1. We analyzed RNR-IRBIT interaction in the absence or presence of RNR nucleotide effectors (fig. S1, A and B). In the absence of nucleotides, IRBIT interacted with RNR directly but with low affinity, whereas the formation of the RNR-IRBIT complex was greatly stimulated in the presence of deoxyadenosine triphosphate (dATP) (fig. S1, B and C).

The region of IRBIT essential for RNR interaction lies within amino acids 64 to 87 (fig. S1D). This fragment was sufficient for complex formation with R1/R2B in the presence of dATP (Fig. 1B). This well-conserved serine- and threonine-rich domain exists in all IRBIT-like molecules (Fig. 1C). Human IRBIT interacted strongly with *Drosophila* R1, whereas *Drosophila* IRBIT (dIRBIT) (CG9977) interacted with human RNR in a dATP-dependent manner (Fig. 1D). Human IRBIT also showed specific recruitment of *Xenopus* R1 from egg extracts (Fig. 1E). RNR bound IRBIT in the presence of ~1 to 3 μ M dATP (fig. S1E), which is below physiological dATP levels (10 to 40 μ M) (7).

dATP binds R1 at two sites: dATP bound to the low-affinity activity site (A-site) inhibits RNR, where-

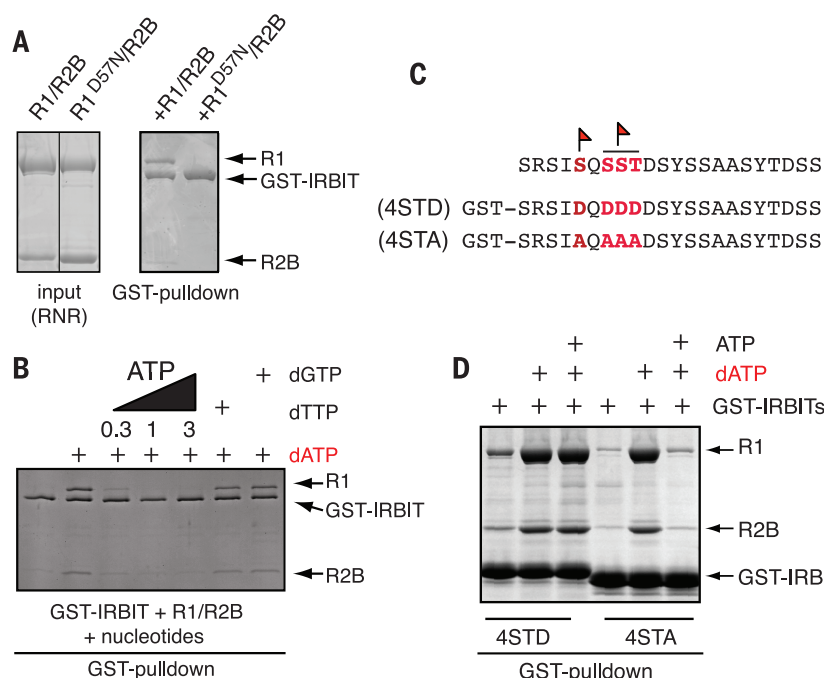


Fig. 2. IRBIT binds RNR when dATP is in the A-site and requires phosphorylation for its full binding activity. (A) dATP occupancy of RNR's A-site is required for IRBIT binding. GST-IRBIT was mixed with either R1/R2B or R1^{D57N}/R2B (input, left) and 10 μ M dATP and retrieved by glutathione-Sepharose (right). (B) IRBIT-RNR interaction is sensitive to ATP competition but insensitive to S-site occupancy. GST-IRBIT was mixed with R1/R2B and 3 μ M dATP followed by addition of 0.3 mM ATP, 1 mM ATP, 3 mM ATP, 1 mM dTTP, or 1 mM dGTP. (C) Endogenous phosphorylation sites that were mapped on IRBIT (flags) and corresponding recombinant phosphomimetic or nonphosphorylatable mutants. (D) IRBIT phosphorylation prevents dissociation of the IRBIT-R1-dATP complex by ATP. GST-IRBIT^{4STD} (STD) or GST-IRBIT^{4STA} (STA) was mixed with R1/R2B and 3 μ M dATP followed by addition of 1 mM ATP. In all panels, complexes were analyzed as in Fig. 1B.

as dATP bound to the specificity site (S-site) promotes production of deoxyuridine diphosphate (dUDP) and deoxycytidine diphosphate (dCDP) (8). We analyzed interaction between IRBIT and a mutant that does not differentiate between dATP and ATP in the A-site [R1^{D57N}], in which asparagine (N) replaces aspartic acid (D) at residue 57] (9).

R1^{D57N} did not stably interact with IRBIT in the presence of dATP (Fig. 2A), which indicated that the A-site is occupied by dATP when R1 is bound to IRBIT. Consistent with this idea, the RNR-dATP-IRBIT complex was not disrupted by deoxythymidine triphosphate (dTTP) or deoxyguanosine triphosphate (dGTP), which can compete only

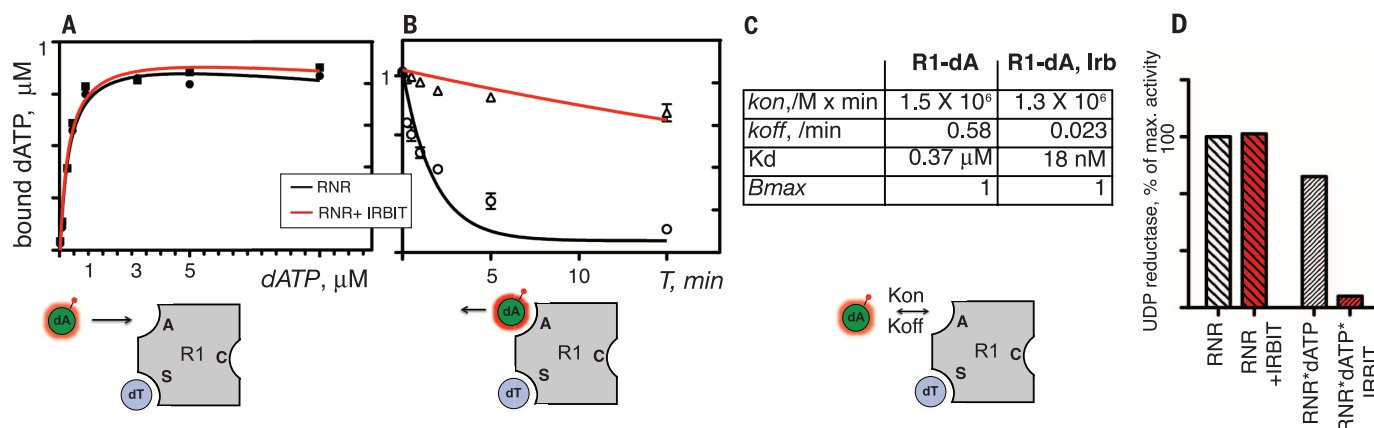


Fig. 3. IRBIT stabilizes and inhibits RNR-dATP complex. (A) IRBIT does not create additional dATP binding sites on RNR. RNR was mixed with increasing doses of [^{32}P]dATP in the absence (circles) or presence (squares) of hIRBIT and in the presence of dTTP (1 mM) at +4°C. (B) IRBIT inhibits dATP release from RNR. Values are means $\pm$ SEM. (C) Rate constants of R1-dATP interaction. B_{max} (maximum number of binding sites) was derived from (A), k_{off} was obtained from (B), and k_{on} was calculated from fig. S2D. K_d , dissociation constant. (D) IRBIT enhances dATP inhibition of RNR. RNR was premixed with or without dATP and with or without 6His-IRBIT and tested for uridine diphosphate (UDP) reductase activity.

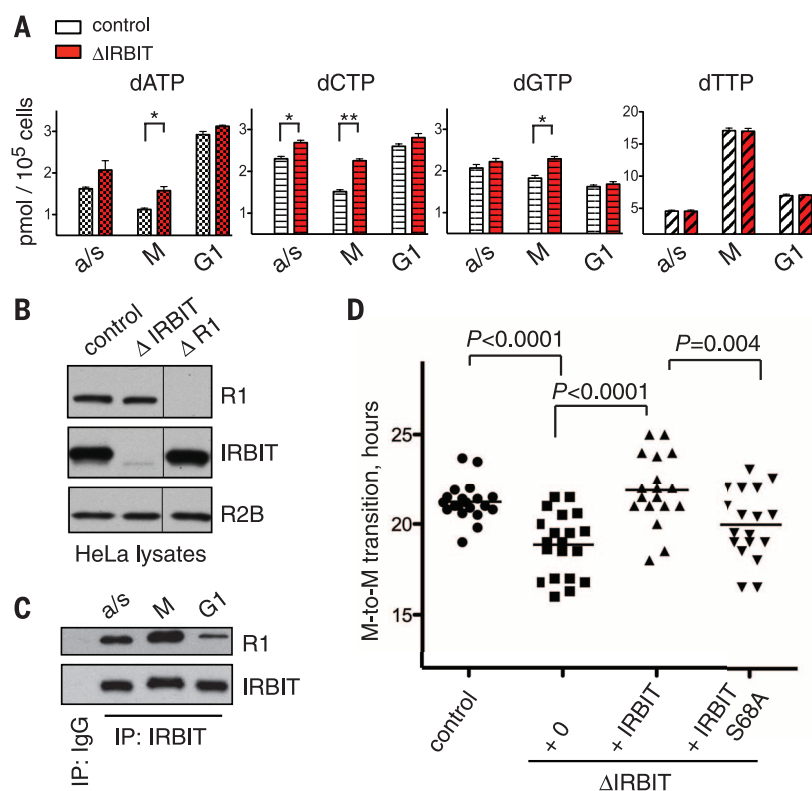


Fig. 4. IRBIT controls the endogenous dNTP pool and is required for normal cell cycle progression. (A) dNTP levels in HeLa and in HeLa Δ IRBIT cells in mitotic (M) or G₁ phases; a/s for asynchronous population. (B) Stability of R1 and IRBIT do not depend on each other. Total lysates of HeLa, HeLa Δ IRBIT, and HeLa Δ R1 cells were analyzed by Western blots using antibodies against IRBIT, R1, and R2B. (C) IRBIT-RNR interaction during the cell cycle. IRBIT was immunoprecipitated from corresponding lysates and analyzed for the presence of R1. (D) Cell cycle progression of HeLa cells with or without IRBIT. M-to-M transition indicates timing between anaphase and prometaphase of the next cell cycle of an individual daughter cell ($n = 20$). Values are means $\pm$ SEM. Statistical differences were evaluated by Student's t test (** $P < 0.01$).

for S-site occupancy, but it was sensitive to ATP (Fig. 2B). In addition, glutathione *S*-transferase fused with human IRBIT (GST-hIRBIT) could bind preformed R1-dATP-dTTP complex (fig. S1F).

Physiological levels of adenosine triphosphate (ATP) (1 to 3 mM) disrupted the IRBIT-RNR-dATP complex, which indicated that RNR bound with IRBIT was still subject to competition between

ATP and dATP for A-site occupancy (Fig. 2B) (10). We suspected that some posttranslational modifications of IRBIT might increase its inhibitory potential. We analyzed the phosphorylation status of endogenous IRBIT by mass spectrometry and found that there are at least two phosphorylation sites. Both of them were mapped to the IRBIT^{G4-S7} domain: Ser68 and an undetermined residue within a SerSerThr motif (residues 70 to 72) (Fig. 2C). We compared complex formation between R1/R2B and either a phosphomimetic (4STD) or a nonphosphorylatable IRBIT domain (4STA) (Fig. 2C). Both domains bound to RNR in a dATP-dependent manner. However, high concentrations of ATP disrupted only 4STA-RNR-dATP complex but not 4STD-RNR-dATP complex, which indicated that IRBIT phosphorylation at these residues stabilizes dATP on R1 (Fig. 2D).

We hypothesized that IRBIT may recognize inactive, dATP-bound R1 and stabilize the enzyme in this state. To test this idea, we performed binding studies in the presence of IRBIT using [α - ^{32}P]dATP and R1/R2B saturated with dTTP in the S-site. The association constant of dATP binding to the A-site of R1 (k_{on}) was virtually unchanged in the absence or in the presence of IRBIT (Fig. 3C and figs. S2D and S3A), and the A-site on R1 was saturated at $\sim 3 \mu M$ of dATP (Fig. 3A). The dissociation rate of R1-dATP (k_{off}) was substantially inhibited in the presence of IRBIT (Fig. 3, B and C, and fig. S3), which indicated that IRBIT stabilizes dATP in the A-site of R1 (11). IRBIT did not substantially alter dTTP binding to the S-site whether dATP was present in the reaction or not (fig. S2C). The fragment of IRBIT that binds R1 (GST-IRBIT^{G4-S7}) was sufficient in itself to increase A-site affinity for dATP (fig. S2A).

To measure the consequences of this binding for RNR function, we analyzed RNR activity in vitro. IRBIT did not affect RNR activity in the absence of dATP (Fig. 3D). Preformed RNR-dATP complexes exhibited 20% less activity than RNR alone. As expected, RNR in the RNR-dATP-IRBIT

complex was substantially inhibited (Fig. 3D and fig. S3E).

To determine the consequences of RNR inhibition by IRBIT, we measured deoxynucleotide triphosphate (dNTP) concentration in HeLa cells that express Tet-inducible short hairpin RNA against IRBIT. IRBIT-depleted asynchronous HeLa cells showed an imbalanced pool of deoxynucleotides (Fig. 4A). These changes in dNTP levels were not due to the altered expression levels of the RNR subunits (Fig. 4B). Note that the sensitivity of dNTP levels to IRBIT depletion was more pronounced in mitosis but was minimal in G_1 cells (Fig. 4A), and coprecipitation experiments showed that IRBIT interacted with R1 more strongly during mitosis than during G_1 phase (Fig. 4C).

We analyzed cell cycle progression in IRBIT-depleted cells through live imaging of HeLa cells expressing histone H2B fused with green fluorescent protein (H2B-GFP). Most of the control HeLa cells underwent mitosis every 22 hours (Fig. 4D). In contrast, depletion of IRBIT resulted in a much greater variation in the duration of interphase between individual cells. Reintroduction of wild-type IRBIT to the depleted cells rescued this phenotype, whereas IRBIT^{S68A} did not (Fig. 4D), which indicated that IRBIT function depends on phosphorylation of this residue, as suggested by our *in vitro* results.

The N-terminal domain of IRBIT belongs to the class of IDP (intrinsically disordered protein or peptide) (12). Budding yeast Smlp is an IDP that binds R1 (13). We noticed that there is some similarity between the IRBIT^{64–87} domain and the central region of Smlp (amino acids 46 to 72), which was not previously implicated in interaction with RNR (fig. S4) (14). Smlp^{46–72}, like IRBIT^{64–87}, interacted with RNR in a dATP-dependent manner. We speculate that Smlp may use this domain to recognize dATP-bound R1 in a manner that may be analogous to that between Smlp and IRBIT (fig. S8).

Altogether, our results demonstrate that IRBIT interacts with RNR in a dATP-dependent manner and stabilizes dATP in the RNR A-site, potentially by stabilizing oligomeric form(s) of R1 formed in the presence of dATP (15–17) (fig. S1G). Because binding of dATP to the A-site is inhibitory to RNR activity, IRBIT inhibits RNR. Under normal physiological conditions, where ATP levels are high, such inhibition could only be achieved when IRBIT's binding is strengthened by phosphorylation. This mechanism is likely to be critical, because cells depleted of IRBIT show substantial alteration in their cell cycle progression and because IRBIT is indispensable for embryogenesis (18). It is also possible that IRBIT-RNR acts as a part of multimeric complexes (fig. S5).

In a larger context, we note that balanced control of deoxynucleotide levels is central to maintaining the genome in an accurate fashion (figs. S6 and S7) (19). Modulation of IRBIT binding offers a fundamentally different mechanism for RNR inhibition that may circumvent toxicity issues of current RNR drugs, and it thus offers a promising target for future drug development.

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ACKNOWLEDGMENTS

We are grateful to B. Cooperman and L. Thelander for reagents and for the help with RNR assay. We are indebted to M. F. Ahmad, I. Arnaoutova, P. Kalab, D. Mukhopadhyay, and C. Dealwis for their help and for the critical reading of the manuscript.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6203/1512/suppl/DC1
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References (20–22)

30 January 2014; accepted 21 August 2014
10.1126/science.1251550

GENE REPRESSION

H3K27me and PRC2 transmit a memory of repression across generations and during development

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For proper development, cells must retain patterns of gene expression and repression through cell division. Repression via methylation of histone H3 on Lys27 (H3K27me) by Polycomb repressive complex 2 (PRC2) is conserved, but its transmission is not well understood. Our studies suggest that PRC2 represses the X chromosomes in *Caenorhabditis elegans* germ cells, and this repression is transmitted to embryos by both sperm and oocytes. By generating embryos containing some chromosomes with and some without H3K27me, we show that, without PRC2, H3K27me is transmitted to daughter chromatids through several rounds of cell division. In embryos with PRC2, a mosaic H3K27me pattern persists through embryogenesis. These results demonstrate that H3K27me and PRC2 each contribute to epigenetically transmitting the memory of repression across generations and during development.

Proper development depends on regulation of gene expression by packaging the genome into expressed and repressed chromatin domains. Our understanding of how that packaging is achieved and maintained is incomplete. Methylation of histone H3 on Lys27 (H3K27me) is a well-established mark of repressed chromatin that is generated by Polycomb repressive complex 2 (PRC2) in diverse phyla. In *Drosophila*, PRC2 and H3K27me maintain repression of important genes, including the Hox genes during somatic development and cell cycle genes during germline development (1–3). In mammals, H3K27me is also present on develop-

mentally important genes in somatic and germ cells (4–6), and PRC2 serves numerous roles, including participation in X-chromosome inactivation and differentiation of embryonic stem cells (7, 8). In *Caenorhabditis elegans*, PRC2 is required only in germ cells where it participates in repression of the X chromosomes (9, 10). A critical question is how H3K27me-repressed chromatin states are passed from mother to daughter cells. One model is that H3K27-methylated histones are passed locally to the two daughter chromatids during DNA replication (11). Another model is that PRC2, but not methylated histones, is passed locally to daughter chromatids and newly establishes H3K27me after each round of DNA replication (12). We tested these models by examining cells containing or lacking PRC2 activity and with differentially H3K27-methylated chromosomes. We present evidence that H3K27-methylated histones transmit the memory of repression transgenerationally and short-term in embryos and

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that PRC2 promotes long-term memory during development.

C. elegans PRC2 is composed of MES-2 [ortholog of E(Z)/EZH2], MES-6 (ortholog of ESC/EED), and MES-3 (worm-specific subunit) and is essential for germline development but not somatic development (9, 13). PRC2 is maternally supplied to progeny and needed for the progeny's primordial germ cells (PGCs) to survive and proliferate. In both naturally occurring sexes in *C. elegans*, hermaphrodites with two X chromosomes (XX) and males with one X (XO), germline development requires repression of the X chromosomes (14). PRC2-generated H3K27me₃ participates in repressing the X chromosomes in XX germ cells: Repressive H3K27me₃ is on all chromosomes but is concentrated on the X chromosomes in germ cells (13), and loss of PRC2 function causes germ lines to up-regulate numerous genes on the X (10). Whether XO germ lines require PRC2 repression has been unclear (15). We demonstrate that PRC2 is the main mode of X repression in XO worms, but that PRC2 function can be bypassed if the single X in XO worms experienced two other repressive conditions in the parental germ line: spermatogenesis and H3K9me.

The X chromosomes in germ cells are globally repressed during most stages of development except during late oogenesis (14, 16). Thus, a sperm-inherited X has a repressed transcriptional "history," whereas an oocyte-inherited X lost repression before fertilization. We analyzed XO *mes* mutant males that inherited their X from these two different backgrounds (Fig. 1 and fig. S1). XO *mes-3* mutants with an oocyte-inherited X (X^{oo}) had a severely underproliferated germ line, lacked sperm, and 0% were fertile. In striking contrast, XO *mes-3* mutants with a sperm-inherited X (X^{sp}) generally had a well-proliferated germ line, and 73% were fertile. Similar results were ob-

served for *mes-2* and *mes-6* mutants (fig. S1, A and D). We tested whether the gamete source of the X correlates with subsequent repression or expression of the X in male germ lines, using an X-linked *lmn-1::GFP* transgene that in wild-type worms is expressed in somatic cells and silenced in the germ line (17) (fig. S2). *lmn-1::GFP* was repressed in 91% of germ lines from XO (X^{sp}) *mes-3* mutants but in only 5% of XO (X^{oo}) *mes-3* mutants (Fig. 1 and fig. S2). These values are similar to the percentages of fertile XO *mes-3* mutants (Fig. 1). Our findings suggest that fertility depends on continued X-chromosome repression in the germ line, which requires inheriting a repressed X. Although PRC2 likely modulates many aspects of gene expression in ways that are not essential for viability or fertility, our finding that XO worms with a sperm-inherited X do not require PRC2 suggests that the only essential role of

PRC2 in worms is repression of the X chromosomes in germ cells.

We reasoned that, in mutants lacking PRC2 and H3K27me, sperm may contribute to embryos an X chromosome repressed by an alternative mechanism, such as H3K9me. H3K27me is generally associated with developmentally regulated repression and H3K9me with repression via heterochromatin formation (18). To test if H3K9me is required for XO (X^{sp}) *mes* mutants to be fertile, we analyzed *mes-3* mutants also mutant for *met-2* and *set-25* (lacking H3K9me) (19). With lack of both H3K27me and H3K9me, XO worms had severely underproliferated germ lines, and 0% were fertile (Fig. 1). Our findings show that H3K9 methylation provides an alternative mode of transmitting X repression to progeny. H3K9me likely enables X^{sp} to retain the heterochromatic state it experienced during spermatogenesis.

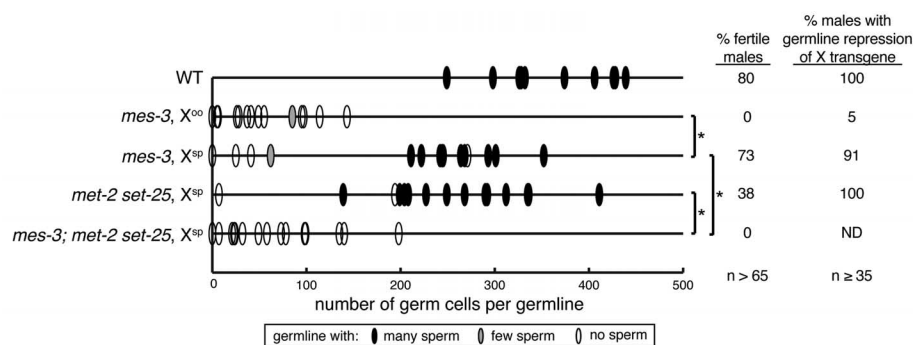


Fig. 1. XO males with a sperm-inherited X do not require H3K27me and rely on H3K9me as an alternative mechanism of X repression. Analysis of germline proliferation, presence of sperm, fertility, and germline repression of an X-linked transgene in males with an oocyte-inherited X (X^{oo}) or sperm-inherited X (X^{sp}): wild type (WT), lacking H3K27me (*mes-3*), lacking H3K9me (*met-2 set-25*), or lacking both. Statistically significant differences between genotypes, * $P < 0.01$ Mann-Whitney U test. Also see figs. S1 and S2.

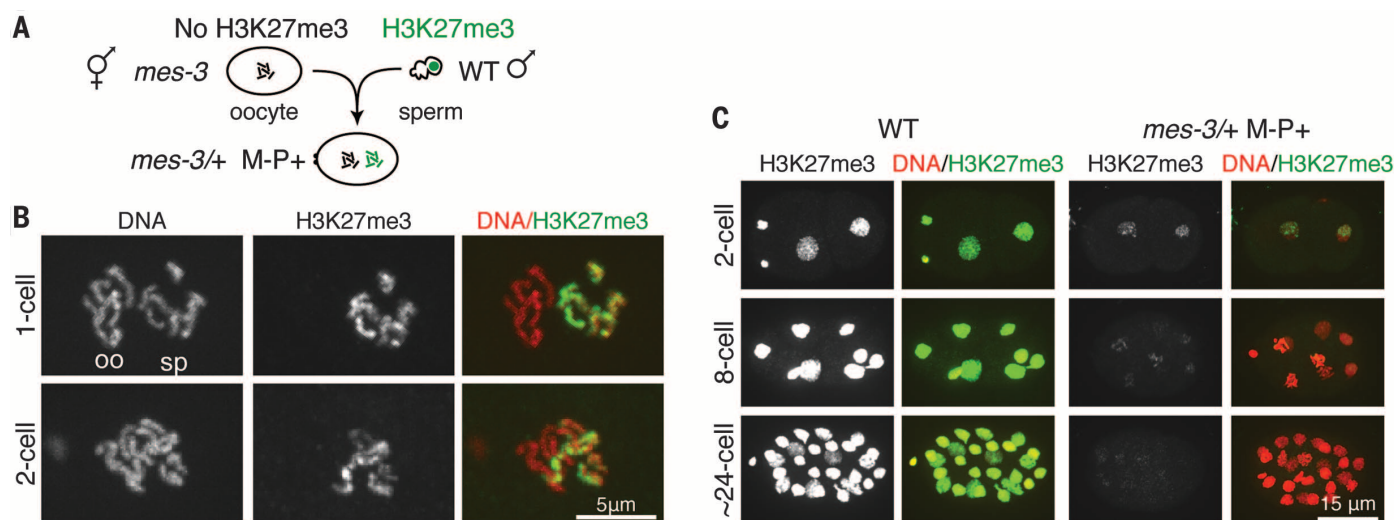


Fig. 2. Repressive H3K27me₃ is transmitted to embryos on sperm chromosomes and without PRC2 is transmitted through cell divisions. (A) Diagram of oocyte and sperm union to generate M-P+ embryos with H3K27me₃ inherited from the sperm (P+) but not the oocyte (M-) and lacking maternal and paternal HMT. (B) Images of the two pronuclei in a one-cell embryo, and a diploid nucleus in a two-cell embryo. Merge panels show DNA (red) and H3K27me₃ (green). The polar bodies [shown in (A)] identify the pronuclei as oocyte-derived (oo) or sperm-derived (sp). (C) Perdurance of paternally inherited H3K27me₃ on a subset of chromosomes until the ~24-cell stage. Also see fig. S5, and fig. S6 for wild type.

Our data suggest that the memory of X-chromosome repression can be inherited through sperm. As sperm mature in *C. elegans*, some histones are exchanged for sperm-specific histone variants and putative protamine-like proteins (20). H3K4me has been detected in *C. elegans* sperm (21). Our analysis revealed that H3K27me3 and H3K9me2 are also both present in mature sperm (fig. S3A) and transmitted to embryos via sperm (Fig. 2). To track the inheritance of sperm histone modifications in embryos, we analyzed embryos that could inherit histone modifications on paternally contributed chromosomes (P+) but not on maternally contributed chromosomes (M-) (Fig. 2A).

In M-P+ one-cell embryos from oocytes lacking H3K27me3 (*mes-3*) fertilized by wild-type sperm with H3K27me3, we observed H3K27me3 on all sperm-contributed chromosomes but not on oocyte-contributed chromosomes (Fig. 2B). In M-P+ one-cell embryos from oocytes lacking H3K9me (*met-2 set-25*) fertilized by wild-type sperm, we observed H3K9me2 lightly on the sperm-contributed autosomes and heavily on the sperm-contributed X, similar to its pattern in the male germ line (22) (fig. S4C). These results demonstrate that the repressive histone marks H3K27me3 and H3K9me2 are transmitted to embryos by wild-type sperm.

Fig. 3. PRC2 maintains the memory of repression on gamete-of-origin chromosomes.

(A) Diagram of oocyte and sperm union to generate M+P- embryos with H3K27me3 inherited from the oocyte (M+) but not the sperm (P-) and containing maternal HMT. (B) Images (as described for Fig. 2B) of M+P- embryos with a III-X-IV fusion chromosome in the oocyte (M+) chromosome set (arrow). See fig. S6 for wild type.

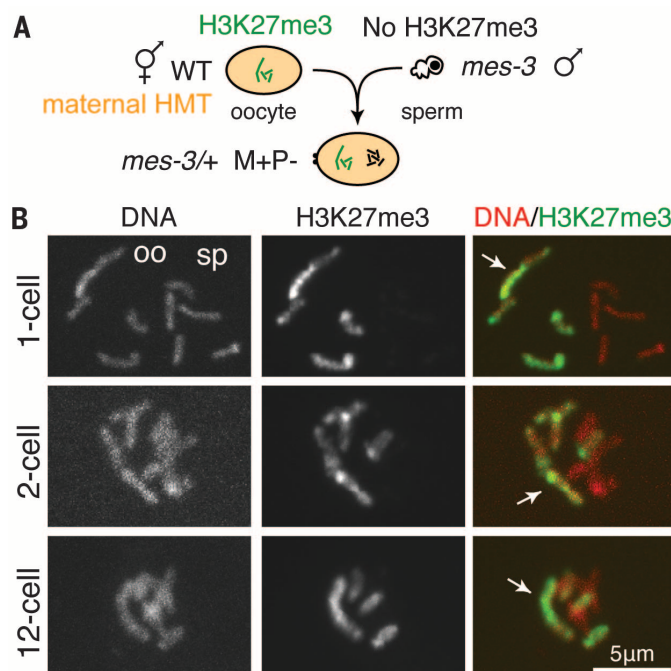
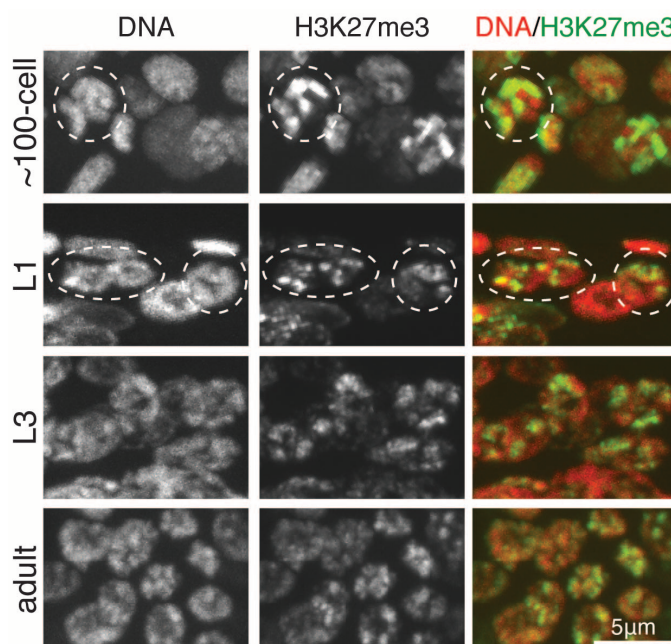


Fig. 4. The memory of repression is maintained by PRC2 during embryogenesis and is newly established during germ cell development in larvae.

Images of worms generated as in Fig. 3. M+P- ~100-cell embryo (one nucleus circled), L1 larva (germ nuclei circled), and L3 and adult germ lines. See fig. S6 for wild type.



mes M-P+ embryos lack a maternal load of PRC2 and do not inherit PRC2 by way of the sperm (fig. S3, B to D), which offers an opportunity to test if H3K27me3 on paternal chromosomes can be passed to daughter chromatids in the absence of histone methyltransferase (HMT) activity. In M-P+ embryos, H3K27me3 declined with age but persisted at easily visible levels on chromatin until the 16- to 24-cell stage, through at least four rounds of DNA replication (Fig. 2C and fig. S5C). H3K27me3 remained associated with a subset of chromosomes, likely sperm-derived chromosomes, and did not detectably spread to all chromosomes in each diploid nucleus (Fig. 2, B and C). We verified that two of the H3K27me3-stained chromosomes were sperm-derived (fig. S5, A and B). A similar pattern was observed for H3K9me2 in two-cell M-P+ embryos lacking MET-2 and SET-25 HMT activity (fig. S4C). Our results separate histone and HMT inheritance and demonstrate that modified histones can remain associated with DNA through several rounds of replication. These results also suggest that the memory of repression inherited on sperm chromosomes is transmitted through early embryo development.

To investigate propagation of repressive marks by ongoing methylation in the embryo, we generated embryos with HMT activity (maternally supplied in early embryos and transcribed from the embryonic genome in later-stage embryos) that contained histone marks on some but not all chromosomes. We analyzed M+P- embryos with H3K27me3 on oocyte-derived chromosomes but not on sperm-derived chromosomes (Fig. 3A). In such M+P- embryos, PRC2 maintained high levels of H3K27me3, and H3K27me3 remained restricted to one set of chromosomes throughout embryo development (Figs. 3 and 4). We verified H3K27me3-stained chromosomes to be oocyte-derived by using a maternally contributed III-X-IV fusion chromosome (Fig. 3). In the germ lines of larvae, we observed H3K27me3 gradually become detectable on all chromosomes as larval development progressed (Fig. 4). This spreading of H3K27me3 to all chromosomes is probably the result of germ cells turning on their transcriptional program and establishing repressed chromatin domains de novo. We speculate that H3K27me3-repressed chromatin is newly established during larval germline development each generation. In contrast to H3K27me3, maternally inherited H3K9me2 in the presence of H3K9 HMT activity spread to all chromosomes by the two-cell stage (fig. S4D). This difference in histone mark propagation may underlie *C. elegans*' reliance on PRC2 for transgenerational regulation of repression. Both H3K27me and H3K9me provide short-term memory, but only PRC2 methylation of H3K27 provides long-term epigenetic memory to embryos.

Our findings demonstrate that PRC2-generated H3K27 methylation is epigenetically transmitted across generations and, in *C. elegans*, is important for transmitting the memory of X-chromosome repression in the germ line. H3K27me3 is delivered to embryos on the chromosomes from both oocyte and sperm. In the absence of PRC2,

H3K27me3 remains associated with gamete-of-origin chromosomes through several rounds of cell division (fig. S7), consistent with passage of modified histones in cis and locally during DNA replication (11, 23). In the presence of PRC2, H3K27me3 is propagated on gamete-of-origin chromosomes through embryogenesis (fig. S7). We speculate that worm PRC2 perpetuates paternally and maternally inherited patterns of repression through embryogenesis using a mechanism similar to mammalian PRC2: recruitment via its EED subunit to preexisting H3K27me3 and the resulting stimulation of HMT activity on neighboring unmethylated H3K27 (24). As in worms, other organisms may use a maternal supply of PRC2 to propagate inherited chromatin repression in embryos (25, 26); in worms, this transmits the memory of gene repression from parent germ lines to PGCs in progeny. As worm PGCs activate their transcription program in larvae, PRC2 likely catalyzes de novo H3K27me3 on all chromosomes in a pattern dictated by transcription factors, non-coding RNAs, nascent RNAs, and antagonism by marks of active chromatin (e.g., H3K4me and H3K36me) (10, 27–29).

Our studies in worms provide precedents for transmission of sperm H3K27me3 patterns to embryos and for important developmental consequences of paternal marking. Paternal inheritance could be similarly important in mammals, where H3K27me3 is present on sperm chromatin in a pattern that suggests involvement in em-

bryogenesis (5, 30). Many of these H3K27me3 targets are also repressed in PGCs (30), where H3K27me3 increases just before epigenetic reorganization of the genome and may facilitate reacquisition of pluripotency by germ cells (31, 32). Thus, two common themes for PRC2 function in germ cells may be transmission of a memory of repression from parents to offspring and protection of the immortal and totipotent properties of germ cells.

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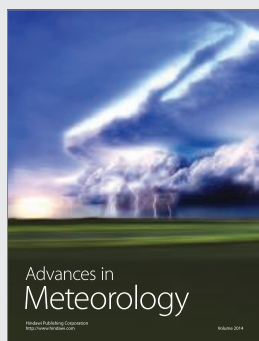
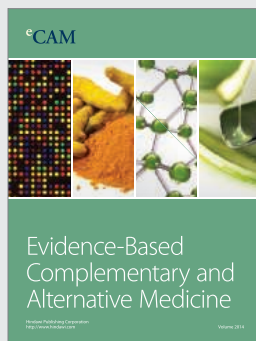
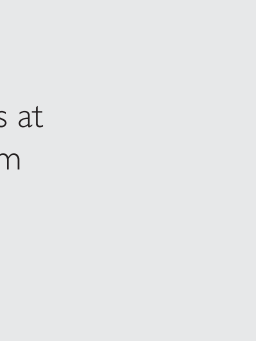
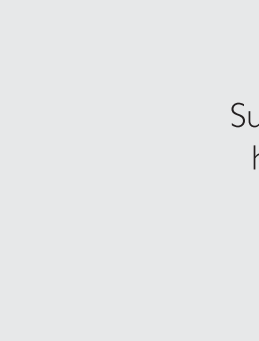
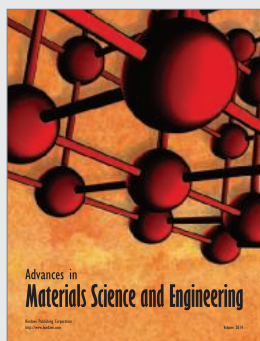
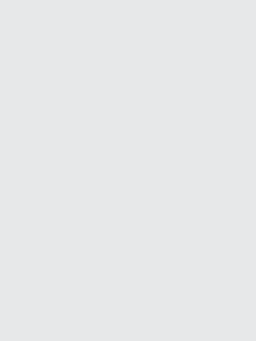
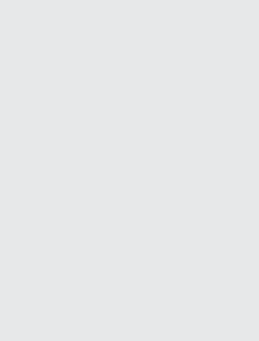
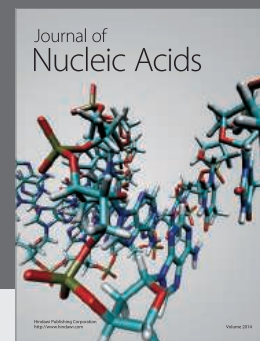
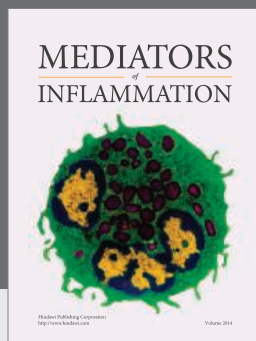
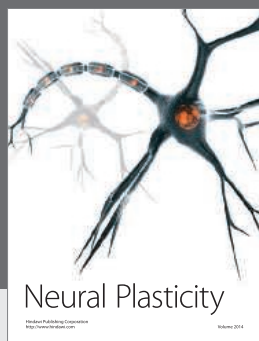
ACKNOWLEDGMENTS

We thank Strome laboratory members, N. Bhalla, and J. Tamkun for helpful discussions; H. Kimura for antibodies; and the *Caenorhabditis* Genetics Center for some strains (funded by NIH-ORIP-P40OD010440). This work was supported by NIH-T32GM008646, UCSC Dissertation-Year Fellowship, and Achievement Rewards for College Scientists Foundation Award to L.J.G. and NIH-R01GM034059 to S.S.

SUPPLEMENTARY MATERIALS

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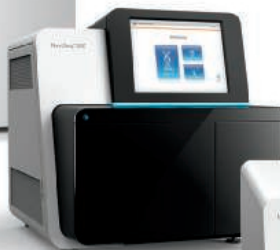
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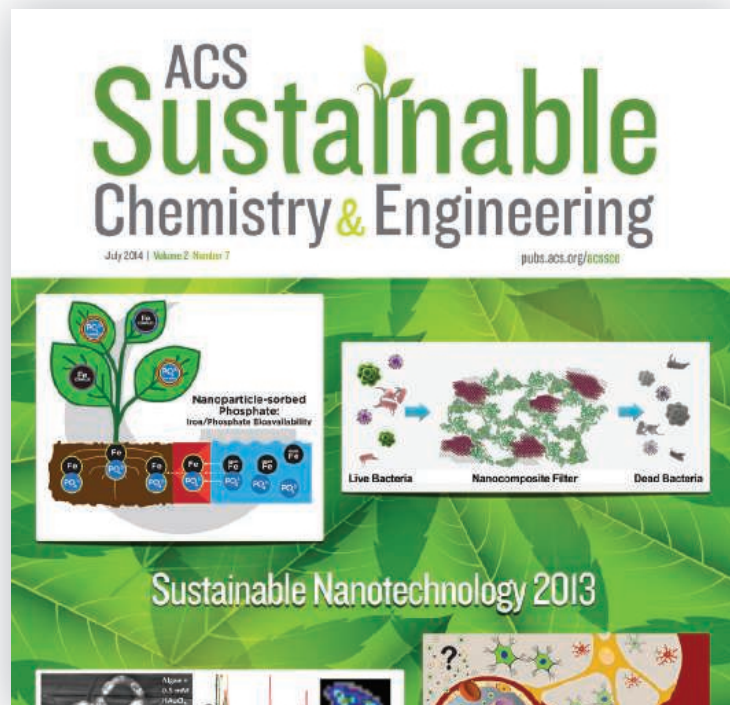
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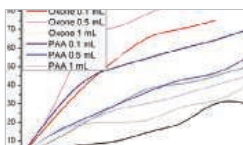
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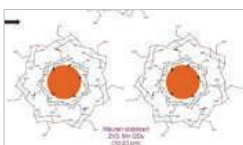
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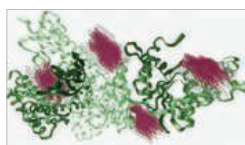
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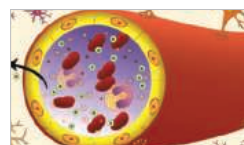
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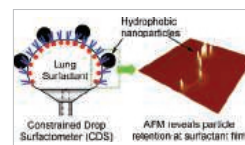
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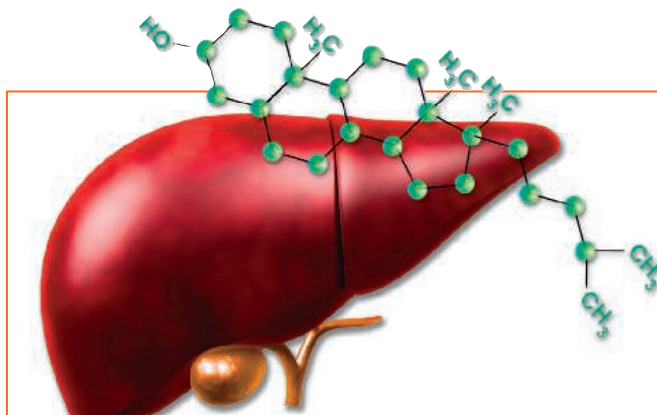


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What's Next in 'Omics: The Metabolome

The youngest sibling in the family of 'omics fields is growing up. Maturing right behind genomics, transcriptomics, and proteomics, metabolomics is the comprehensive analysis of small molecule metabolites. Since most metabolites are generated by enzymatic proteins that result from gene expression, and metabolites give organisms their biochemical characteristics, the metabolome links genotype with phenotype. Metabolomics is still developing, though, as vendors adapt separation and detection instruments to meet its challenges and the research community interprets and integrates the complex data they are acquiring. **By Chris Tachibana**

We're just a bunch of chemical reactions, says David Wishart, professor of biological science and computing science at the **University of Alberta, Canada**, "so the genome and proteome evolved to support the metabolome, not the other way around." Metabolomics offers a more immediate measure of physiology than other 'omics approaches, says Wishart. The metabolome responds to nutrients, stress, or disease long before the transcriptome or proteome. This makes metabolomics an attractive approach for multiple fields: environmental toxicology, evolution and development, disease diagnosis and treatment response, and the development of drugs, pesticides, and herbicides. Metabolomic flux analysis aids synthetic biologists by revealing how genetic changes affect pathways and products.

Metabolomics, the youngest field in the 'omics family, is growing rapidly. In 2014, the International Metabolomics Society

held its 10th annual conference in the same city in Japan as the first conference: Tsuruoka. "The first year, we had about 150 people," says Ute Roessner, **University of Melbourne** associate professor and leader of the Metabolomics Australia node at her university. "This year we had more than 500 registrants. And metabolomics is becoming part of every researcher's toolkit. Young researchers now routinely do metabolomics as part of their Ph.D."

The major challenges of metabolomics stem from its advantages. Metabolomic data are powerful because organisms have many metabolites including related precursors, derivatives, and degradation products with concentrations that vary dramatically and change rapidly. This complexity demands sophisticated separation and detection methods. Metabolomics includes analysis of lipids, a developing area with tremendous potential, says Yingying Huang, strategic marketing manager at **Thermo Fisher Scientific**. "Lipidomics is directly related to cardiovascular disease, cancer, diabetes, and obesity," she says, "but lipids have huge diversity in structure and concentrations that vary 10-fold in the body." Many physiological lipids and other metabolites share a chemical composition, differing only in arrangement, which led **University of California Davis** Professor and West Coast Metabolomics Center Director Oliver Fiehn to call metabolomics, "the science of isomers."

The instrument decision tree

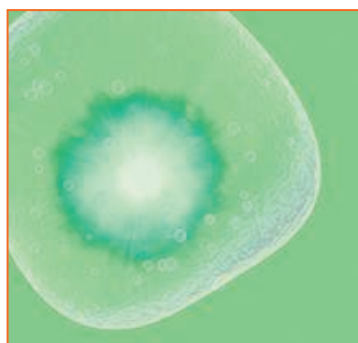
Rising interest in metabolomics has led vendors of molecular separation and detection equipment to develop hardware, software, and support specifically for metabolomics. Researchers now have a complex decision tree to navigate when adding this technology to their laboratory. The first branch is untargeted vs. targeted analysis. The untargeted approach gathers data on all possible compounds. Targeted analysis focuses on known compounds, often to quantitate changes, for example in response to stress or disease.

Nuclear magnetic resonance (NMR) spectroscopy is particularly powerful for targeted analysis because it is quantitative, reproducible, and suitable for complex samples such as blood, urine, or tissue extracts with little or no processing. Peter Würtz, head of molecular epidemiology, **University of Oulu, Finland**, recently coauthored a paper that applied NMR metabolomics using Bruker instruments to thousands of Estonian and Finnish biobank samples. The research team discovered that four biomarkers, when combined, are significantly associated with increased risk of short-term death from all causes. The study was observational, with no exploration of mechanisms, notes Würtz, but shows the power of NMR for biomarker analysis. However, use of NMR for metabolomics is limited because its sensitivity is orders of magnitude lower than the more popular metabolomics method: mass spectrometry (MS).

MS requires sample processing. Compounds are often ionized and usually undergo separation, with gas chromatography (GC) and liquid chromatography (LC) the most common **continued**

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Capillary electrophoresis gives excellent separation of polar metabolites and can be used with samples as small as a single cell.

LC-MS is particularly suitable for large, thermo-unstable organic molecules including many secondary metabolites, larger carbohydrates, and lipids. Advances such as ultraperformance LC (UPLC)—originally developed by Waters—supercritical fluid chromatography, and ion mobility separation have increased speed and separating power, including for nonpolar molecules and compounds with many isomers and isobars such as lipids. Separation methods are coupled to mass analyzer options such as time-of-flight (TOF) detection MS, which is fast and has high resolution over a large mass range. Adding quadrupole or linear ion traps allows selection of particular compounds or fragments of compounds generated during ionization. Once researchers have sorted their metabolomics priorities, they can choose between buying equipment and sending samples to a service.

Vendor and service options

Agilent offers GC-MS, LC-MS, CE-MS, and NMR instruments. “We are in the unique position of offering all these technologies from a single vendor,” says Sana, “including hardware, software, and technical support.” (Shimadzu also offers multiple systems that researchers recommend as inexpensive starter setups.) Users compliment Agilent on their intuitive software and Sana confirms this is a company priority. An example is the Agilent GeneSpring-Mass Profiler Professional software for integrated analysis of multiple ‘omics data. “GeneSpring lets you take disparate data from RNA, proteins, and metabolites and dynamically map them on biological pathways like those from KEGG [a metabolic pathway tool],” says Sana. GeneSpring supports preprocessed results from other vendor platforms, for example as a spreadsheet. Visualizing results from a large number of experiments using a pathway tool is useful for gleaning biological insights and planning subsequent experiments, says Sana.

AB SCIEX specializes in LC-MS instruments that researchers praise for fast data acquisition, excellent dynamic range, and

methods. Capillary electrophoresis (CE) gives excellent separation of polar metabolites and can be used with samples as small as a single cell. CE-MS is a specialty of Human Metabolome Technologies (HMT), but is also offered by others such as **Agilent** and **AB SCIEX**. However, CE requires specialized expertise and powerful software to account for drift in migration times, says Theodore Sana, metabolomics program manager at Agilent.

GC-MS is powerful for resolving compounds that are volatile or can be volatilized through derivation such as the small molecules of primary central metabolism.

quantitation and accurate mass capabilities (generally defined as accuracy to five parts per million or lower). The company also offers CE and ion mobility options. For metabolomics, AB SCIEX recommends the Triple TOF and QTrap systems. “Our Triple TOF is a high-resolution, accurate mass instrument for qualitative and quantitative analysis, suitable for discovery metabolomics,” says Fadi Abdi, senior market manager. “The Qtrap is for sensitive, targeted, quantitative workflows.” The high acquisition speed of the Triple TOF series allows simultaneous MS and tandem MS (MS-MS) from a single injection in an LC timescale, explains Abdi, for identification, confirmation, and quantitation of compounds. Qtrap multiple reaction monitoring provides data on a parent compound and fragments successively generated by MS, simplifying data processing and giving researchers confidence about their data, says Baljit Ubhi, staff scientist in metabolomics and lipidomics applications at AB SCIEX.

Researchers looking for broad metabolite coverage can consider packages that combine high-resolution LC-MS systems from AB SCIEX with high-resolution GC-MS systems from **LECO**. The partnership offers clients “the best of both technologies,” says Jeff Patrick, LECO director of marketed technologies. The packages include software from Genedata that integrates data from the two platforms for comprehensive metabolomics analysis.

Other popular, versatile LC-MS options are the Thermo Fisher Scientific orbitrap-based instruments. Orbitrap mass analyzers are commonly used for proteomics but the high resolving power is particularly suitable for metabolomics when combined with an advanced separation system such as UPLC, says Huang. Parallel detection eliminates multiple runs for faster sample analysis. The newest system in the Thermo Scientific line is the Orbitrap Fusion Tribrid with three mass analyzers. After LC separation, sample filtering occurs in a quadrupole with parallel analysis possible in the subsequent orbitrap and linear ion trap. The Tribrid system was designed to offer researchers flexibility along with accurate mass capability, says Huang.

Thermo Fisher, AB SCIEX, and Bruker also offer instruments for imaging MS, also called MALDI imaging. Roessner explains that this method overcomes a metabolomics limitation: metabolite analysis is usually done on homogenized samples or extracts, but specialized cells and tissues have different metabolite profiles. Imaging MS gives data on the spatial distribution of metabolites from a thin tissue section, for example from an organ or plant tissue. The process is laser desorption of a matrix-treated sample to ionize compounds that are then analyzed by MS. A reconstructed two-dimensional image of the compounds provides the location of metabolites across the tissue.

“It’s still early, but it’s an exciting method,” says Roessner, who uses a Bruker system for this technique. “It can be used to see the distribution of drug-related metabolites in a liver sample or to follow alterations in metabolite distribution in tissues after environmental stimuli.” The Thermo Scientific Orbitrap is advantageous for this method, Huang says. “Since the ions are coming from the tissue with no LC separation, you need an ultrahigh-resolution instrument like the Orbitrap.”



Researchers who aren't ready to purchase a system or prefer the convenience of a service have several options. Biocrates and **Metabolon** offer MS metabolomic analysis worldwide. Metabolon is also involved in large, long-term collaborations, such as Craig Venter's Human Longevity project, says Chris Bernard, Metabolon senior vice president,

sales and marketing. Collaborating is important for Metabolon, says Bernard, for seeing how metabolomics is practically implemented and how it is used in a range of projects.

Scientists interested in the advantages of NMR metabolomics—true quantitation without standard curves, sample preservation and minimal sample processing, and detection of all metabolites with structural information in a single run—might be hindered by the startup costs. Researchers can access this method through services, for example from Chenomx. Würtz and colleagues also offer NMR metabolomic services and collaborations through **Brainshake**, founded on their experience using NMR for biomarker research and linking metabolomics data to genome-wide association studies that connect genetic variation with disease risk.

Metabolomics services are increasingly available through national and academic core facilities. In Australia, Roessner established one of the first government-funded national metabolomics service facilities. Canada is also funding national metabolomics initiatives and other countries are following suit. In the U.S., the National Institutes of Health (NIH) Common Fund has been establishing regional metabolomics cores since 2012. Fiehn leads the West Coast program. "The cores increase metabolomics capacity and services for both academics and industry and they have different specialties," he says, "such as metabolic flux in the Kentucky center and NMR in North Carolina." The NIH core facilities also offer short courses for scientists new to metabolomics.

The next challenges

When asked about the major challenges facing metabolomics, researchers and vendors consistently name three issues: identification of unknowns, development of standardized data repositories that can be queried like the genomics resource GenBank, and integration of metabolomics and other systems-wide data.

"MS is so sensitive that we get lots of compounds and we don't know what they all are," says Wishart. To help researchers with metabolite identification, Agilent has curated GC-MS and LC-MS spectral libraries for metabolite identification with MS-based techniques. LECO offers a GC-TOF library that Patrick says can be used with any GC data, although it is optimized

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for LECO instruments. LECO is currently exploring opportunities to collaborate on high-resolution accurate mass libraries.

Wishart notes that most unknowns are metabolites of metabolites: "breakdown products and molecules transformed by enzyme or microbial activity." This molecular similarity can be the key to identifying them. The Fiehn group, which devel-

oped several of the commercially available MS libraries, is also producing virtual libraries. Starting with known spectral data on compounds, the researchers predict spectra of similar or modified variants. LipidBlast is the Fiehn group's free downloadable MS-MS virtual spectra library for more than 200,000 lipids.

To meet challenges of searchability and data integration, the metabolomics community has several initiatives to establish data repositories. Examples are Metabolights in the United Kingdom, supported by the European COSMOS (COordination of Standards in MetabOmicS) consortium that is developing metabolomics data standards, and Metabolomics Workbench, which aims to be the database for NIH-funded metabolomics projects. Thermo Fisher collaborates with Fiehn on mzcloud.org, a free community database that includes actual and virtual MS spectra with unknown compounds to be annotated as they are identified.

Even after the metabolomics community has databases to corral its data, Patrick notes that "an overarching challenge for systems biology is biological inference"—giving context and meaning to large amounts of data. The solution, he says is software, which "is driving the direction of metabolomics." Bernard agrees, saying that the number-one request of Metabolon's clients is help in understanding the biological meaning of results. "MS is wonderful for generating data," he says. "The challenge is separating signals from noise: 85% of MS data points is noise." Metabolon clients have access to the MetaboLync portal which includes a list of metabolites in their sample with comparison to an in-house library of over 14,000 compounds for automatically verifying metabolite identification, says Bernard, adding that the most powerful feature of the portal is the ability to visualize and explore data at the pathway level.

As the field grows, vendors and service providers are eager to collaborate with and hear from users about their metabolomics research, not just for sales but to stay current. "We collaborate closely with several investigators," says Sana. "Researchers are frequently the source of novel ideas for advancing the field of metabolomics."

Chris Tachibana is a science writer based in Seattle, USA, and Copenhagen, Denmark.

DOI: 10.1126/science.opms.p1400088



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DIRECTOR Julius L. Chambers BIOMEDICAL/BIOTECHNOLOGY RESEARCH INSTITUTE

North Carolina Central University (NCCU) invites applications for the position of Director, Julius L. Chambers Biomedical/Biotechnology Research Institute (BBRI). The Director of BBRI reports to the Vice Chancellor for Research and Economic Development; and, provides leadership for a broadly based research portfolio which includes cancer, cardio-metabolic disorders, neuroscience and nutrition. BBRI's research programs are conducted on the University's main campus in Durham, NC and the North Carolina Research Campus in Kannapolis, NC. The incumbent must hold the earned doctorate degree (Ph.D.) or equivalent doctoral degree in the biomedical sciences; or (M.D.) degree with relevant research experience; and have experience as a researcher and administrator. The incumbent must have a strong track record of grant funding from major funding agencies (e.g., NIH, NSF, ACS, DOD), and research publications. Due to the proximity of NCCU to North Carolina's Research Triangle Park and other colleges and universities, the Director is expected to forge additional long-term successful partnerships among various academic units and faculty at NCCU in the biomedical, behavioral and social sciences as well as with agencies, corporations and academic research institutions within Research Triangle Park, throughout the state of North Carolina and beyond.

Established in 1999, the BBRI facility provides 40,000 sq. ft. of basic research space. The BBRI is an innovative research and training institute dedicated to the advancement of fundamental knowledge of human diseases, particularly those that disproportionately affect underrepresented minority groups.

This is a twelve-month administrative research appointment with the opportunity to maintain and continue a sustained robust research agenda post-administrative appointment. Rank and salary depend upon qualifications and experience. Review of applications will begin on **October 28, 2014** and will continue until the position is filled. Please send a letter of application, curriculum vitae, names and contact information of five references to:

Carol Hicks, Executive Assistant
Division of Research and Economic Development
North Carolina Central University
Room 309 Hubbard-Totton Building
1801 Fayetteville Street
Durham, NC 27707
or via Email:
Carol.Burnette@nccu.edu

UC DAVIS FACULTY POSITIONS THE CENTER FOR COMPARATIVE MEDICINE Schools of Medicine and Veterinary Medicine, University of California, Davis

The Center for Comparative Medicine (<http://ccm.ucdavis.edu>), a University of California-Davis center engaged in investigative research involving animal models of human disease, and cosponsored by the Schools of Medicine (<http://www.ucdmc.ucdavis.edu/medschool>) and Veterinary Medicine (www.vetmed.ucdavis.edu), is seeking candidates for 2 full-time academic positions at the rank of Assistant or Associate Professor in the Ladder Rank and In-Residence series. Successful candidates must have a PhD, and/or MD (School of Medicine, 1 position) or PhD and/or DVM (School of Veterinary Medicine, 1 position) and postdoctoral experience and a record of publication in high-quality journals. Applicants must have active research programs involving human diseases and/or the use of animal models of human diseases with an emphasis on infectious disease and host-responses to infection. Candidates are expected to have or to establish and maintain a strong extramurally funded research program and to actively participate in the academic and service missions of the Center and the University. Candidates must possess excellent interpersonal and communication skills and demonstrated ability to work with others in a collegial team atmosphere. Ample office and laboratory space is available in the Center (including access to BSL2 and BSL3 laboratory space), with state-of-the-art facilities, instrumentation, and administrative support. Center research and teaching programs interdigitate with other campus-wide programs and resources in the Schools of Medicine and Veterinary Medicine, the Mouse Biology Program, the California National Primate Research Center, and the Cancer Center. Each appointment will be in an appropriate academic department within the School of Medicine or School of Veterinary Medicine and will receive 0.5 FTE salary support and start-up funds to support the research program. The remainder of the salary support is expected to be recovered through the candidates extramurally supported research program. Review of applications will commence immediately until the position is filled. Priority will be given to applications received by **October 31, 2014**. Please use the links provided below to submit applications with a letter of interest, curriculum vitae, concise statement of present and future research plans, summary of teaching experience, 3 representative reprints, and names of 3-5 references (including addresses, telephone numbers and e-mail addresses),

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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 over 27,800 undergraduate and postgraduate students who are recruited globally, and more than 2,000 members of academic staff coming from 50 countries, 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, 2015.

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:

- Real Estate and Construction
- School of English
- Faculty of Dentistry
- Faculty of Education
- Civil Engineering
- Electrical and Electronic Engineering
- Mechanical Engineering
- Centre for Cancer Research
- School of Chinese Medicine
- Research Centre of Heart, Brain, Hormone and Healthy Aging
- Medicine
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- Public Health Research Centre
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- School of Biological Sciences
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- The State Key Laboratory of Emerging Infectious Diseases
- The State Key Laboratory for Liver Research

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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 webpage at <http://jobs.hku.hk/> to view the list of the Faculties/Departments/Schools/Centres 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 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>. Further particulars can be obtained at <http://jobs.hku.hk/>. **Closes October 15, 2014.** The University thanks applicants for their interest, but advises that only shortlisted applicants will be notified of the application result.

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Please refer to the following web page <http://apptkr.com/502153> for eligibility requirements and instructions on how to apply. When applying and prompted, please mention where you saw this ad. The deadline for applications is November 1, 2014. LLNL is operated by the Lawrence Livermore National Security, LLC for the U.S. Department of Energy, National Nuclear Security Administration. We are an equal opportunity employer with a commitment to workforce diversity.



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The Monash Faculty of Biomedical and Psychological Sciences is sponsoring a total of four new EMBL Australia Group Leader positions in Electron Microscopy, Protein Crystallography, Developmental Biology, Bioinformatics and Regenerative Medicine.

Group Leaders in Electron Microscopy and Protein Crystallography (2 positions):

We seek future research leaders in Electron Microscopy (EM) and in protein crystallography. In the EM field we are interested in recruiting scientists with expertise in correlative light and electron microscopy (CLEM), high-resolution electron tomography or in determining protein structures at high-resolution using EM. In the protein crystallography field we particularly encourage researchers with outstanding track records in working with challenging membrane protein systems to apply. The new recruits will form a key part of the Biomedical Discovery Institute being developed within the Faculty.

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More details are available at www.imagingcoe.org, and <http://www.med.monash.edu.au/biomed-psych/>

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We seek applicants with research interests in broad aspects of development and regeneration, including but not limited to applications of evolution, cancer, immunology, mechanisms of stem cell action and pluripotency, adult stem cell biology and therapies, applying bioengineering, bioinformatics or systems approaches to conduct interdisciplinary research in areas of regenerative medicine.

Successful applicants will have access to advanced gene editing and genetic modeling in mammals, world class zebrafish and axolotl facilities, monoclonal antibody production, state-of-the-art light and electron microscopy, a dedicated medical beamline at the adjoining Australian Synchrotron and excellent computational resources.

The Faculty's Australian Regenerative Medicine Institute (ARMI) seeks to exploit the extraordinary evolutionary variation in regenerative capacity amongst animal species and in our own embryonic development to uncover fundamental attributes of effective organ and tissue repair, applied to the restoration of damaged tissues and regenerating the ageing human body.

ARMI is the national headquarters of EMBL Australia, the Systems Biology Institute- Australia and the Monash headquarters of Stem Cells Australia. ARMI researchers benefit from national collaborative agreements with neighbouring CSIRO, Australian Nuclear Science and Technology Organisation, Australian Institute for Marine Sciences, and international partnerships with RIKEN (Japan) and The Jackson Laboratory (USA)

More details are available at www.armi.org and <http://www.med.monash.edu.au/biomed-psych/>

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An initial contract of 5 years will be offered to the successful candidate. This is foreseen to be extended to a maximum of 9 years, subject to an external review. The EMBL Australia Partner Laboratory is based on the EMBL model, with distributed, highly integrated research nodes focusing on complementary aspects of biological research. Currently there are three nodes: at Monash University's Australian Regenerative Medicine Institute, the University of Queensland's EMBL Australia Bioinformatics Resource and at SAHMRI in South Australia. Current research partners in EMBL Australia include the Group of Eight universities, CSIRO and SAHMRI. More details at www.emblaustralia.org

To apply for a position

Please email (in English) a cover letter clearly stating position(s) being applied for, your CV, names and contact details of 3 referees, and a summary of current and future research interests to: positions@emblaustralia.org

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**National Institute for Basic Biology
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The National Institute for Basic Biology (NIBB), Okazaki, Japan, is inviting applications for three full professorships. Since being established following a recommendation by the Science Council of Japan in 1977, NIBB has been conducting research activities as a center for biological sciences. Such research activities are among the highest ranked in the field of biology in Japan. To continue efforts to serve as a national center for biology, NIBB recruits leading scientists not only from Japan but also from abroad. We seek candidates with excellent research records who can internationally lead the field of basic biology, in particular those answering fundamental questions in biology, specifically in:

- Plant science
- Neuroscience

In addition, we also welcome applications from candidates of any fields that have excellent potential to advance basic biology.

Each professor plays an important role in NIBB's continuing efforts to serve as a national research center for basic biology and is appointed as an adjunct professor of the Graduate University of Advanced Studies (SOKENDAI) and as such should be actively involved in the education of graduate students. The candidate should be fluent in either Japanese or English.

Application deadline: October 31, 2014.

Detailed Information available at:

<http://www.nibb.ac.jp/en/about/recruit/>

NIBB observes the Equal Employment Opportunity Law of Japan, and is dedicated to promotion of gender equality.

Department of Biology

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TWO ASSISTANT PROFESSOR POSITIONS IN PLANT BIOLOGY

The Department of Biology at the University of Minnesota Duluth (UMD) invites applications for two tenure-track Assistant Professor positions beginning August 2015. We seek candidates with expertise in one or more of the following areas: plant cell/molecular biology, plant physiology, and plant or fungal diversity. The positions are part of a broader initiative to build a strong program in plant sciences in the UMD Biology Department. Depending on the candidates' particular research experience in the above areas, they may instruct lecture and laboratory courses in cell biology, physiology, or taxonomy and diversity, and will also be expected to develop at least one advanced course in their area of specialization. The area of specialization within the above areas is open. The successful candidates will establish independent, externally funded research programs involving undergraduate and graduate students. Service to the department, college and University is also expected. Opportunities exist for collaboration with researchers at UMD's Natural Resources Research Institute, Large Lakes Observatory, College of Pharmacy, School of Medicine and the EPA Mid-Continent Ecology Division. State-of-the-art research and instruction facilities and competitive startup funding are available.

Required Qualifications (must be mentioned on application/ curriculum vitae) include: Ph.D. or terminal degree in the biological sciences from a regionally accredited institution, evidence of potential for achievement in teaching appropriate for appointment at the Assistant Professor level, peer-reviewed publication record, and excellent written communication skills. The University of Minnesota requires that you apply online for this position. For a complete description of the positions and information on how to apply online, visit <http://employment.umn.edu/>, and search for **Job Requisition 192180**. Complete applications will be reviewed beginning **October 20, 2014** and continue until the positions are filled.

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Faculty Positions Open

Marine Science
Medical Physics
Biology

Okinawa Institute of Science and Technology (OIST) is a new, English-language graduate university offering a world-class research environment and a highly diverse, international research community with faculty, staff and students from over 40 countries. It is located in Okinawa on a beautiful campus overlooking the East China Sea.

Suitably qualified candidates will be appointed as Professors, Associate Professors or Assistant Professors. Professors and Associate Professors are usually appointed with tenure; Assistant Professors are tenure-track appointments, with an initial period of 7 years and a tenure review in the 5th year. The scope of this broad search is guided by the following:

- ♦ *At least one tenure-track professor in **Marine Science**, including physiology, marine genomics and coastal ecology (Reference V1401)*
- ♦ *Two appointments, at least one of which is a tenured professor, specializing in the **Medical Physics** area covered by Radiation Physics, Radiobiology and Medical Imaging (Reference V1402, V1403)*
- ♦ *At least one appointment, to include a tenured professor, in **Biology**, including cell biology, mammalian developmental biology and stem cell biology (Reference V1404)*

For all positions we seek applicants with strong interdisciplinary interests and outstanding scientific records of creativity and productivity. Detailed descriptions of all posts and the application procedure may be accessed at <https://groups.oist.jp/facultypositions>

Inquiries about any of these positions should be directed to Professor Ken Peach, Dean for Faculty Affairs, OIST, faculty-recruiting@oist.jp

Applications should be made online before 30th November 2014.

OIST Graduate University is an equal opportunity, affirmative action educator and employer and is committed to increasing the diversity of its faculty. We strongly encourage women and minority candidates to apply.



Are you a creative investigator who excels at working collaboratively and pushing disciplinary boundaries?

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The College of Biological Sciences at the University of Minnesota is hiring scientists for interdisciplinary research clusters in these emerging areas of biology:

- **biology education**
- **cellular biophysics**
- **computational and genome-enabled biology**
- **synthetic biology**

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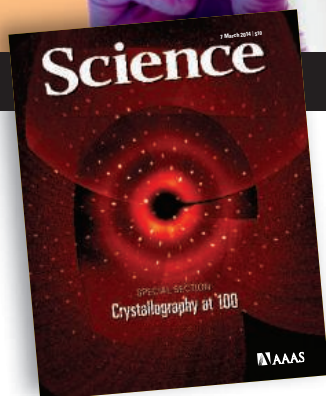
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Faculty Career Feature

October 3, 2014

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Science Careers

From the journal *Science*



ScienceCareers.org



Lahore University of Management Sciences



Syed Babar Ali School of
Science & Engineering

Abdus Salam Chair & Siemens Chair

The **Syed Babar Ali School of Science & Engineering (SBASSE)** at LUMS invites applications at the level of full professor with tenure to occupy two recently established Endowed Chairs.

The **Abdus Salam Chair** in **Physics and Mathematics** and **The Siemens Chair** in the area of **Energy and Power Systems** will be awarded to exceptional scholars and practitioners in their respective fields.

The successful candidates will be internationally recognised in their chosen field of research, have participated in the design and delivery of novel academic programmes and demonstrated exceptional professional leadership. These endowed positions carry highly competitive salaries and include a research fund.

Established in Lahore in 1986, LUMS is ranked among the top institutions of Asia. Its 100-acre lush green campus includes modern facilities, infrastructure and on-campus faculty housing. It offers an excellent work environment that is conducive to professional growth. Candidates wishing to be a part of the LUMS family are encouraged to submit their application comprising a detailed curriculum vitae including the names of three professional referees along with a research statement and teaching philosophy to: deansbasse@lums.edu.pk. Nominations are also encouraged.

Application deadline is **September 30, 2014**. However this search will continue until the appropriate candidate is identified.

Senior Faculty Position in Proteomics Research

The Icahn School of Medicine at Mount Sinai is seeking outstanding applicants for a senior faculty position in proteomics-based research. We invite applications from established investigators applying mass spectrometry techniques to understand basic disease-related mechanisms and using quantitative mass spectrometry tools to characterize proteins and their post-translational modifications. State-of-the-art infrastructure and support is available in the school.

Appointments are available at the level of associate or full professor. The position offers a highly competitive salary and start-up package. Individuals must have a PhD or equivalent degree in a relevant research field, and applicants with current research funding will be given preference. Successful candidates will be expected to maintain National Institutes of Health (NIH)-funded and innovative programs of research and to establish strong collaborations within the medical school.

The school is one of the nation's leading medical schools, ranked in the top 20 schools for NIH funding. The school is undergoing rapid expansion and offers an outstanding intellectual and multidisciplinary environment with a commitment to translational research.

Interested candidates should submit their curriculum vitae, a brief description of their research accomplishments, and proposed research program. Complete contact information for at least 3 professional references should also be provided. Materials should be sent as a single PDF file to Nora McLaughlin at nora.mclaughlin@mssm.edu. Dr. Paul J. Kenny, Professor and Chairman, Department of Pharmacology and Systems Therapeutics and Dr. Ramon Parsons, Professor and Chairman, Department of Oncological Sciences will oversee the search.



Icahn
School of
Medicine at
Mount
Sinai

ETH zürich

Professor of Computer Science (Medical Informatics)

→ The Department of Computer Science (www.inf.ethz.ch) at ETH Zurich invites applications for a Professor of Computer Science in the area of Medical Informatics.

→ Applicants should have an excellent record of internationally recognized research, which demonstrates a strong link of core computer science (information systems & machine learning) and applications in medicine and life science. Examples for the computer science expertise of the successful candidate include, but are not limited to, medical data analytics, data management, cloud computing, computational medicine, clinical care systems, possibly complemented by computational science or medical imaging.

→ The professor is expected to establish and lead a research group in the Department of Computer Science with close links to the University Hospital Zurich. Active involvement in the network "Life Science Zurich" is also envisioned, especially with the Competence Center for Personalized Medicine. The professorship is embedded in a vibrant research and teaching community for interdisciplinary medical and life science research that benefits from the proximity of the University Hospital Zurich and ETH Zurich and ranges from biology, systems biology and biotechnology to life science, medicine, and health research in various engineering departments.

→ Candidates are expected to supervise graduate students, to teach undergraduate level courses (German or English) and graduate level courses (English) in his/her own field of research, and to participate in core courses of computer science. The expectation is to fill the position with a tenured full professor but excellent applications at the (tenure track) assistant professor level will also be considered.

→ Please apply online at www.facultyaffairs.ethz.ch

→ Applications should include a curriculum vitae, a list of publications, a statement of future research and teaching interests and the names of at least five references. The letter of application should be addressed to the **President of ETH Zurich, Prof. Dr. Ralph Eichler**. The closing date for applications is **15 December 2014**. ETH Zurich is an equal opportunity and family friendly employer and is further responsive to the needs of dual career couples. We specifically encourage women to apply.

UCLA

Life Sciences Open Rank Tenure Track Faculty Position

The Division of Life Sciences in the UCLA College of Letters and Sciences announces the continuance of its special initiative to recruit excellent research scientists or scholars with a strong commitment and history of mentoring students from underrepresented and underserved populations. Candidates should have outstanding records of scholarly publications, research support, and teaching, and be eligible for Academic Senate appointment in one of the following departments of Life Sciences (www.lifesciences.ucla.edu): Ecology and Evolutionary Biology; Integrative Biology and Physiology; Microbiology, Immunology, and Molecular Genetics; Molecular, Cell and Developmental Biology; Institute for Society and Genetics; and Psychology. The successful candidate will be expected to continue his/her active research program. Teaching and service expectations will be adjusted so that the successful candidate can maintain research excellence while pursuing his/her mentoring vision. The candidate may choose to participate in campus-wide and departmental programs that provide research and professional development opportunities for our diverse student body (e.g. <http://ugresearchsci.ucla.edu>). Faculty appointment will be made at a professorial rank commensurate with current academic standing and achievement. UCLA offers competitive salaries, research set-up funds, and recruitment allowances.

Questions should be sent to: **Professor Stephanie White** (sawwhite@ucla.edu). Application packages should be submitted online through <https://recruit.apo.ucla.edu/apply/JPF00398> and include the following: (1) curriculum vitae; (2) statement of research interests; (3) statement of teaching interests; (4) statement of commitment to mentorship, including formal and informal mentorship activities targeting underrepresented and underserved populations and/or vision for future mentoring activities; and (5) cover letter that includes names of referees that can be contacted for letters. Items 3 and 4 should be distinct documents. Review of applications will begin on **2 November 2014**, and continue until positions are filled.

UCLA is California's largest university, with an enrollment of nearly 38,000 undergraduate and graduate students. The UCLA College of Letters and Science and the university's 11 professional schools feature renowned faculty and offer more than 323 degree programs and majors. The Biosciences at UCLA has more than 300 faculty members, includes many top ten ranked departments, and is consistently in the top ten in NIH funding. The UCLA College has a tradition of outstanding teaching and mentorship of undergraduates that the Division of Life Sciences will enhance through this special initiative.

As a campus with a diverse student body, we encourage applications from women, minorities, and individuals with a history of mentoring under-represented demographics in the sciences.

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, 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



FACULTY POSITION BIOCHEMISTRY IMAGING MOLECULES

Breakthroughs in the imaging of molecules at increased spatial or temporal resolution have linked classical biochemistry with studies at the cellular and organismal level. The Biochemistry Department at the University of Wisconsin-Madison (www.biochem.wisc.edu) seeks to hire an Assistant Professor (tenure track) in this area. The successful candidate will utilize state-of-art imaging methods to advance important areas in biochemistry, structural biology, cell biology or physiology. Approaches include but are not limited to: cryo-EM or correlative light-EM; electron tomography; super-resolution, single molecule or light sheet microscopy; atomic force microscopy; imaging with novel fluorescent or optical approaches. We seek individuals who employ these methods to address key issues concerning biological molecules, machines, and structures. The successful candidate will be expected to develop a vigorous, extramurally-funded research program, and to participate in teaching in the Department. Attractive recruitment packages include start-up funding, salary support, state-of-art core facilities, exceptional laboratory space, access to vibrant graduate training programs, and a collegial and collaborative environment. Applicants should submit PDF files of curriculum vitae with summary of research accomplishments, description of future research program and teaching interests, and letters from three references to jobs@biochem.wisc.edu by **October 15, 2014**.

Science Careers

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Science

POSITIONS OPEN

TWO FACULTY POSITIONS in
Quantitative Systems Biology
University of California Santa Barbara
Division of Mathematical
Life and Physical Sciences
Job # JPF00382

The University of California Santa Barbara invites applications for two tenure-track faculty positions at the **ASSISTANT PROFESSOR** Level. These positions are part of a multi-year, multi-departmental initiative to establish an interdisciplinary program in the area of Quantitative Systems Biology, emphasizing quantitative experimentation and theoretical analysis in the study of living systems. Subjects of interest include, but are not limited to: quantitative studies of development, microbial biology and dynamics of evolution, genomics and population genetics. While faculty may be associated with more than one department, the primary appointment would be in the academic unit most closely corresponding to the candidate's research focus and teaching qualifications. Preference will be given to candidates with broad scientific interests, a record of research excellence and creativity, and a strong commitment to undergraduate and graduate teaching. Interdisciplinary educational background combining biology with physical and mathematical sciences and/or a strong record of active interdisciplinary collaboration will also be valued. Candidates must possess a Ph.D. by September 2014. Appointment begins July 1, 2015.

Applicants should submit a cover letter, curriculum vitae, a statement of research and teaching interests and arrange for three letters of recommendation. Materials should be submitted electronically via **website: <https://recruit.ap.ucsb.edu/apply>**. Applications received on or before December 15, 2014 will be given full consideration.

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. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, or any other characteristic protected by law including protected Veterans and individuals with disabilities.

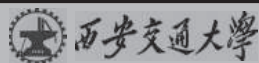
TENURE-TRACK ASSISTANT PROFESSORSHIP University of Minnesota – Twin Cities

The Department of Biochemistry, Molecular Biology and Biophysics invites applications for a full-time, tenure-track Assistant Professor position to begin on or around July 1, 2015. We are particularly interested in candidates with expertise in transcriptional or post-transcriptional gene regulation as well as all aspects of chromatin modification and regulation, although all types of biomedical research will be considered. The successful candidate will be expected to develop a strong, externally funded research program and contribute to the interdisciplinary undergraduate, graduate, and professional teaching programs. Cross-disciplinary collaboration is also an asset. All candidates must have a Ph.D. and/or M.D. degree, applicable postdoctoral experience, and a strong publication record. The successful candidate will receive a start-up package and a salary commensurate with education and experience.

The deadline for submitting applications is November 15, 2014. Please apply online at **website: <http://employment.umn.edu>**, click on "Search Postings", and enter **194164** into the requisition number field. Please attach full curriculum vitae, a two-page research overview, and a one-page teaching statement. Three letters of recommendation that consider both research and teaching potential should be sent directly from the referee to: **Faculty Search Committee, c/o Ms. Ann Johnson, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 6-155 Jackson Hall, 321 Church Street S.E., Minneapolis, MN 55455** or as an attachment to e-mail: mbsearch@umn.edu.

The University of Minnesota is an Equal Opportunity Educator and Employer. The Department of Biochemistry, Molecular Biology and Biophysics strongly encourages all individuals to apply regardless of gender, ethnicity or background and is committed to a diverse research and teaching environment.

RECRUITMENT NOTICE


Position Offered: Deputy Dean, Fuli School of Food Science and Engineering (FSFSE) of Xi'an Jiaotong University (XJTU)

Xi'an Jiaotong University (XJTU), known as Nanyang College founded in 1896, is one of the nation's most renowned higher institutions. XJTU was among the first group of universities in China funded by the Project 211 and Project 985, with the goal of becoming a world famous university.

Fuli School of Food Science and Engineering (FSFSE) of XJTU is jointly-established by XJTU and the Fuli Charitable Fund of Zhejiang Province. In line with the fast development of food industry and the great demand of high-calibers in the field of food safety, FSFSE aims to cultivate innovative and inter-disciplinary talents with specialized knowledge of both food science and mechanical engineering. To further promote the development of FSFSE, XJTU offers a Deputy Dean position for an open worldwide application and welcomes elites to join us.

Position Responsibilities:

1. Assist the Dean in charge of discipline construction, responsible for discipline development planning and orderly promotion and step-by-step implementation as planned.
2. Assist the Dean in charge of scientific research, responsible for making scientific research management regulations to standardize management and its practice.
3. Take charge of international cooperation and academic exchanges to expand the influence of the school domestic and overseas.
4. Take charge of laboratory construction.
5. Complete other tasks assigned by the leaders.

Recruitment Requirements:

1. Love education, strong dedication and sense of responsibility, good morals and ethics, and deep understanding of policy, Abide strictly by academic norms, rigorous academic research attitude, and follow regulations and disciplines.
2. Work experience in administrative management in college is mandatory requirement, Strong organization, coordination, communication skills, and team and service awareness.
3. With educational background of process equipment and control, precision instrument, food science and engineering or relevant disciplines. Outstanding performance in relevant academic fields, and have become academic leader or possess high academic influence.
4. Capable of taking a full-time job at XJTU.
5. With high professional title and degree of Ph.D. under the age of 50 in good physical health.

Salary and Welfare

1. **Annual Basic Salary:** RMB 340,000 (before tax)
2. **Annual Medical Insurance:** RMB 2,000 (Comprehensive Medical Insurance for visitors from foreign countries, Hong Kong, Macau, and Taiwan)
3. **Annual Accommodation Subsidy:** RMB 50,000 (Furnishings and electric appliances provided)
4. **Annual Bonus:** RMB 100,000 (before tax)
5. **Vacation:** Paid (summer vacation, winter vacation and national holidays)
6. **Work Visa:** processed and paid by XJTU (RMB 1000)
7. **Children Education:** Children could be placed at XJTU Affiliated kindergarten and schools
8. **Research Start-up Funding:** offered in accordance with XJTU Faculty Recruitment Regulation

Application Guide

1. **Materials:** make sure to send via e-mail the Registration Form (Attached) to the contact before September 30, 2014.

Applicant from other university need to provide additional following application materials :

Professor/Senior Researcher Employment Certificate

Doctoral Terminal Degree Certificate

Research publications, academic monographs, projects and awards in recent five years

Three recommendation letters

2. **Interview:** potential applicants whose materials are well received will be invited to XJTU for an interview. International airfare, room & board expenses will be covered by XJTU.

Contact Persons

XING Xuning MA Jin

Tel: +86-29-82668242 E-mail: dwzzb@mail.xjtu.edu.cn


Faculty Positions Available at The Institute for Advanced Studies (IAS) and The Medical Research Institute (MRI), Wuhan University, Wuhan, China
Faculty Positions

Two newly founded institutes at Wuhan University in China, the Institute for Advanced Studies (IAS) and the Medical Research Institute (MRI), cordially invite applications for ~50 each, open-rank faculty positions in Biology, Chemistry, Physics, Material Sciences, and Medical Sciences.

All applicants must have a Ph. D or MD and a successful postdoctoral experience. Successful candidates will be expected to establish an active research program in relevant disciplines. We offer internationally competitive recruitment packages.

The applicants should submit, electronically, a full CV, a research statement and contact information of three referees in a single PDF file to wdggy@whu.edu.cn (for IAS positions) or shuoffice@whu.edu.cn (for MRI positions).

Applications that apply for both institutes at the same time will not be accepted and further processed.

<http://hr.whu.edu.cn/>


Southwest Jiaotong University, P.R.China Anticipates Your Working Application

Southwest Jiaotong University (SWJTU), founded in 1896, situates itself in Chengdu, the provincial capital of Sichuan. It is a national key multidisciplinary "211" and "985 Feature" Projects university directly under the jurisdiction of the Ministry of Education, featuring engineering and a comprehensive range of study programs and research disciplines spreading across more than 20 faculties and institutes/centers. Boasting a complete Bachelor-Master-Doctor education system with more than 2,500 members of academic staff, our school also owns 2 first-level national key disciplines, 2 supplementary first-level national key disciplines (in their establishment), 15 first-level doctoral programs, 43 first-level master programs, 75 key undergraduate programs, 10 post-doctoral stations and more than 40 key laboratories at national and provincial levels.

Our university is currently implementing the strategy of "developing and strengthening the university by introducing and cultivating talents". Therefore, we sincerely look forward to your working application.

More information available at <http://www.swjtu.edu.cn/>

I. Positions and Requirements
A. High-level Leading Talents

It is required that candidates be listed in national top talents programs such as *Program of Global Experts*, *Top Talents of National Special Support Program*, *"Chang Jiang Scholars"*, *China National Funds for Distinguished Young Scientists* and *National Award for Distinguished Teacher*.

Candidates are supposed to be no more than 50 years old. The limitation could be extended in the most-needed areas of disciplinary development.

Candidates who work in high-level universities/institutes and reach the above requirements are supposed to be no more than 45 years old.

B. Young Leading Scholars

Candidates are supposed to be listed in or qualified to apply for the following programs:

• *National Thousand Young Talents Program*

• *The Top Young Talents of National Special Support Program (Program for Supporting Top Young Talents)*

• *Science Foundation for the Excellent Youth Scholars*

Candidates should have good team spirit and leadership, outstanding academic achievements, broad academic vision and international cooperation experience and have the potential of being a leading academic researcher.

C. Excellent Young Academic Backbones

Candidates under 40 years old are expected to graduate from high-level universities/institutes either in China or other countries. Those who are professors, associate professors and other equal talents from high-level universities/institutes overseas could be employed as professors and associate professors as well.

D. Excellent Doctors and Post Doctoral Fellows

Candidates under 35 years old are supposed to be excellent academic researchers from high-level universities either in China or other countries.

II. Treatments

The candidates will be provided with competitive salaries and welfares that include settling-in allowance, subsidy of rental residence, start-up funds of scientific research, assistance in establishing scientific platform and research group as well as international-level training and promotion. As for outstanding returnees, we can offer further or specific treatments that can be discussed personally.

III. Contact us:

Contacts: Ye ZENG & Yinchuan LI Telephone number: 86-28-66366202 Email: talent@swjtu.edu.cn

Address: Human Resources Department of SWJTU, the western park of high-tech zone, Chengdu, Sichuan, P.R.China, 611756

<http://www.swjtu.edu.cn/>

POSITIONS OPEN

DUKE UNIVERSITY Department of Chemistry

Duke University, Durham, North Carolina. The Department of Chemistry invites applications and nominations for a tenure-track position (at the ASSISTANT or recently promoted ASSOCIATE PROFESSOR level) to begin July 1, 2015. In addition to a commitment to teaching, candidates should have research interests in experimental physical/biophysical chemistry; we are particularly interested in enhancing interactions with our strong interdepartmental centers and institutes, such as the Fitzpatrick Institute for Photonics, the Duke Cancer Center, and the Center for Molecular and Biomolecular Imaging. Applications received by November 1 will be guaranteed consideration, although extraordinary applicants will be considered after this date. To guarantee consideration, all applicants should submit a curriculum vitae, list of publications, description of research interests and the names and e-mail addresses of three references (for requesting letters of recommendation) via the website: <https://academicjobsonline.org/ajob/jobs/4354>. *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, or sexual orientation. Veterans are strongly encouraged to apply.*

ASSISTANT PROFESSOR Princeton University Department of Chemistry

The Department of Chemistry at Princeton University invites applications for two tenure-track Assistant Professor positions in chemistry with an emphasis on theory, inorganic materials, and organic chemistry. We seek faculty members who will create a climate that embraces excellence and diversity with a strong commitment to teaching and mentoring that will enhance the work of the department and attract and retain students of all races, nationalities, and genders. We strongly encourage applications from members of all underrepresented groups. Candidates are expected to have completed the Ph.D. in chemistry or a related field at the time of appointment. Applicants should submit a description of research interests, curriculum vitae, a list of publications, and contact information for three references online at website: <http://jobs.princeton.edu/applicants/Central?quickFind=65639>. The deadline for applications is October 15, 2014. *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.*

ASSISTANT PROFESSOR OF BIOLOGY Faculty Position in Microbiology

The Biology Department at Missouri State University anticipates an August 2015 opening for a tenure-track Assistant Professor in Microbiology. Requirements include a Ph.D. in Microbiology or related area, peer-reviewed publications in microbiology and excellent communication skills. Duties include teaching courses in microbiology, pathogenic microbiology, and specialty; graduate and undergraduate advisement; and externally funded research. A letter of application (including a commitment to working with diverse student populations), curriculum vitae, names and contact information for three references, a statement of teaching experience and interests, a statement of research interests, and copies of all university transcripts should be submitted online at website: <https://jobs.missouristate.edu>. Employment will require a criminal background check at University Expense. Application review begins on 23 October 2014 and continues until position is filled. Starting date is 10 August 2015. Direct queries to e-mail: pauldurham@missouristate.edu. *Equal Opportunity/Affirmative Action/Minorities/Females/Veterans/Persons with Disabilities.*

POSITIONS OPEN

TENURE-TRACK IMMUNOLOGIST Assistant Professor Position in Immunology

The Department of Pathology, Microbiology, and Immunology at the University of South Carolina's School of Medicine invites applications for a tenure-track Assistant Professor position in Immunology. The successful candidate is expected to develop a strong extramurally funded research program, and must participate in the teaching mission of the department. Outstanding applicants working in an area complementing our existing faculty research interest (website: <http://pmi.med.sc.edu/>) will be considered. The departmental strengths include the NIH-funded Center for Complementary and Alternative Medicine, and the COBRE on Dietary Supplements and Inflammation. Candidates must have a Ph.D. or equivalent, and at least three years of postdoctoral research experience. Preference will be given to a candidate who has shown evidence of independence with currently active grant funding. Competitive salary and startup funds are available. Please submit curriculum vitae, teaching philosophy, and statement of research plans to: Dr. Mitzi Nagarkatti, Chair, Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC 29208 or e-mail: pmi.immunology@uscm.edu. Kindly arrange to submit three letters of recommendation upon request. The search will start immediately and will continue until the position is filled. *USC Columbia is an Equal Opportunity/Affirmative Action Employer, encourages applications from women and minorities, and is responsive to the needs of dual career couples.*

FACULTY POSITION in Physical Chemistry University Of Oregon

The Department of Chemistry and Biochemistry (website: <http://chemistry.uoregon.edu>) invites applications for a tenure-related position in experimental physical chemistry (broadly defined) beginning fall 2015 at the anticipated rank of ASSISTANT PROFESSOR. A Ph.D. is required and postdoctoral experience is preferred. The potential for establishing a vigorous independent research program in physical chemistry and active participation and excellence in teaching at the undergraduate and graduate levels will be the most important criteria for selection. In addition to membership in the Department of Chemistry and Biochemistry, the successful candidate will have the opportunity to pursue research in a variety of interdisciplinary institutes see website: <http://chemistry.uoregon.edu/research.html?institutes>. To ensure consideration, please submit application materials by October 24, 2014 at website: <https://academicjobsonline.org/ajob/jobs/4651> and upload curriculum vitae, a statement of research plans and objectives, and a brief statement of teaching philosophy or interests. The candidate should also arrange for three letters of recommendation to be submitted directly by the recommenders via the preceding URL link. The position will remain open until filled. *The University of Oregon is an Equal Opportunity/Affirmative Action Institution committed to cultural diversity and compliance with the ADA. The University encourages all qualified individuals to apply, and does not discriminate on the basis of any protected status, including veteran and disability status.*

ASSISTANT PROFESSOR in Neuroscience

The Department of Psychology at New Mexico Tech seeks an applicant for a tenure-track position with expertise in Neuroscience. The successful candidate holds a Ph.D. in Neuroscience, Psychology or a related field and must be qualified to teach Psychology and upper-division courses in Neuroscience. The candidate will be involved in our interdisciplinary Biomedical Science and Bioengineering programs and will establish a research program in any area of Neuroscience, which may include Bioengineering. Collaboration with the Biology department and other departments at NMT is expected, as is the involvement of students in research. For more information, including how to apply for this position, please visit website: <http://www.nmt.edu/hr-jobs-at-nmt>.

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C A R N E G I E I N S T I T U T I O N

At the Frontiers of Science

President's Science Deputy

**Carnegie Institution for Science
1530 P St. NW
Washington D.C.**

Andrew Carnegie founded the Carnegie Institution in 1902 as an organization for scientific discovery. His intention was for institution to be home to exceptional individuals—men and women with imagination and extraordinary dedication capable of working at the cutting edge of their fields. The primary mission of the Institution remains exploration, discovery, and scientific understanding.

Today, Carnegie scientists work in six scientific departments: the Geophysical Lab and Department of Terrestrial Magnetism in Washington, D.C., the Department of Embryology in Baltimore, the Observatories in Pasadena, California, and the Departments of Global Ecology and Plant Biology on the Stanford University campus in California. The approximately 500 people of Carnegie carry out a remarkable range of projects from astronomy and cosmology to earth science, plant & animal biology, and ecology. With superb facilities such as giant telescopes in Chile, diamond anvils for reproducing conditions of the deep earth, an airborne observatory for mapping Amazon forests, and many instruments for seismic measurements and microscopy, CIS people explore the history of the universe, the history of the earth, and the history of life to understand our present and inform our future. The Carnegie Academy for Science Education teaches science to children. The Math for America program prepares high school teachers for advanced degrees and for improving math education. Programs for to inform the public about scientific discoveries, including lectures and conferences, are organized by CIS. CIS has its central headquarters in a grand building at 1530 P Street NW in Washington D.C., a short distance from the Smithsonian and from Dupont Circle. From there the operations of the six departments are coordinated, the teaching programs are carried out, and new projects, teaching initiatives, and public outreach events are created and managed.

For more information, please visit www.carnegiescience.edu.

Position: The Science Deputy will report directly to the President. S(he) will serve as a liaison between the President and the staff of the six Carnegie Departments: Embryology, Global Ecology, Geophysical Lab, Observatories, Plant Biology, and Terrestrial Magnetism. S(he) will sometimes work with the Carnegie education programs. S(he) will work closely with the president to plan research plans, scientific conferences, lecture series, exhibits, and online presentations. S(he) will research and analyze specific areas of science to assess potential new projects and directions for the Institution. S(he) will sometimes be asked to investigate important science policy questions that relate to publicly funded and/or privately funded research enterprises.

The primary work site will be the Carnegie headquarters in Washington, address above, and residence in Washington or within commuting distance is required.

S(he) will make presentations to staff members based on this research and analysis. S(he) will draft reports and public statements about policy or science for the President. Excellent writing ability is therefore important. S(he) may be asked to attend science or policy conferences to report back on them and/or to represent Carnegie Science.

Other key attributes include people skills such as being a good listener, empathizing with other points of view, comprehending the different personalities and traditions in different scientific fields, being patient, and warmth and humor. The job will require a lot of energy and a positive attitude. Enjoyment of travel will help, since Institution departments are in four locations: Washington DC, Baltimore, Pasadena, and Stanford. Part of the work will occur at these sites. The Institution also manages projects in foreign countries and some travel to them may be required.

Requirements: Ph.D. in geology, earth science, physics, astronomy, cosmology, chemistry, or related fields. Interest in a broad range of science reflecting the rich diversity and history of Carnegie Science. A commitment to at least two years in the job is required. The job is suitable for someone planning a career in science management, policy, or foundation work in government, academia, or the private sector. The job will bring chances to work with outstanding scientists in fields from cosmology to geophysics to plant & animal biology and ecology. There will be much to learn and many exciting projects, and it will be fun.

Salary will be commensurate with experience. Carnegie Institution for Science is an equal opportunity employer committed to increasing representation of women and minorities in science and science administration. All qualified applicants will receive consideration for employment and will not be discriminated against on the basis of race, color, religion, sex, national origin, veteran status, or disability.

Interested applicants should send a full C.V., including names and contact information of three people who can provide recommendations, and a cover letter explaining interests and career goals to jobs.carnegiescience.edu.

By Sandy Becker

Rhubarb pie and science

When I am asked how I became a developmental biologist, I say, “Jim hired me for my rhubarb pie and my Phi Beta Kappa key, and the rest is history.” ■ I did not set out to become a scientist. I majored in history as an undergraduate—at Pomona College in Claremont, California, class of 1963—then bailed on graduate school and became an elementary school teacher in a vain effort to calm a boyfriend’s jealousy. (He feared competition from my graduate school classmates.) A few years later, I married my high school crush and put him through college and medical school by teaching fifth grade. His medical practice under way and our younger child in kindergarten, I began to think about going back to work—but back to what? I was burned out on public school teaching.

My daughter had become friends with the daughter of a professor in the biology department at Wesleyan University in Middletown, Connecticut, near where we live. Our two families had frequent potluck dinners, and Jim—the biologist—came to be fond of my rhubarb pies. He concluded, I guess, that I knew how to follow a recipe and probably had enough brains to follow one written in metric units, because he offered me a job as a technician in his lab.

When I first started working, I had no training in biology except what I had picked up by reading my husband’s textbooks while he was in college and medical school. If asked, I would probably have answered that a molar solution is something used to rinse your back teeth. In Jim’s lab, I learned to make primary cultures of *Drosophila* embryos, run Northern gels, and extract polytene chromosomes. (This was the 1980s.) It was indeed a lot like baking pies: If you carefully followed the recipe—the protocol—your experiment would likely work, although the data might not tell you what you were hoping to hear. I took my first biology course: genetics. I got a B.

A perk of being a Wesleyan employee is that you get to take courses for free, so I gradually filled the holes in my scientific education. Over the years, I’ve taken 17 biology courses, if you count organic chemistry, and I now have three Wesleyan graduate degrees, two in biology and one in science journalism.

I’ve thought once or twice about getting a Ph.D., but I haven’t done it. I guess I just don’t want my boss’s job. Principal investigators (PIs) slave away, teaching and writing grants, and the data come to them secondhand. For me, the



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fun part is doing the experiments!

When Jim closed his lab, I moved upstairs to another cell biology lab, using mouse cells instead of *Drosophila*. I have stayed in Laura’s lab for more than 20 years, doing embryonic stem cell culture, preparing cells for transplant into mouse brains, and immunostaining. Sometimes my job is to be skeptic-in-chief, and sometimes I’m the head troubleshooter. I read the literature, train students—graduate and undergraduate—and try to make sense of it all.

Being an academic research technician is not a very profitable career—not, at least, at a small private university. In 2005, I left the university to work for a biotechnology company in Massachusetts and

was paid twice as much. That biotech company may cure macular degeneration someday—I hope they do because I got stock options—but what I really want to do is find stuff out. So, after 3 years, I returned to Laura’s lab for another few years of sometimes exasperating but always engrossing basic research.

My name is on many of the papers that have come out of the lab. I’m seldom the first author and never the last, but along with the students, I am always the first to see the data, and when they have something important to say, I’m often the first to recognize it. You might say that Laura and I have a symbiotic relationship: I need a PI to lead and fund the lab, and she needs me to run it. ■

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