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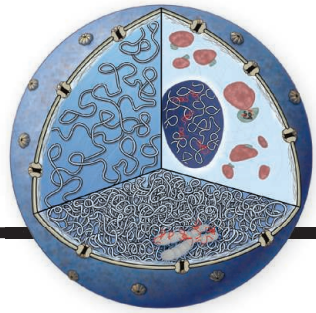

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ON THE COVER

A woman cares for her 5-year-old grandson, who has malaria, in a hospital in northwestern Zambia. An upsurge in funding for global health over the past 15 years has brought dramatic declines in deaths and illness from malaria and other diseases of the poor. Countries like Zambia are now trying to preserve those gains while tackling new challenges. See page 1256.
Photo: © John Stanmeyer/VII/Corbis

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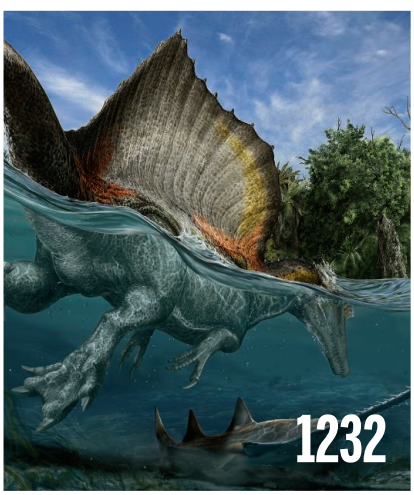

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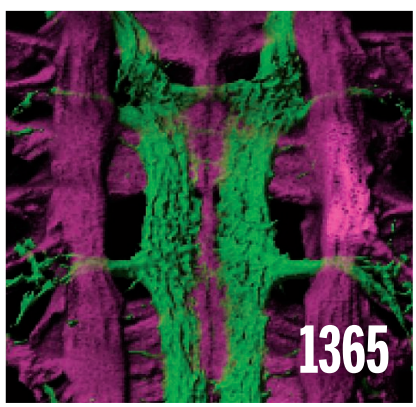

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SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals mail postage (publication No. 484460) 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). Domestic institutional 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 #1254 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.

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EDITORIAL

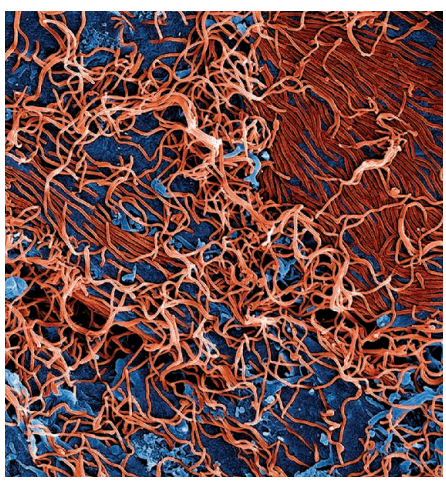
Ebola's perfect storm

The devastating Ebola epidemic in West Africa is the result of a perfect storm: dysfunctional health services as the result of decades of war, low public trust in government and Western medicine, traditional beliefs and even denials about the cause or existence of the virus, and burial practices that involve contact with contagious Ebola-infected corpses. There are now five affected West African countries: Guinea, Liberia, Nigeria, Sierra Leone, and most recently, Senegal. Ebola has killed around 2000 and infected more than 3500, with over 40% of cases occurring within the past few weeks. The World Health Organization (WHO) predicts that 20,000 may become infected. This fast pace of Ebola's spread is a grim reminder that epidemics are a global threat and that the only way to get this virus under control is through a rapid response at a massive global scale—much stronger than the current efforts.

West African governments and the international community have been slow to act in a way commensurate to a major threat to health, economies, and societal stability. It took nearly 4 months after the first patient died in December 2013 before the outbreak was confirmed as being caused by the Ebola virus. Despite multiple calls by Médecins Sans Frontières (MSF), WHO and the governments concerned only declared the epidemic a public health emergency in August 2014. Finally, national authorities and international organizations, including WHO, the United Nations, the U.S. Centers for Disease Control and Prevention, and MSF are intensifying their efforts, hopefully in a coordinated manner, with WHO releasing a roadmap to contain Ebola within 9 months.

The immediate needs are enormous. International assistance to the growing local efforts must include support for disease-control activities such as the provision of protective equipment, patient care, and addressing the health, nutritional, and other needs of populations in quarantine. This must be done while dealing with other endemic health challenges: Uninfected people are dying from treatable diseases because of closed or abandoned health facilities, the cancellation of international flights to the infected countries is creating an obstacle to international support, and there are growing concerns about sending medical help without a plan of treatment for these workers (around 150 doctors and nurses have died of Ebola, and 240 medical staff are infected).

This is an opportune time to accelerate clinical evaluation of experimental therapies, vaccines, and diagnostics, while respecting ethical and scientific standards for such trials. Human trials of Ebola vaccines and therapies are about to start. WHO has announced that compassionate use of experimental therapies is ethically justified, even if they have not been tested in humans. An exceptional crisis requires an exceptional response. One of the lessons from the AIDS response is that prevention has little credibility if treatment for those infected is not available. Let us hope that this is the last Ebola outbreak where all we have to offer is isolation and quarantine, instead of a vaccine and treatment.



"Let us hope that this is the last Ebola outbreak where all we have to offer is isolation and quarantine, instead of a vaccine and treatment."

The impact of this epidemic will last long after its end. Health systems will need to be rebuilt, disease surveillance systems established, trust in health services and authorities rebuilt, orphans educated and protected, and economic losses restored. More Ebola outbreaks will follow, because the populations at risk in Africa are growing, as is their mobility. There is an urgent need to clarify the ecology and reservoir of the Ebola virus, as well as the human behaviors that trigger epidemics.

The tragedy in West Africa demonstrates that where there is a fertile ground of poverty, dysfunctional health systems, and slow response, local outbreaks can turn into major epidemics with major casualties.

– Peter Piot

Peter Piot is director and professor of Global Health at the London School of Hygiene & Tropical Medicine, London, UK. E-mail: director@lshtm.ac.uk

10.1126/science.1260695

PHOTOS: (INSET) NIAID/FLICKR; (RIGHT) HEIDI LARSON

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NEWS

> “In Monrovia, taxis filled with entire families … crisscross the city searching for a treatment bed. There are none.”
>
> **A World Health Organization report** from 8 September on the rapidly worsening Ebola outbreak in Liberia. For more on Ebola, see pages 1228 and 1229 in this issue, and visit **http://scim.ag/ebola14.**

IN BRIEF

No dumping in Great Barrier Reef

The Great Barrier Reef’s coral formations won’t be flooded with dredge spoil.

Plans to dump dredge spoil inside Australia’s Great Barrier Reef off the Queensland coast have been blocked by the state’s government. Last December, federal Environment Minister Greg Hunt approved a plan by the business consortium building one of the world’s largest coal ports at Abbot Point to dispose of the spoil offshore in the World Heritage Site. The state’s about-face follows intense public and scientific criticism as well as a call last week by a federal Senate committee for a total ban on disposal of any dredge spoil in reef waters. On 8 September, Queensland Deputy Premier Jeff Seeney announced that he would ask Hunt to fast-track a new plan to dispose of the anticipated 3 million cubic meters of spoil on land.

AROUND THE WORLD

New cancer drug approved

SILVER SPRING, MARYLAND | A new type of cancer drug that harnesses the body’s immune system to fight tumors has won approval from the U.S. Food and Drug Administration. Merck’s Keytruda (pembrolizumab) is an antibody that blocks a protein on T cells called programmed death receptor 1 (PD-1); tumors use the protein to hide from the immune system. Approved for patients with advanced melanoma, it will cost $12,500 a month. Keytruda is less toxic than so-called CTLA-4 inhibitors, a similar type of melanoma drug already on the market. They are among a wave of cancer treatments targeting the immune system that have prolonged the lives of some patients far longer than conventional therapies. In 2013, *Science* named cancer immunotherapy the Breakthrough of the Year.

U.K. backs animal research

LONDON | Embattled U.K. biomedical researchers can find comfort in a new survey showing that a sizable majority of the public continues to support the use of animals in research. But the government’s decision this year to field two almost identical surveys on the topic reveals that the question’s wording can influence the answer. For example, “animal research” garners more support than “animal experimentation,” and “medical research” is much more popular than “research.” Also, more people see its merits if told there are no other choices. Bottom line: Some 68% of adults say “I can accept the use of animals in scientific research as long as it is for medical research purposes and there is no alternative.” **http://scim.ag/anipoll**

Australia upholds gene patents

SYDNEY, AUSTRALIA | Bucking last year’s decision by the U.S. Supreme Court, Australia has greenlit gene patenting. Australia’s Federal Court last week upheld a lower court’s verdict that handed private companies the right to own human genes, arguing that isolating genetic material

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A bird to watch

Like all 32 other species of forest birds in Hawaii, the ‘I‘iwi, a honeycreeper, is faced with shrinking habitat and threats from introduced species. They need conservationists’ attention, according to *The State of the Birds 2014: United States of America*, a report released this week by the U.S. Committee of the North American Bird Conservation Initiative, a partnership of 23 government agencies, academic labs, and conservation organizations. Based on long-term data from bird surveys, including the Christmas Bird Count and the North American Breeding Bird Survey, 230 of the 720 breeding bird species in the United States are on the report’s watch list, and 33 others are still common but their numbers are dropping fast. Shorebirds and birds in arid lands are in the most trouble. On a positive note, conservation has helped wood ducks, ring-necked ducks, and other waterfowl increase their numbers.
http://scim.ag/USbirdreport

PHOTOS: (TOP TO BOTTOM) © PHOTO RESOURCE HAWAII/ALAMY; © STEPHEN FRINK COLLECTION/ALAMY

creates something new and patentable. Last year, cancer survivor Yvonne D’Arcy and advocacy group Cancer Voices Australia challenged the validity of patents, held by U.S. firm Myriad Genetics and Melbourne-based Genetic Technologies Ltd., covering DNA sequences linked to breast and ovarian cancer. Myriad has developed diagnostic tests for breast cancer based on partial sequences of the *BRCA1* and *BRCA2* genes. Critics of the decision contend it will slow the quest for new treatments. As the plaintiffs weigh an appeal, advocates are pushing the federal government to sidestep the court and ban gene patents.

Anglers dispute records’ impact

DANIA BEACH, FLORIDA | A prominent trophy angling group has rejected a call from fisheries scientists to stop awarding weight-based world records for threatened species—and challenged the numbers behind a recent scholarly paper that argued the record-keeping encourages people to kill the largest, fittest fish. The International Game Fish Association (IGFA) in Dania Beach maintains records for some 1200 species. Last month, researchers reported in *Marine Policy* that 85 of those species have been judged vulnerable, endangered, or critically endangered. They urged IGFA to shift to length-based records for the threatened fish. But anglers have submitted just 15 potential world records for the threatened species since they were listed, IGFA

Anglers say records for trophy fish like the goliath grouper don’t impact their numbers.

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Flowing over the ice

Somewhere in the Arctic Ocean, two Norwegian scientists are adrift on an ice floe, equipped with a year's worth of food and fuel—and one research hovercraft named *SABVABAA* (Inuit for "flows swiftly over it"). University of Bergen/Nansen Environmental and Remote Sensing Center professor emeritus Yngve Kristoffersen, 72, and crew member Audun Tholfsen established ice drift station FRAM-2014/15 on the 1.1-meter-thick floe on 30 August, when it was 280 kilometers from the North Pole. Over the next few months, they will drift northward along the submarine Lomonosov Ridge, taking sediment cores to learn about the polar environment more than 60 million years ago. It's the hovercraft that makes the setup truly unique: Using *SABVABAA*, they can travel up to 100 kilometers from their floating base, assessing ice properties, currents, and water temperatures. The hovercraft—the brainchild of Kristoffersen and geophysicist John K. Hall, 74, of the Geological Survey of Israel—also makes it possible to conduct a year-round study, Hall says. The ridge is covered by thick multiyear ice, forbidding to icebreakers, but *SABVABAA* (pictured) "allows you to have boots on the ground."

Using *SABVABAA* (*background*), scientists monitored earthquakes along the Gakkel Ridge in 2012.

PHOTOS: (TOP TO BOTTOM) YNGVE KRISTOFFERSEN; NASA/JPL-CALTECH/SPACE SCIENCE INSTITUTE

BY THE NUMBERS

$350 million

Donation by real estate tycoon Gerald Chan to the Harvard School of Public Health, to fund research on obesity, cancer, infectious pandemics, and more.

2.9

The rise in atmospheric carbon dioxide, in parts per million, from 2012 to 2013, according to the World Meteorological Organization's annual *Greenhouse Gas Bulletin*, released 9 September. It's the largest annual increase since 1984.

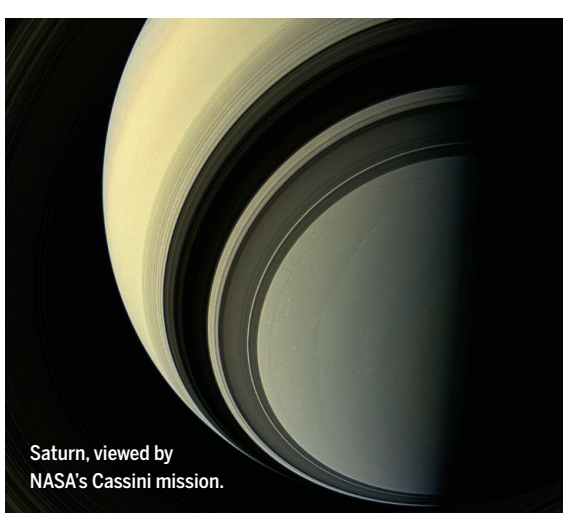
266

Minimum number of different food items—including ants, dandelions, algae, and even dirt—that grizzly bears in Yellowstone Park are known to eat, according to a study in *Ursus*.

argued in a response earlier this month, suggesting trophy fishing has "a disproportionately low impact" on population declines. http://scim.ag/anglingrec

NASA extends seven missions

WASHINGTON, D.C. | NASA will extend seven ongoing planetary science missions, based on a review by senior scientists, NASA officials revealed 3 September at a meeting of a planetary science advisory committee. Earlier this year, there were fears that two long-standing missions—the Lunar Reconnaissance Orbiter and the Mars rover Opportunity—might be shut down. But everyone appears to have escaped the knife. The highest ranked extension proposal came from Cassini, which will get 3 more years to explore the Saturn system. The lowest grade went to the Mars rover Curiosity; the panel was disappointed that the rover team was planning to drill and analyze just eight more samples during its extended mission, prompting NASA to ask the Curiosity team to revise its science plan. http://scim.ag/NASAmissions



Saturn, viewed by NASA's Cassini mission.

NEWSMAKERS

2014 Lasker prizes awarded

Five researchers who study a cellular system for fixing misfolded proteins, deep brain stimulation for Parkinson's disease, and breast cancer genetics have won this year's prestigious Lasker Awards. The award for basic medical research goes to **Kazutoshi Mori**, 56, of Kyoto University in Japan and **Peter Walter**, 59, of the University of California, San Francisco, for work on the unfolded protein response. **Alim Louis Benabid**, 72, of Joseph Fourier University in Grenoble, France, and **Mahlon DeLong**, 76, of Emory University in Atlanta won the clinical award for research on deep brain stimulation. And **Mary-Claire King**, 68, of the University of Washington, Seattle, won the special achievement award for her work on breast cancer genetics and DNA techniques for identifying people. http://scim.ag/Laskers2014

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A health care worker in Liberia helps transport a person suspected of having Ebola to a clinic. Ebola vaccines contain the virus's surface protein (*below*).

INFECTIOUS DISEASE

Ebola vaccines racing forward at record pace

Rushing them into widespread use without large-scale trials raises hopes and concerns

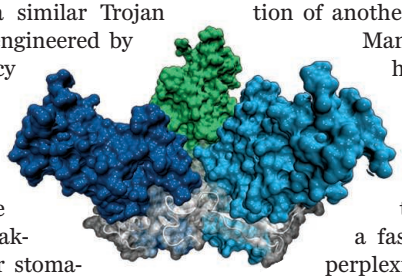
By **Jon Cohen**

On 2 September, a 39-year-old woman in Bethesda, Maryland, received a novel Ebola vaccine never given to humans before. In as little as 2 months, this same vaccine may go into the arms of thousands of health care workers and other first-line responders to the Ebola epidemic now wreaking havoc in West Africa. No experimental vaccine has ever been on a faster track toward widespread use. "It's absolutely unprecedented," said Marie-Paule Kieny, an assistant director-general at the World Health Organization (WHO).

On 5 September, more than 200 "technical experts" convened by WHO recommended bypassing the usual time-consuming regulatory pathways for potential Ebola vaccines and therapies. "We have to change the sense that there is no hope in this situation to a realistic hope," Kieny said. No one expects a vaccine will slow the epidemic directly, yet it could help keep and attract workers leading containment efforts.

The Bethesda woman is taking part in a phase I trial at the U.S. National Institutes of Health (NIH), one of several small studies getting under way in four countries to assess this vaccine's safety and the immune responses it triggers. The genetically engineered vaccine she received contains genes for a surface protein from two different strains of Ebola stitched into a harmless chimpanzee adenovirus that serves as a vector. Pharmaceutical giant GlaxoSmithKline (GSK), which is developing the vaccine in collaboration with NIH's National Institute of Allergy and Infectious Diseases (NIAID), may produce as many as 10,000 doses by the end of the year.

A second Ebola vaccine starting human tests this month uses a similar Trojan horse strategy. Initially engineered by the Public Health Agency of Canada and now being developed by NewLink Genetics of Ames, Iowa, this vaccine contains the Ebola surface protein gene inside a weakened version of vesicular stomatitis virus (VSV), which commonly infects farm animals. Most of the 1500 doses of this vaccine that exist will be used in clinical studies, a company representative says.



Both vaccines have protected monkeys and smaller animals in lab experiments. A study published online on 7 September in *Nature Medicine* shows that monkeys primed with the NIAID/GSK preparation and boosted by yet another vaccine that uses a vector known as MVA—a modified version of the smallpox vaccine virus—were protected from an Ebola virus "challenge" 10 months after their last vaccination. The researchers, led by NIAID's Nancy Sullivan, claim this is the first demonstration of "durable" protection from Ebola virus.

If the ongoing safety trials are encouraging—and results will be in as soon as November—health care workers and first responders would be the first to get the vaccines, following the recommendation of another WHO panel in August. Many of these responders have been killed and sickened by the virus, and recruiting people to fight the epidemic has been difficult. But putting the vaccines on such a fast track raises a host of perplexing issues "that the field has to think through very carefully," says John Mascola, who heads NIAID's Vaccine Research Center (VRC).

Typically, vaccines that pass phase I safety trials move into expanded phase II trials meant to answer the same questions in more people, finally moving into phase IIb and phase III tests to determine whether they actually work and should go to market. Mascola and other Ebola vaccine developers have

PHOTOS: (TOP TO BOTTOM) JOHN MOORE/GETTY IMAGES; CHRISTINA CORBACI/JEFF LEE/ERICA OLLMANN SAPHIRE, TSRI

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long contended that traditional efficacy trials could not be done because past outbreaks have ended quickly with the help of standard infection control procedures such as isolating confirmed cases, testing their contacts, and making sure that all health care workers wear personal protective equipment.

Instead, researchers had planned to take advantage of what's known as the Animal Rule at the U.S. Food and Drug Administration (FDA). The rule says when efficacy studies "are not ethical or feasible," FDA will license vaccines for diseases if they work in two animal models and if large-scale phase II studies conducted in humans prove they are safe and trigger immune responses that mirror those in protected animals.

But with today's fast-moving epidemic, which has spread to five West African countries and killed more than half of the roughly 4000 cases, talk about large phase II studies in unaffected populations has gone out the window. Now, the push is to vaccinate people at high risk and try to gain interpretable data, says Adrian Hill, director of the Jenner Institute at the University of Oxford in the United Kingdom. Hill's group will soon launch a small safety study of the NIAID/GSK vaccine, and he is confident it will pass muster. "Then it's decision time," he says. "Not just whether we go forward but how to go forward."

Traditionally, efficacy trials randomly assign participants to receive the vaccine or a dummy shot. That's clearly not ethical here, so some researchers are calling for a "stepwedge" trial, which analyzes what happens to people at similar risk who receive the vaccine at different times. That way those who have been vaccinated can be compared with others who have yet to receive their shots. "You can't give everyone the vaccine the same day," Hill notes.

But a step-wedge design could face serious limitations. The rate of spread can differ between sites because one might have, say, better or worse personal protection measures, which could cloud analyses of a vaccine's shortcomings or strengths. Researchers might also have trouble detecting efficacy if a vaccine offers only partial protection, NIAID's Mascola says. "If there's a 50% mortality with Ebola and you use the vaccine and it's 40% protective, what does that mean?" he asks.

Impassioned debates also surround which experimental vaccine to deploy. As the *Nature Medicine* study shows, the chimp adenovirus vaccine worked best when boosted with the MVA-Ebola construct. But that's not being assessed in the initial human trials. Mascola says a company has made the MVA preparation in bulk but it's not ready for testing.

Thomas Geisbert, a researcher at the University of Texas Medical Branch in Galveston who helped develop several VSV Ebola vaccines, including the one made by NewLink Genetics, contends "it's a much stronger vaccine system." The VSV replicates, unlike the chimpanzee adenovirus vector, stimulating an immune response that Geisbert argues is as good as the one achieved by the prime-boost approach NIAID has championed. "In the context of an outbreak, where you are going to put first responders on an airplane, you don't have time for a prime-boost," he says. "You need a single injection."

But even though this VSV is weakened, safety concerns remain about the possibility that it could cause neurological disease, as it has in some animal studies, or infect livestock. And Anthony Fauci, who heads NIAID, says that the chimpanzee adenovirus vaccine may work well enough without the extra boost, noting that a single injection protected 100% of monkeys challenged with Ebola virus at 5 weeks. "The vaccine was developed to be able to induce immunity really fast," he says.

No experimental vaccine has ever been on a faster track toward widespread use. "It's absolutely unprecedented," says WHO's Marie-Paule Kieny.

Fauci and his NIAID colleagues say it makes sense to test different vaccine strategies in parallel. "We've been fooled by trying to translate monkey findings to humans before," says Fauci, who notes that an AIDS vaccine worked well in monkeys and actually increased HIV infection rates when tested in humans.

Gary Nabel, who headed NIAID's VRC before leaving in 2012 to join Sanofi in Cambridge, Massachusetts, says manufacturers should massively scale up production now, before trial results are in. "If it were me personally, I'd err on the side of caution, and I'd think carefully about stockpiling doses," Nabel says. "If it isn't safe, you throw away those lots. In the worst case you've wasted some money."

If the virus spreads even farther and one of these vaccines does prove safe and effective but is not available, "I think there'll be a lot of finger-pointing," Nabel says. "Extraordinary times call for extraordinary measures, and this is an extraordinary time." ■

INTERVIEW

Ebola: 'Wow, that is really tough'

WHO's Bruce Aylward says international action is needed right now

By **Leslie Roberts**

Bruce Aylward is used to mobilizing armies of health workers. An assistant director-general at the World Health Organization (WHO) in charge of polio and emergencies, he leads the massive global effort to eradicate the poliovirus. But Aylward says he has never encountered a challenge as great as the Ebola outbreak in West Africa, which has infected more than 4000 people and killed more than 2000. Margaret Chan, who heads WHO, asked Aylward to help with the response in August; since then, he has been running operations and helped draw up WHO's Ebola Response Roadmap, released on 28 August. He spoke with *Science* on 4 September. (This interview has been edited for clarity and brevity.)

Q: Margaret Chan has said that all organizations involved in the outbreak, including WHO, underestimated its complexity and magnitude. How did this happen?

A: I didn't live through it all, but as I've gone back and asked what was happening, clearly these guys [in the response effort] have been flat-out on this for 6 months. And they've put 450 people in the field. Those are unheard-of numbers in responding to Ebola. But the virus got ahead of them.

Could the response have been scaled up faster? Maybe they were off by 2 weeks at one point here or there. As Margaret says, you're always a couple of weeks behind this virus, and there are so many reasons why. It's a dangerous pathogen. Foreign medical teams and NGOs [nongovernmental organizations] are used to dealing with trauma and primary health care; they're not trained to deal with pathogens.

Q: I've heard there are tensions between WHO and Doctors Without Borders (MSF), the organization that has treated more patients than any other. They have criticized WHO for being too slow and doing too little.

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A: Probably at local levels there's some tension in some places, but certainly not here, in Geneva at senior levels. There's a great respect for the organization. MSF is great at two things: They're fantastic at their field operations and at telling the rest of us how bad we are at them. Sure, people are going to go out and say, "Oh, that's unfair." You have to have a tough skin. You're in the World Health Organization; you're dealing with a major international threat, and our job is to be accountable. And if MSF feels that this is public accountability, it's their right.

Q: Why is stopping this outbreak so hard compared with controlling polio?
A: The polio program is really tough because of the level of programmatic perfection you need. You have to get to every single kid with vaccine over a huge geography and in very challenging environments. But even if you don't, you still have a level of control over the virus. Now, when I look at Ebola, you need a whole other level of perfection.

You have to do perfect contact tracing, because one contact can blow open a whole new chain of transmission. You have to get your burials perfectly safe. You have to get your laboratory testing right. There isn't a lot of capacity in the world on that. You have to get your social messages right. You have to be perfectly safe and protect health care workers. And you have to do all this in incredibly weak environments, in three countries that are near the bottom of the development index, and also deal with the embers that land in Nigeria or in Senegal. Wow, that is really tough.

Q: Do you think it's still possible to contain the outbreak with the standard procedures—isolating patients, tracing contacts, burying the dead safely?
A: Absolutely. But with an important difference. What's happened is you've got a caseload that far exceeds the capacity of the standard Ebola strategies to manage them, so you have to innovate on these strategies. Each infected person is having a heap of contacts because they're basically being left in their communities for long periods of time. What you've got to do is first of all cut down the outward spread from every patient, which means you've got to get many new Ebola treatment centers up. And you've got to adapt your strategies in a way that communities can play a much bigger role and help them scale up their own Ebola community care units. This is absolutely critical and must be done in September.

Will it be done? Well, that's going to depend on whether the international community will put the money on the table, help the people get in, and understand the conditions they need to operate.

A massive influx of money and people is needed immediately to stop Ebola, says Bruce Aylward.

Q: WHO's Ebola road map calls for the epidemic to be ended in 6 to 9 months. Isn't that overly optimistic?
A: I don't know, because no one's ever had to do something on this scale before. What I do know is if this road map is not implemented, you're not going to stop it in 6 to 9 months. In 1 month you need at least 10 new facilities operational with additional bed capacity and teams on the ground, and money so that people doing this stuff are getting paid, and a way to evacuate the responders that get into medical trouble there. You have to start implementing the road map today.

But the usual relief organizations aren't lining up to do it. These aren't bad people and these aren't cowards. These are people who go into the most dangerous operating environments—wars and natural disasters. But they don't normally deal with hazardous pathogens.

Q: So you need more people and you need more money, but it's simply not coming?
A: Not yet, but I'm optimistic. I think it's taking the world time to grapple with this. It is so new, and it plays to people's deepest fears and their greatest uncertainties. People will learn MSF is going to stay on the ground there. And then one or two NGOs are going to go in and run a facility, and they're going to do fine. And then it's going to escalate. The world doesn't want to be beaten by a pathogen. But the question is: Are they going to do it fast enough?

Q: So who are the players that you hope will come to build and run treatment centers?
A: A lot of foreign medical teams are linked to governments, and some affected countries have deep relationships: the U.S. with Liberia; the U.K. with Sierra Leone; France with Guinea. Now those countries are very keen to look at what they can do and how to do it, but they are having trouble mobilizing. They might be able to put up a field hospital, but can they staff it? Because a field hospital that's not staffed is just one more building, that's not an Ebola treatment center.

Q: Two vaccine candidates are soon going to be tested in phase I studies and may be deployed later this year (see p. 1228). How important do you think they and candidate drugs will be for ending the epidemic?
A: You want to have as many tools as possible to help drive down that reproductive number to where you can manage it with traditional strategies. You want to do both things in parallel and go flat-out. The vaccines and new therapies would be hugely helpful—they would help get responders in and keep the responders that are there healthy. That may give us an edge to shut this thing down more quickly. But if we say we need these drugs and vaccines, then you're setting yourself up for defeat because you might not get them. And then you also have the risk of people saying: "There's going to be a vaccine or med, let's wait," and then an awful lot of people are going to die. I'm not going to sit around twiddling my thumbs waiting to find out, and neither is my organization. ■

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The fate of the U.K. Astronomy Technology Centre in Edinburgh is up in the air.

U.K. POLITICS

Secession vote rocks science

Vying camps make bold claims about sovereignty as a blessing—or a curse—for science

By **Daniel Clery**

On 18 September, the people of Scotland will vote on whether their nation should separate from the United Kingdom and become independent. With the margin of victory now expected to be razor-thin, the debate among researchers is growing more strident over whether independence will ring in a golden era for Scottish science—or cripple it for years to come.

Researchers opposed to independence say a split will harm science, depriving it of funds and talent. "There's no clarity at all. We have no idea where our research funding will be coming from," says Richard Cogdell, director of the Institute of Molecular, Cell and Systems Biology at the University of Glasgow. "Yes" campaigners counter that the Scottish government has vowed to protect science during the transition and to maintain funding at least at current levels. "Scotland is a rich country. We want to do it and we can do it," says Bryan MacGregor, head of physical sciences at the University of Aberdeen.

Sorting out the competing claims is hard because of the complex daisy chains connecting Scottish research and the U.K. system. Following the devolution of some powers to Scotland in 1999, the Scottish government became responsible for education and universities. As in the rest of the United Kingdom, university research has several income streams: Lab overheads are supported by grants to institutions from the Scottish Funding Council, project grants are provided by seven research councils that operate U.K.-wide, and additional funding comes from industry, research charities, and the European Union.

A strong science record is at stake. Five of Scotland's 15 universities are in the top 200 worldwide—that's more elite universities per capita than in any other country. In 2012 to 2013, Scottish university researchers won 13.1% of the grant funding from the U.K. research councils, even though Scotland accounts for only 8.3% of the U.K. population. They had similar success in other areas, winning 11.2% of the U.K. grants from the E.U. Seventh Framework Programme, for instance.

According to the "No" campaign and the U.K. government, which opposes independence, an independent Scottish government would have to create a new grant funding system and stump up £307 million to match Scotland's research council take in 2012 to 2013. The U.K. government has also said that it could not guarantee scientists in an independent Scotland access to research facilities in England, such as the Diamond synchrotron and the ISIS neutron source. The fate of the few U.K. facilities north of the border, including the U.K. Astronomy Technology Centre and the Roslin Institute, where Dolly the sheep was created, would be up in the air. And U.K.-based charities like the Wellcome Trust have indicated that they would need to review their support for research in what would become a foreign country.

Meanwhile, it's uncertain whether an independent Scotland would automatically be a member of the European Union—as the Scottish government asserts—or be forced to apply as a new member. That ambiguity is worrisome, says astrophysicist Avery Meiksin of the University of Edinburgh, because "more and more funding in physics and astronomy is coming from European grants." And Scotland would have to pony up to join international research organizations such as CERN, the European Space Agency, the European Southern Observatory, and the European Molecular Biology Laboratory.

A divorce could also threaten international collaborations and the ability to attract research talent to Scotland, opponents say. *The Guardian* reported last month that Scottish universities are bracing for a brain drain of top talent if Scotland secedes. "If we can't attract people, how will we recover?" Cogdell asks.

Proponents of independence paint a very different picture. To prevent independence from equating to isolation for scientists, the Scottish government proposed in April a "common research area" with the United Kingdom, with shared research councils funded by both U.K. and Scottish taxpayers. That idea would require the U.K. government to agree, however, and taxpayers in the south are unlikely to accept researchers in an independent Scotland winning as large a share of the funding pot as they do now. It is one of several issues that would only be resolved in negotiations following a "Yes" vote.

Even if Scotland remains in the United Kingdom, science is in for a rough ride, MacGregor warns. He points out that whatever party wins the U.K. general election in 2015 must take drastic action to reduce the national debt. "We're heading for more cuts and the science budget won't be protected," he contends. Independence, he says, would allow Scottish researchers to dodge that bullet. ■

Scotland by the numbers

8.3
Percentage of the U.K. population made up by the 5.3 million Scots

13.1
Percentage of U.K. funds for grants and fellowships spent in Scotland in 2012–13

11.2
Percentage of the U.K.'s €4.5 billion share of European FP7 funds won by Scotland from 2007–13

CREDITS: (PHOTO) SCIENCE & TECHNOLOGY FACILITIES COUNCIL; (DATA) *SCOTLAND ANALYSIS: SCIENCE AND RESEARCH*, NOVEMBER 2013

Downloaded from www.sciencemag.org on September 12, 2014

Published by AAAS

The aquadino *Spinosaurus* dives for prey in an artist's reconstruction.

PALEONTOLOGY

Giant dinosaur was a terror of Cretaceous waterways

Fossils offer first evidence that some dinosaurs could swim

By **Michael Balter**

Spinosaurus was a brute of a dinosaur. It weighed up to 20 metric tons and stretched 15 meters long, and its long jaws housed at least 2 dozen sharp, piercing teeth. It may have been the biggest carnivorous dinosaur ever; in the film *Jurassic Park III*, a *Spinosaurus* clamps its long jaw on the neck of a *Tyrannosaurus rex* and quickly snaps it.

In reality, however, this fearsome beast lived 30 million years before *T. rex*, and it may have been more of a danger to fish than to terrestrial rivals.

The most complete known skeleton of *Spinosaurus*, published online this week in *Science* (http://scim.ag/NIbrahim), shows clear adaptations to life both in the water and on land, according to a team led by paleontologists Nizar Ibrahim and Paul Sereno of the University of Chicago in Illinois. The bones, from 97-million-year-old freshwater sediments in eastern Morocco, include parts of the skull, vertebral column, pelvis, and limb bones. They provide "compelling skeletal evidence for a lifestyle linked to water," says paleontologist Lindsay Zanno of the North Carolina Museum of Natural Sciences in Raleigh, and make *Spinosaurus* the first dinosaur known to swim. (The dinosaurlike marine reptiles called plesiosaurs and mosasaurs actually belonged to different orders.)

Researchers knew that a number of dinos spent lots of time hanging around lakes and rivers. But clear fossil evidence that dinosaurs could swim has been lacking. *Spinosaurus* first surfaced in 1912 in Egypt and was described by the celebrated German paleontologist Ernst Stromer, who marveled at its size but didn't recognize it as possibly aquatic. Then, in April 1944, British bombers blitzed Munich's state paleontology museum and sent *Spinosaurus* and most of the rest of Stromer's fossil collection into oblivion.

In the years since, evidence that some dinos might have swum has surfaced, including claimed "swim tracks"—scrape marks from the bellies of dinos—from lake and river bottoms in Utah and Spain. In 2010, a team led by Romain Amiot of the University of Lyon in France argued in *Geology* that oxygen isotopes suggest that dinosaurs closely related to *Spinosaurus* (known collectively as spinosaurs) were likely to have been aquatic.

In the new *Spinosaurus* skeleton, dug up over several years, Ibrahim and Sereno found signs of watery adaptation not seen in other dinosaurs: a small nostril located far back on the head, apparently to limit water intake; relatively long forelimbs; big flat feet suitable for paddling as well as walking on muddy ground; and very dense limb bones, which would have allowed *Spinosaurus* to submerge itself rather than float at the surface. The bone density in particular resembles that of early whales and today's hippopotamus and makes a strong argument for semiaquatic life, says anatomist J. G. M. Thewissen of Northeast Ohio Medical University in Rootstown.

Sereno says that *Spinosaurus*'s crocodilelike snout, together with its long neck and body, would have made it look "like a duck with the tail of an alligator attached to it." The spiny sail on its back was probably employed for sexual display rather than as an aid to swimming, the team surmises. Amiot adds that the new discoveries could help explain how a variety of different carnivorous dinosaurs could have coexisted in Africa, Europe, and Asia. The competition among them might have been diminished if the mighty *Spinosaurus* exploited freshwater lakes and rivers, eating fish, while other dinos hunted prey on land, he suggests.

All the same, Sereno and other team members think that amphibious life was not widespread among dinosaurs, but rather represented an "extreme evolutionary experiment" that did not leave descendants, as co-author Cristiano Dal Sasso of the Natural History Museum of Milan in Italy puts it. But James Kirkland, a paleontologist at the Utah Geological Survey in Salt Lake City and co-discoverer of some of the dino swim tracks found in the state, disagrees. "I would not consider *Spinosaurus* a fluke," he says, pointing to evidence (such as the wear patterns on their teeth) that not only spinosaurs but also other carnivorous dinosaurs crunched into scaly fish.

If humans had been around to see it, Kirkland adds, *Spinosaurus* would have been a pretty sight as it swam along. "Those sails certainly would have looked brilliant in the tropical sunset." ■

ILLUSTRATION: DAVIDE BONADONNA/NIZAR IBRAHIM/SIMONE MAGANUCO

Downloaded from www.sciencemag.org on September 12, 2014

Published by AAAS

ATMOSPHERIC SCIENCE

China blamed for U.S. ozone

Western states caught between rising background levels and more stringent federal limits

By **Eric Hand**

On 10 August 2012, temperatures rose above 40°C in the San Joaquin Valley, a natural bowl in California's midsection. The blistering heat, along with trapped air pollution, was perfect for the production of ozone, an oxygen molecule that corrodes the lungs. By the afternoon, an air quality monitor in Fresno signaled that the region was in violation of one of the legal limits set by the U.S. Clean Air Act and enforced by the Environmental Protection Agency (EPA). Combined with other violations, the event meant that the region would have to pay an annual $29 million fine.

In May, however, regional air quality officials petitioned EPA to let them off the hook for that bad ozone day. Their excuse? It was China's fault. That August day, on a rural ridge top 168 kilometers to the west of Fresno, a sensor had picked up high levels of background ozone wafting in from the Pacific, presumably all the way from East Asia, where ozone-creating emissions are booming. The petition argued that about 10% of the ozone pollution on that day—more than enough to put the San Joaquin Valley over the limit—came from abroad.

The petition is the first time a U.S. region or state has pointed the finger at China for ozone pollution, says Seyed Sadredin, the executive director of the San Joaquin Valley Air Pollution Control District in Fresno. But as background ozone levels rise and federal standards tighten, it will not be the last, especially in high-altitude western states. "This is not just going to be a San Joaquin Valley problem," Sadredin predicts.

On 29 August, citing the latest health research, EPA staff recommended that the agency lower the ozone limit from the current 75 parts per billion to somewhere between 60 to 70 parts per billion. For areas with high background ozone levels, that leaves "very little wiggle room," says Owen Cooper, an atmospheric scientist at the Cooperative Institute for Research in Environmental Sciences in Boulder, Colorado.

Given the squeeze, the Clean Air Act needs to evolve to reflect pollution blowing across borders, contends Daniel Jacob, an atmospheric chemist at Harvard University. "It's really important that we start to take our air quality policies beyond the state and national level and start to think about air quality on an international level."

Ozone levels in the United States and Europe have dropped significantly in recent decades as clean air policies cut pollution from cars and power plants. Not so in fast-developing China, where levels of nitrogen oxides—key ozone precursors—have more than doubled between 1995 and 2010, according to a study published in October 2013 in *Atmospheric Chemistry and Physics*.

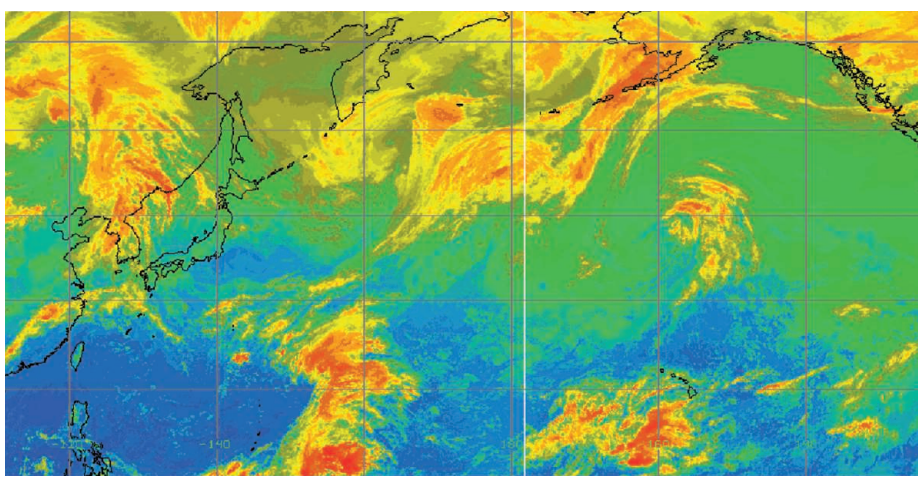
And this pollution moves. Studies have found that both the nitrogen oxides and locally produced ozone can survive in the atmosphere for days, if not weeks. During this time, the pollution can travel across the Pacific in a Northern Hemisphere "conveyor belt" of westerly winds several kilometers up. The U.S. mountain west lies right in their path—and because of its altitude it also gets doses of ozone from the stratosphere, where the "good" ozone that protects Earth from ultraviolet light is made naturally.

Over the past 15 years, the western United States has experienced an overall rise in springtime background ozone of nearly a half a part per billion per year at altitudes of between 3 and 8 kilometers, according to one recent analysis of balloon and aircraft measurements. The background trends are more significant in the springtime, when conveyor belt winds from East Asia are strong and direct. "As the standard goes down, spring is going to become more important," says Dan Jaffe, an atmospheric chemist at the University of Washington, Bothell, who in June published a study online charting the rise of background ozone at a remote site atop Mount Bachelor in Oregon. "Traditionally cities only worry in summer," when hot weather promotes ozone formation.

Tracing background ozone to a specific source is a challenge. Not all is humanmade: Forest fires and soil emissions contribute, and weather events can allow ozone from the stratosphere to mix with the lower atmosphere. In fact, in May, EPA granted the state of Wyoming a first-ever exemption for an ozone violation because of a stratospheric intrusion event. Climate change is another factor, because rising temperatures can enhance ozone production.

But it is clear that emissions from overseas lead to a significant part of the background, even if "things really get muddled when you try to get down to which city contributed what," Cooper says. In Europe, ozone pollution blowing from North America or even China is a major factor in rising background levels, notes Paul Monks, an atmospheric chemist at the University of Leicester and chair of the United Kingdom's Air Quality Expert Group. "The benefits of emission controls could be significantly counterbalanced by increasing background ozone levels," he says. "It's the elephant in the room."

Sadredin is already grappling with that elephant. If EPA accepts his arguments, taxpayers in his district will stop having to pay the annual penalty. But he worries about meeting future standards. Already, there are hundreds of regulations on the books to control ozone-generating pollution—rules that govern everything from bakery ovens to brandy aging. Sadredin is running out of regulatory room. "We're down to regulating people's barbecues and hairsprays," he says.■



In a satellite map, storm systems march across the Pacific, driven by winds that also carry pollutants.

IMAGE: OWEN COOPER/COOPERATIVE INSTITUTE FOR RESEARCH IN ENVIRONMENTAL SCIENCES (CIRES)/UNIVERSITY OF COLORADO/NOAA EARTH SYSTEM RESEARCH LABORATORY

Downloaded from www.sciencemag.org on September 12, 2014

Published by AAAS

FEATURES

Young blood

Young animals' blood holds rejuvenating powers. Amy Wagers wants to know why

By **Stephen S. Hall**

Amy Wagers was about halfway through a 90-minute talk to a group of Boston-area science teachers last July when she showed her Arnold Schwarzenegger slides. One picture depicted the former governor of California as a buff young bodybuilder; the other showed a more recent, flabbier, and stooped version of the Terminator playing tennis. If a picture is worth a thousand words, the slide served as a two-volume treatise illustrating the point that Wagers, a Harvard University stem cell scientist, wanted to make: As humans age, our muscles fail to maintain and regenerate as they once did.

"I'm not saying aging is a disease," Wagers hastened to add, once her audience had stopped laughing. "But it is associated with increased incidence of particular types of diseases."

Wagers's quiet voice barely carried over an aspirating aquarium at the side of the science lab at Dover-Sherborn High School. But the 30 or so educators gathered at the Summer Science Institute avidly followed the narrative of her recent work—especially when Wagers moved on to her "fountain of youth" slides.

In a series of experiments that have captivated both the field of regenerative medicine and its many lay spectators, Wagers and a diverse army of collaborators have shown that when the blood of a young mouse circulates through the murine equivalent of an old geezer, startling physiological changes occur. Many of the trademark depredations of old age—withering muscles; stiff, oversized hearts; cognitive decline; and even the fraying of the myelin coating that insulates nerve fibers—are slowed, repaired, or even reversed.

"We became convinced that there was something in the blood" responsible for the dramatic effects, Wagers told the teachers. Indeed, after a difficult search, she and colleagues have recently isolated a molecule from "young blood," growth differentiation factor 11 (GDF11), that appears to rejuvenate the architecture of the heart, the vasculature of the brain, and the bulk of skeletal muscle—at least in animals. As Wagers told the group, "GDF11, which is this 'fountain of youth' kind of factor for the heart, is also a fountain-of-youth factor for the skeletal muscle and for the brain. It is generally a good protein for rejuvenation."

"Rejuvenation" has always been a loaded crossover term in biology. Quickly raising her hand, one young high school science teacher from Walpole could barely spit out the question on everyone's mind. "You have to wonder, given what you're seeing … I mean,

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it seems like—well, when are you going to market this!"

The teachers also circled around an issue that has dogged stem cell researchers ever since the isolation of the first human embryonic stem (ES) cells in 1998 suddenly made the potential of regenerative medicine seem real. "Are we talking about extending life," asked another teacher, "or are we talking about improving the quality of life? The ethical issues are huge!"

"Yeah, yeah," Wagers nodded in agreement, before quickly walking the conversation back to biology. "I don't want to leave you with the impression that this molecule explains everything," she said. "It's absolutely certain that it does not." Indeed, other groups studying the rejuvenating properties of young blood are eyeing different proteins, factors, and pathways. Still, Wagers predicted GDF11 would have some sort of role in medicine within 4 years. "I guess the overarching image that I want to leave you with," she said, "is that perhaps aging is an imbalance of signals, and maybe we can restore the balance."

Balance may be the most important word in Wagers's vocabulary these days. She is tending a raft of collaborations, which produced 14 papers last year. She is still struggling to regain her own balance since an episode of misconduct in 2010 rocked her lab and resulted in the retraction of two high-profile papers. And, as an inveterate skeptic who made an early mark by challenging overstated claims by other researchers, she is aiming for balance between scientific enthusiasm about the "rejuvenation factor" and realism about what it might mean for medicine.

WAGERS, 41, IS OFTEN DESCRIBED as a "rising star" in the field, but it has been a challenging ascent. By her own admission, Wagers was "pathologically shy" as a child—to the point where she refused to speak to people in public, including restaurant waiters and store clerks, using her younger sister to communicate for her. The first time she gave a scientific talk, as a teenager, she "fainted dead away" and dropped to the floor. "If anyone had told me, 'By the way, Amy, you'll be speaking in front of huge audiences pretty much every week,' I would have run screaming from this career at that point," she admits.

She attributes the shyness, in part, to a peripatetic childhood in which her family relocated almost once a year because of her father's job as an electrical engineer in the telecommunications industry. Although nominally from Ohio, her family hopscotched around the country. "Sometime in the fourth grade, when I was at the playground, I wouldn't play with anybody," Wagers recalls. "The teachers asked me why, and I said, 'Oh, we're just going to move in a year. It doesn't really matter.' "

Wagers received her undergraduate degree in biology from Northwestern University in 1994 and continued her graduate work there in the laboratory of immunologist Geoffrey Kansas, where she earned her Ph.D. in 1999. She had contacted a number of labs about a postdoctoral fellowship, but didn't hear back from the one she most wanted to join: the Stanford University laboratory of noted stem cell scientist Irving Weissman in Palo Alto, California. "Geoff Kansas called me up," Weissman recalls, "and said, 'Irv, you're going to make the worst mistake of your life' if you don't talk to her."

He did, and she joined his lab. Although Weissman found Wagers "sort of quiet and unassuming" at first, he quickly realized that she was "terrific at managing collaborations" and taking on projects. "I could not saturate her with potential ideas or experiments or where we would go. She would just take on one after another, and get the results in a very clear way."

"It's obviously a fascinating idea that something in the blood can potentially reverse aging. But you'd like to know how these things work."

Toren Finkel, National Heart, Lung, and Blood Institute

By that time, a national debate was raging over the ethics of studying ES cells. The bearded and burly Weissman became one of the most implacable and articulate scientific voices defending the use of ES cells in research. Working with Weissman, Wagers says, "landed me squarely in the middle of that debate."

A key ethical flashpoint was whether adult stem cells, such as the hematopoietic cells that form blood, could function just as well as embryo-derived cells in potential therapeutic applications. Several prominent groups had claimed that these bone-marrow cells could differentiate into other tissues, including muscle, heart, and brain cells. To test the claims, Wagers introduced a marker into mouse hematopoietic stem cells and transplanted just one of the cells into a recipient mouse. She could then see whether any new brain, heart, or muscle cells were descended from the transplanted cell.

Aside from what turned out to be rare "fusion events," where blood-forming stem cells and muscle-forming stem cells physically merged, Wagers and Weissman saw no sign that adult hematopoietic stem cells from bone marrow could form muscle. "Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells," declared their 2002 *Science* paper (27 September, p. 2256). ("Irv originally wanted it to be 'No Evidence,' " Wagers says, "but I think I talked him into 'Little Evidence.' ")

"My work was basically refuting those who were saying, 'Well, we don't need embryonic stem cells because we have adult stem cells,' " Wagers says.

"It was deeply unpopular," Weissman recalls. "What Amy showed was that not only was she smart and able to do experiments, but strong and courageous about standing up for what she did."

The "little evidence" paper was among the first of what Wagers now laughingly refers to as her "negative data" oeuvre—studies that challenged or disproved previously published claims. That body of work even inspired a bit of endogenous slang in the Weissman lab: To "wagerize" was to rigorously refute a research claim. The "negative data" papers also marked a turning point in Wagers's career, because some of the work involved pairs of mice with linked circulatory systems. That was her introduction to a curious 19th century laboratory creation that is now playing a starring role in aging research: the parabiotic mouse.

IN 1864, A FRENCH ZOOLOGIST named Paul Bert described an unusual surgical technique that merged the circulation systems of two animals into one. The technique involved making facing incisions in the flanks of two animals (mice, for example), and then sewing the skin of the two together, so that their flesh would be in contact. As the wound healed, capillaries from one animal would infiltrate the tissue of the other, Bert observed, "so as to create an exchange of nutrients by establishing a common circulatory system." Nearly a century would pass before scientists realized that parabiosis, as the technique became known, offered a powerful approach to studying aging and regeneration.

During the 1950s, Cornell University researcher Clive McCay, famous for having shown in the 1930s that caloric restriction extended lifespan in laboratory animals, and colleagues reported that stitching a young mouse to an old mouse (the technical term is heterochronic parabiosis) seemed to rejuvenate the older animals. But in an age before high-powered molecular analysis, the Cornell researchers had little chance of explaining

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this surprising effect. Weissman himself had published parabiosis experiments as a high school student growing up in Great Falls, Montana, but for the most part the idea of using parabiosis to study the biology of aging fell out of fashion.

That began to change about 10 years ago, when Stanford biologist Thomas Rando and Irina Conboy, a postdoc in his lab, in collaboration with Wagers and Weissman, resurrected the technique with the thought of looking at how young blood affected the function of aging adult stem cells, such as the "satellite cells" that help maintain and repair muscle. These cells become less effective at regenerating tissue as an animal ages. The Stanford group sewed young mice together with old mice to see if there was anything in the blood that influenced the stem cells.

"We didn't invent parabiosis," Rando says, "but we revived the use of the technique." The results, published in 2005 in *Nature*, confirmed its power: The Stanford team found that sharing blood with young mice caused muscle stem cells in old mice to recapture their youthful ability to proliferate and regenerate muscle.

What remained unclear was the trigger—did something in young blood spark rejuvenation, or did something in old blood inhibit it? Rando, Tony Wyss-Coray, also at Stanford, and Conboy, who established her own lab at the University of California (UC), Berkeley, have pursued the answer ever since. So has Wagers, who moved to Harvard Medical School in 2004. As soon as her lab was up and running at Harvard's Joslin Diabetes Center, she launched a broad campaign to identify what she and others in the field coyly began to refer to as "systemic factors" in young blood.

Wagers set up multiple collaborations to explore the effects of those factors in different organs and tissues—with Richard Lee, a cardiologist at Brigham and Women's Hospital in Boston, to look at the heart; with Robin Franklin, whose lab at the University of Cambridge studies spinal cord injuries and ways to restore the myelin coating to nerve fibers; and with Lee Rubin, who studies neurodegenerative diseases at Harvard.

Just as the work was taking off, however, she had to take an involuntary and unofficial detour to deal with what she now describes as "the darkest time in my career, if not my life."

IN THE SUMMER OF 2010, Wagers became aware of a potential problem in a figure that had appeared as part of a *Nature* paper published earlier that year. First author Shane Mayack, a postdoc in her lab, had led the study, which claimed that the young blood phenomenon also affected hematopoietic stem cells in old mice, enhancing their blood-forming ability. One illustration in the supplemental material appeared to duplicate data from a 2008 *Blood* paper by Mayack and Wagers.

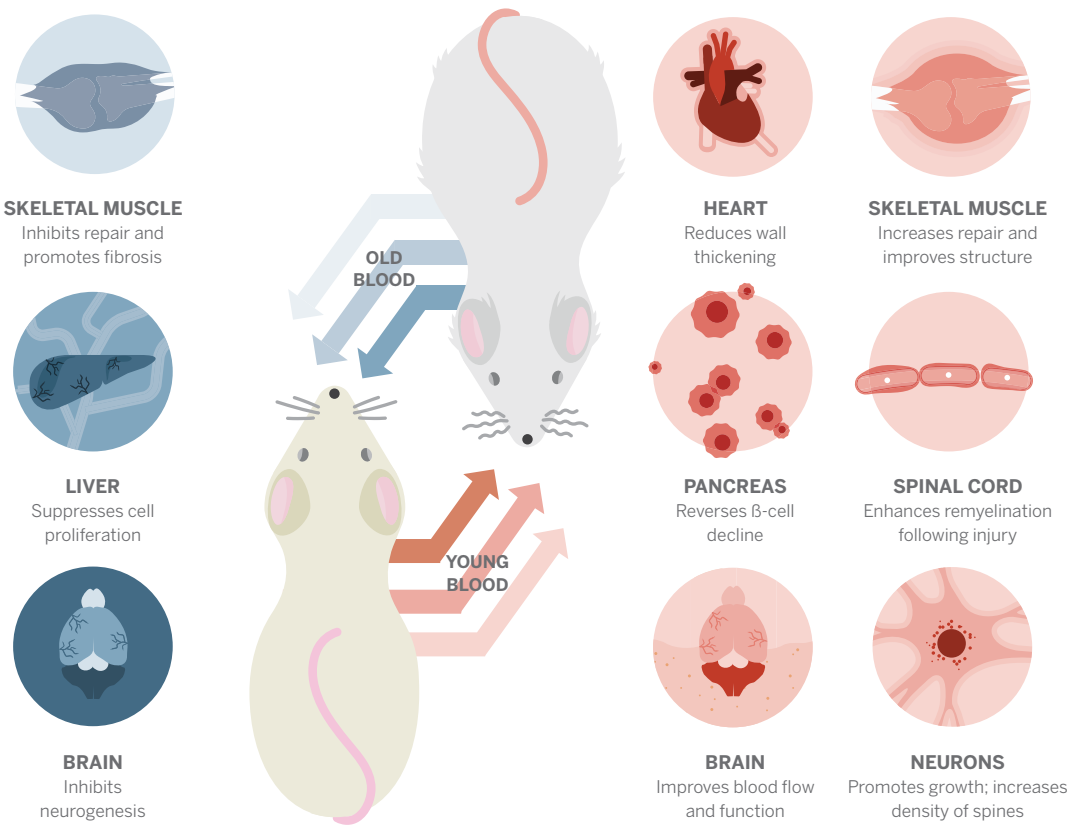
The possibility of impropriety hit Wagers like "a punch to the gut." She alerted Harvard Medical School officials, who launched an inquiry. Without waiting for the outcome, however, Wagers moved immediately to retract the *Nature* paper and contacted editors at *Blood*. "The first thing I thought was: I have to fix this."

A subsequent 2012 analysis by the federal Office of Research Integrity (ORI) concluded that Mayack had "engaged in research misconduct"; among the transgressions identified by ORI was the use of a figure from another, unrelated paper "to falsely represent [Mayack's] own experiment" and the use of an illustration from an online source as original data. The ORI report said Mayack also "falsely" relabeled identical flow cytometry plots to represent them as different experimental results.

The episode left Wagers psychologically bruised and scientifically sobered. "All of the waste in the research dollars; all of the waste in the time and effort of people in our lab and in other labs. And then the enormous amount of waste of the efforts of the people who track it down and document it. It's just horrible." Several online commentators at the website Retraction Watch faulted Wagers for lax oversight, but she wonders how lab chiefs can guard against the kind of false representations documented by ORI. "You can try very hard not to be deceived, but if you are, you are," she says. (According to the *Federal Register*, Mayack "neither admits nor denies" the ORI findings, and

Young blood versus old blood

Factors in "young blood" activate stem cells and rejuvenate organs and cells in old mice. Factors in "old blood" appear to inhibit regenerative capacity in young mice.



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Mayack later issued a statement admitting mistakes but denying misconduct.)

Despite coverage of the retractions in *The Boston Globe* and *The New York Times*, the episode of misconduct did not dim Wagers's star at Harvard; the university granted her tenure in 2012, and her former mentor Weissman applauded her quick reaction to the incident. "To her credit," he says, "she didn't try to wait the fuss out."

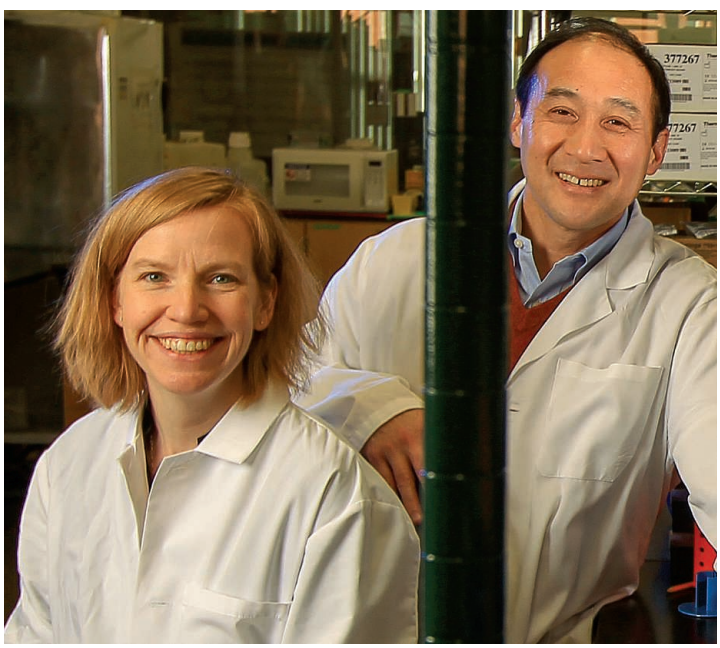
DURING THIS DIFFICULT TIME, the search for rejuvenating "systemic factors" in young blood went on, in Wagers's lab and elsewhere. But as she told the group of schoolteachers, "It's really hard to find something in the blood."

The Harvard researchers looked at numerous possibilities: proteins, fats, hormones. "It was all coming up negative," Lee recalls. "After about a year of work, we were starting to get concerned that we were never going to find this factor." Then the group enlisted the aid of a biotech firm in Boulder, Colorado, called SomaLogic Inc. The company used an advanced protein-capture technology to produce a list of 13 possible factors that seemed to be present at high levels in young blood and diminished levels in old blood. Out of this baker's dozen, the group ultimately zeroed in on GDF11.

"It's not a well-studied molecule," Wagers says. The protein is a member of the transforming growth factor β superfamily, a large group of related proteins that perform a wide variety of signaling functions regulating growth, differentiation, and immune function. Wagers's lab is still trying to figure out which cells make GDF11 and why levels decline with age. But using commercially available supplies, her team proceeded to test the rejuvenating potential of the molecule by injecting it into old mice.

The results were striking. In 2013, Wagers, Lee, and their colleagues reported in *Cell* that injection of GDF11 alone has the same effect on the hearts of old mice as an infusion of young blood: It significantly reversed cardiac enlargement (hypertrophy), which often causes the kind of heart failure commonly seen in older people. And this spring, in two papers in *Science*, Wagers, Lee, and their colleagues showed that raising GDF11 to youthful levels rejuvenated muscle stem cells, reversing age-related impairments and producing greater strength and endurance in older mice. Working with Rubin, they also reported that GDF11 bolstered circulation in older mouse brains, which promoted the formation of new neurons and ultimately improved brain function.

The reaction outside the lab has run the gamut from cautious excitement to over-the-top cultural enthusiasm (online commentators gushed about the potential for "vampire therapy"). "It's obviously a fascinating idea that something in the blood can potentially reverse aging," says Toren Finkel, who heads the Center for Molecular Medicine at the National Heart, Lung, and Blood Institute in Bethesda, Maryland. "That's sort of the holy grail for aging research. … But you'd like to know how these things work, what the mechanism is."



Amy Wagers and Richard Lee studied the rejuvenating effect of GDF11, a blood-borne factor.

PHOTO: *THE BOSTON GLOBE*/GETTY IMAGES

Other researchers caution that the work still needs to be reproduced and note that growth factors have the potential to initiate or accelerate cancers.

Wagers is the first to acknowledge that the biology is far from settled. "This is a complicated and robust system of regulation, so there's likely multiple signals." Indeed, Rando's lab at Stanford has been pursuing blood-borne factors in older mice that seem to suppress stem cell activity and blunt their regenerative capacity. Conboy's lab at UC Berkeley has recently reported that levels of the hormone oxytocin in the blood decline with age, and increased amounts of oxytocin seem to play a major role in activating adult muscle stem cells and improving muscle regeneration. And Wyss-Coray and colleagues reported this past May in *Nature Medicine* that infusions of "young blood plasma" reversed the neural and cognitive impairments of old mice—largely by rejuvenating the function of synapses. "Interestingly, none of us ended up with the same pathway in any of this," Wagers says.

Despite her team's impressive rodent results, Wagers suspects injecting GDF11 directly into patients would be "not ideal," because such an approach would bypass the tight biological regulation governing the molecule and perhaps increase the risk of side effects. "Minimally, we could use it as a biomarker for predicting outcome" and monitoring other treatments for age-related conditions, she says. Citing unpublished data on nearly 2000 elderly heart patients followed for roughly 9 years, for example, Peter Ganz of UC San Francisco and colleagues have reported at meetings that lower levels of GDF11 in the blood predicted higher rates of heart attack, stroke, congestive heart failure, and overall mortality.

Harvard has filed patents on the GDF11 work, and other groups are quickly moving their own young blood findings toward the clinic. Stanford doctors plan to begin transfusing plasma from young blood donors into patients with Alzheimer's disease, and Conboy says clinical testing of oxytocin, already a Food and Drug Administration–approved drug, and other blood-borne factors are under discussion by UC Berkeley scientists.

Wagers is acutely aware of the dangers of going overboard about GDF11's promise. But she has also been chastised, on the most personal level, for being too negative about potential therapies. Ten years ago, when one of her "negative" studies shot down the notion that bone marrow cells could treat heart attack victims, she got an earful from an outraged heart attack patient: her father. "He was angry at me for publishing that work. That kind of helped me understand the depth, the real power of hope in that way," Wagers recalls.

"You want to be enthusiastic about the potential, the real potential of the science," she continues. "On the other hand, you have to turn around and say, 'But not yet.' It's just a really hard message—to be clear that the hope is real, but that it will take time, and we can't tell you the path to get there." ■

Stephen S. Hall is a science writer in Brooklyn, New York.

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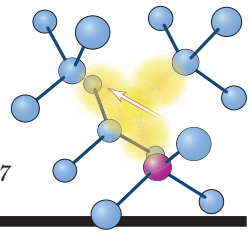
On the science of morality *p. 1242*

Diamond — a gem of a quantum memory *p. 1247*

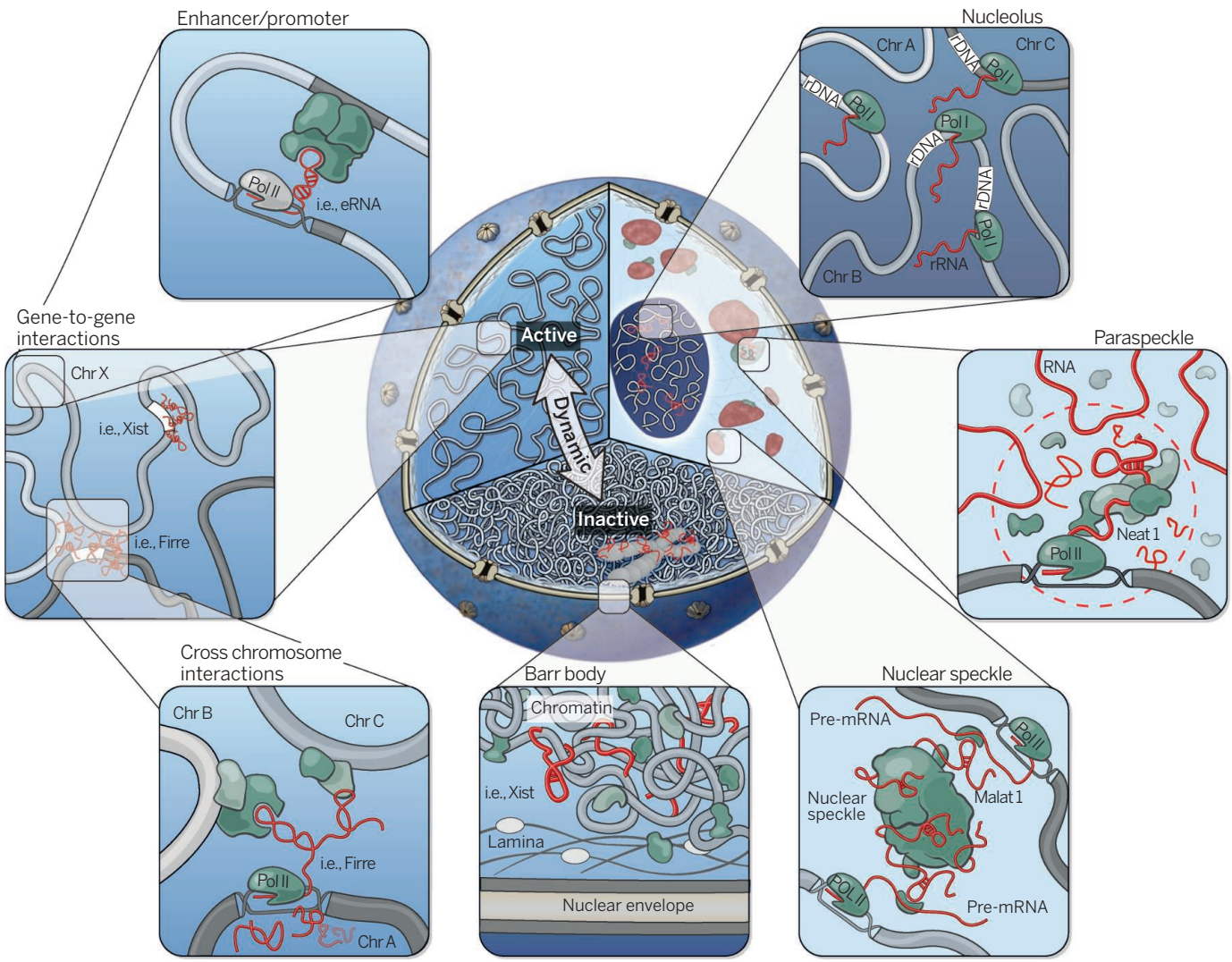


PERSPECTIVES

RNA FUNCTION

RNA and dynamic nuclear organization

Long noncoding RNAs may function as organizing factors that shape the cell nucleus



An RNA roadmap to nuclear organization. Cutaway of the nucleus highlighting three organizational levels: active (left) and inactive (bottom) regions, and nuclear bodies (right). Clockwise from right to left: The nucleolus is formed around actively transcribed rRNA sites; paraspeckles are formed by the Neat1 lncRNA; the Malat1 lncRNA is present within the nuclear speckle, and actively transcribed genes are repositioned close to nuclear speckles; the inactive X chromosome (Barr body) is coated by the Xist lncRNA and dynamically repositioned from the active to inactive compartments where it is localized to the periphery of the nucleus; lncRNAs can mediate gene-gene interactions across chromosomes (bottom panel inset) and within chromosomes (top panel inset).

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By **John Rinn**[1,2,3] *and* **Mitchell Guttman**[4]

The human genome consists of more than 2 m of linear DNA, which is packaged into a three-dimensional structure in the nucleus of each cell. To ensure proper cell-type–specific gene regulation, each cell must organize its DNA, RNA, and protein within the nucleus in ways that differ in each cell type. It had long been suspected that RNA itself might be a key organizing factor that shapes this dynamic nuclear floor plan (*1*), with recent research pointing to a role for nuclear-retained long noncoding RNAs (lncRNAs) in organizing nuclear architecture. Here we provide a perspective on the classical and newly emerging role of RNA in this process.

The nucleus is organized into domains generally associated with shared functional and regulatory roles (*2*, *3*) (see the figure): For example, with ribosomal RNA (rRNA) processing and biogenesis (*4*), mRNA splicing factors (*5*), and domains of genes that are regulated by specific transcription factors (*6*) and chromatin regulators (*3*).

More than 25 years ago, it was first noted that RNA was associated with the "nuclear matrix" (*1*). Digesting or stopping the production of RNA, but not protein, resulted in disorganized chromatin regions inside the nucleus (*1*). Initial insights into the role of this RNA component came from studies of the nucleolus. The nucleolus forms a specific domain where rRNA gene loci, which are spread across multiple chromosomes, coalesce into a spatially organized compartment. Here, the rRNA genes are coordinately transcribed and processed (*4*). The act of transcribing a rRNA gene is sufficient to reposition the gene locus into the nucleolus (*4*).

The relationship between transcription and nuclear organization is not restricted to the nucleolus. For example, there are many genomic regions, often on different chromosomes, that when transcribed by a specific transcription factor are brought into spatial proximity within a common "transcription factory" domain (*6*). In addition, the act of transcription is sufficient to reposition genomic DNA close to the nuclear speckle (*5*), a nuclear domain enriched with pre-mRNA splicing factors.

Recent studies have shown that several lncRNAs actively assemble nuclear domains. For example, the Neat1 lncRNA is necessary for the assembly and maintenance of the paraspeckle (*7*), a nuclear domain that is thought to be the site of nuclear retention of adenosine-to-inosine edited mRNAs. Moving the Neat1 transcription locus is sufficient to form new paraspeckles at the integration locus (*7*). Importantly, Neat1 requires active transcription to "tether" the lncRNA to its own transcription locus in order to carry out this role (*7*).

Another example is the Xist lncRNA, which is essential for silencing, compaction, and repositioning of the X chromosome to the nuclear periphery of the nucleus (*8*, *9*). This large-scale restructuring is dependent on Xist because expression of Xist in male cells or on autosomes, where it is not normally expressed, is sufficient to cause formation of a repressed nuclear compartment.

"...it is becoming increasingly clear that lncRNAs are important in all levels of nuclear organization—exploiting, driving, and maintaining nuclear compartmentalization."

Xist will only form this repressed nuclear compartment in proximity to its integration site (*10*). The ability of Xist to reposition active genes into this compartment is dependent on the same RNA domain required for silencing transcription (*8*, *10*).

These examples and others (*11*) suggest that many lncRNAs may function as nuclear organization factors that can establish nuclear domains. lncRNAs may even serve as a layer of specificity—distinguishing the DNA and RNA that will be contained within one nuclear compartment from those that are contained within a different nuclear compartment. Because lncRNAs very often exhibit cell-type and context-specific expression patterns, this may explain how cell-type–specific nuclear domains are established.

In addition, lncRNAs can also use the existing three-dimensional organization of the nucleus to locate specific DNA target sites. For example, Xist spreads across the entire X chromosome by first localizing at genomic sites that are in three-dimensional nuclear proximity to its own transcription locus (*10*, *12*). Simply moving Xist to a different genomic location leads to its relocalization to new genomic target sites that are also defined by their close spatial proximity to the new Xist integration site (*10*). Other lncRNAs also use spatial proximity to identify target sites (*11*). This interplay between the spatial position of a lncRNA's transcription locus and its localization targets is not restricted to interactions on the same chromosome, but can also occur across chromosomes (e.g., the Firre lncRNA) (*11*, *13*, *14*).

Many nuclear-retained lncRNAs may work through a proximity-guided search, either within or across chromosomes. This may be a general search strategy for lncRNAs because they can retain "positional identity" by functioning immediately upon transcription in proximity to its encoded genomic locus. Since genomic locations that are on different chromosomes can be in close spatial proximity within the nucleus, this proximity-guided model would explain the observations of both cis- and trans-mediated regulatory effects of lncRNAs. This model would also explain how lncRNAs, which are generally of lower abundance relative to mRNAs, can reliably identify their target genes by searching in spatial proximity—and at a high effective concentration—near their transcription locus.

Collectively, these studies suggest that lncRNAs may shape nuclear organization by using the spatial proximity of their transcription locus as a means to target preexisting local neighborhoods (*10*, *14*). lncRNAs can in turn modify and reshape the organization of these local neighborhoods to establish new nuclear domains by interacting with various protein complexes, including chromatin regulators (*7*, *10*). Once established, a lncRNA can act to maintain these nuclear domains through active transcription and recruitment of interacting proteins to these domains (*7*, *13*). While the mechanism for how lncRNAs establish these domains is not fully understood, it is becoming increasingly clear that lncRNAs are important at all levels of nuclear organization—exploiting, driving, and maintaining nuclear compartmentalization. ■

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10.1126/science.1252966

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BEHAVIOR

Morality beyond the lab

Much of moral life involves attending to our own good deeds and to the bad deeds of others

By **Jesse Graham**

Morality may be of the highest importance to us, as Einstein put it. But how often do we actually experience morality in our day-to-day lives, and what forms does this experience take? The science of morality has flourished in recent years as methods from social psychology, behavioral economics, neuroscience, experimental philosophy, and cultural anthropology have been applied to the study of moral thought and behavior (*1*). So far, most of this work has used artificial stimuli, such as hypothetical dilemmas about runaway trolleys or sex with a dead chicken, in artificial environments such as laboratories or medical imaging scanners. On page 1340 of this issue, Hofmann *et al.* (*2*) move morality science out of the lab and to the street, office, kitchen, bar, or wherever people happen to be when their cell phone rings.

Prompted at random intervals over 3 days, participants recorded any moral or immoral events they experienced: acts that they committed or were the target of, that they witnessed directly, or that they heard about. Using this approach, the authors replicate several findings from laboratory studies, including moral contagion (being the target of a good deed makes us pay it forward, subsequently doing good for others), moral licensing (doing good makes us feel entitled to do bad later in the day), and political differences in moral values and concerns.

The study also sheds light on the mysteries of morality. For instance, laboratory studies have shown a "holier-than-thou" effect, in which people overoptimistically predict their own future moral behavior but accurately predict the not-so-moral future behavior of others (*3*). Hofmann *et al.* show that this asymmetry does not only apply to predicted behaviors. Whereas the moral far outweighed the immoral in participants' reports of their own acts, this was not the case for reports of others' acts. In fact, the study suggests that moral life can largely be characterized by two kinds of events: noting one's own good deeds and gossiping about the bad deeds of others.

The results also affirm the value of moral pluralism (*4*), the idea that there are many different moral values, which may come into conflict between cultures or individuals and even within a single person; this is in contrast to moral monism, the idea that all morality can be reduced to a single principle. Analysis of the reported events revealed a rich variety of both moral and immoral acts. Whereas moral psychology has concentrated on immoral acts (particularly those involving harm or unfairness), participants most often encountered acts of care, generosity, and kindness.

Here the study introduces some new mysteries for future research. Although care far outweighed harm in participant reports, the immoral outweighed the moral for every other type of moral concern (unfairness outweighs fairness, dishonesty outweighs honesty, etc.). Why is this? Are we typically noting minor acts of kindness or helpfulness when noting our own good deeds, but including all the intensity and variety of lies, cheating, betrayals, and acts of degradation in our gossip about others' misdeeds? Would other people be more likely to agree with our moral assessments of others' behavior than our assessments of our own behavior? Would others even categorize our acts of self-assessed moral kindness as morally relevant at all?

Some of these questions may be answered with further analyses of Hofmann *et al.*'s rich (and publicly available) data set, but the study also points to future research directions. First, although the authors' use of experience-sampling methods and open-ended responses provided a much more ecologically valid picture of moral life, the study nevertheless relied on self-reports. Future investigations could get around self-report biases with unobtrusive observations of daily life (*5*), informant reports (*6*), or text analyses of online behavior (*7*). Second, surprisingly little is known about the relations between moral judgment and moral behavior (*8*). Experimental manipulations applied with Hofmann *et al.*'s smartphone app could elucidate whether and how moral judgments affect behavior; for example, people could be randomly assigned to attend to either moral or immoral events, to test whether this affects subsequent actions and perceptions. Similarly, field experiments using this method could clarify the causal directions among daily moral events, happiness, and sense of purpose.

By taking the study of morality out of the lab and into the stream of life—and by leaving it to participants to define "moral" and "immoral" themselves—Hofmann *et al.* have moved the field forward while demonstrating the generalizability of past work. Moral events are indeed commonly experienced in everyday life, and these events are consequential for both self and others. Future work using this technology and approach can begin to unpack how moral events are construed by individuals and cultures and which conditions foster different forms of moral behavior. ■

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10.1126/science.1259500

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Changing behaviors to prevent noncommunicable diseases

Altering physical and social environments can change behaviors to improve population health

By **Dave A. Chokshi**[1] *and* **Thomas A. Farley**[2]

Noncommunicable diseases (NCDs)—largely comprising cardiovascular disease, cancer, chronic pulmonary disease, and diabetes—are the most important global health issues of the 21st century, as measured by both mortality and morbidity (*1*, *2*). These diseases are as preventable across entire populations as are infectious diseases, but require a different approach—one that involves policy change to promote healthy behaviors.

Citing their impact on human development, the United Nations General Assembly convened a high-level meeting on prevention and control of NCDs in 2011, resulting in a political declaration and voluntary country commitments. In 2012, the World Health Organization (WHO) adopted the goal of reducing global NCD mortality by 25% in 2025—"25 by 25"—among adults 30 to 70 years of age. By 2013, 50% of countries around the world had an operational NCD policy with a budget for implementation, up from 32% of countries in 2010 (*3*). Rigorous investigation has demonstrated that prevention of NCDs is possible, particularly through population-based approaches (*4*).

The United Nations Secretary-General's report on prevention and control of NCDs is remarkably clear in recommending that "the greatest reductions in non-communicable diseases will come from…population-wide interventions" that address the risk factors of tobacco use, unhealthy diet, lack of physical activity, and harmful use of alcohol (*5*). Trends in these risk four factors demonstrate geographic divergence in some cases and convergence in others. Some high-income countries have made progress in tobacco control (e.g., Australia and Canada) and moderation of alcohol use (e.g., France and Italy), but those health gains are threatened by dramatic increases in overweight and obesity. Meanwhile, most of the 1 billion smokers worldwide live in low- and middle-income countries such as India and China, which simultaneously face the challenge of weight gain and associated metabolic diseases like diabetes.

The four key risk factors reflect human behaviors, but because people's behavior is profoundly shaped by their physical and social environment, population-level impact can best be achieved by changing environments to make healthy choices easier. Tobacco control—as agreed upon by the global Framework Convention on Tobacco Control and encapsulated in the WHO-endorsed package of evidence-based interventions known as MPOWER—exhibits the potential of country-level implementation of effective interventions to change behaviors and improve health. MPOWER, launched in 2008, uses public policies such as taxes, worksite and public-space smoking bans, advertising bans, and counter-advertising to shape the environment. By enacting MPOWER measures, Turkey and Uruguay demonstrated sharp declines in smoking prevalence (*6*). Population-based improvement in diet has succeeded in Finland, where actions to reduce sodium intake, increase fresh fruit and vegetable consumption, and substitute healthier fats have helped to reduce cardiovascular disease morbidity and mortality (*7*). For instance, national salt-labeling regulations for manufactured food items led to reformulation of high-salt products.

The examples of tobacco and cardiovascular risk reduction also show that population-based approaches to change behaviors are often more cost-effective than individual interventions (*8*). This is because it can be far less expensive to alter an environmental element to which many people are exposed (e.g., a ban on "trans" fats) than to interact with each person directly. Even if the effect of an altered environment on each person is small, the cumulative population effect can be large—that is, cost-effectiveness can be favorable because the cost per person reached is even smaller. Some mechanisms, such as excise taxes (often included in the price of the product), may generate government revenue that can offset costs of enforcing a population-wide strategy. Despite this, such approaches must surmount obstacles, the most important of which is political opposition. Cigarette taxes, for example, have proven difficult to pass over industry opposition in low- and middle-income countries despite their demonstrated health benefits.

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Although a changing environment is the predominant source of the global epidemic of overweight and obesity, there is a dearth of proven interventions to reverse it. Excess weight is responsible for about 3.4 million deaths annually and contributes to disability from diseases as varied as diabetes, stroke, cancer, liver disease, and osteoarthritis. Globally, the greatest gain in overweight and obesity happened between 1992 and 2002, mainly among adults aged 20 to 40 years, and no country has demonstrated a significant decrease in obesity prevalence in the past 33 years (*9*). This rise in body weight is largely a consequence of the increasing availability, low cost, and promotion of calorie-rich, highly processed food. Although intensive individual lifestyle counseling can lead to short-term weight loss (*10*), there is scant evidence for population-level interventions that lead to sustained aggregate weight loss, such as physical activity programs or promotion of healthier diets. Physiologically, the fact that losing weight appears to be more difficult than avoiding weight gain underscores the importance of preventive approaches. One of the few positive studies of obesity prevention, conducted in schoolchildren in the Netherlands, showed that blind replacement of sugar-containing beverages with sugar-free beverages modestly reduced weight gain and fat accumulation in normal-weight children (*11*).

Poor diet, tobacco use, and alcohol misuse—three of the four major risk factors for NCDs (the fourth being physical inactivity)—are susceptible to industry marketing of unhealthy products. Because of tobacco control policies and saturation of the processed food and soft drink market in high-income countries, corporations are

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looking to low- and middle-income countries as growth markets for their products. For instance, between 1997 and 2009, the annual growth rate of volume consumption per person for soft drinks was 2.4% in high-income countries and 5.2% in low- and middle-income countries (*12*). A cross-national analysis of 75 countries showed that each 1% rise in soft drink consumption was associated with an additional 4.8 overweight adults per 100 and an additional 2.3 obese adults per 100 (*13*). Out of concern for the public health effects of these trends, Mexico introduced a tax on soft drinks and "junk" food in 2013. Preliminary evidence indicates that the tax is helping to curb consumption. For example, the Coca-Cola Company reported reduced sales volumes of 5 to 7% attributed to the tax (*14*). More generally, policy approaches to counter the marketing of unhealthy products include limits on advertising, regulating locations of sale, and counter-advertising.

Ultimately, global efforts to prevent NCDs depend on successful national and local implementation. As an example, Brazil, in response to the 2011 U.N. political declaration, launched a national NCD plan of action. The plan includes promoting smoke-free environments, sharply increasing cigarette taxes, and banning tobacco advertising; signing agreements with the food industry to reduce sodium content and eliminate trans fats; and promoting and studying urban physical activity interventions (*15*).

Meeting the global "25 by 25" goal of NCD prevention will require rapid dissemination of proven interventions like MPOWER, as well as investment in designing and evaluating new approaches to promoting physical activity, reducing harmful alcohol use, and altering unhealthy diets. This in turn will require governments to redirect their public health systems toward population-level environmental interventions that prevent NCDs. ■

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10.1126/science.1259809

SCIENCE COMMUNICATION

Legislators learning to interpret evidence for policy

Training for making health policy that has the best impact on a population

By **Anne Cockcroft**,[1] **Mokgweetsi Masisi**,[2] **Lehana Thabane**,[3] **Neil Andersson**[4,5]

Should decision-makers allocate budgets for health services, using the best available evidence? Although the answer seems obvious, most resource allocation is unrelated to evidence of what works best. The well-recognized knowledge-to-action gap (*1*) has spawned a complex industry of lobbying and knowledge translation. Bridging the gap is a business opportunity for lobbyists, who help to translate evidence in ways that best present a particular interest. Partly to offset these selective, if not frankly biased, interpretations, the last decade has seen an industrial-scale thrust toward systematic reviews to synthesize available evidence (*2*, *3*). But systematic reviews mostly systematize what has been published. For many decisions on health services, there is simply no evidence with the quality one gets from a randomized controlled trial (RCT). Thus, policy decision-makers have to find their way through evidence of varying quality and relevance, only rarely packaged to clarify the population health impact of different choices. A 2012 international forum on evidence-informed health policy-making in low- and middle-income countries called for building the capacity of potential research users to evaluate and use research evidence (*4*). There are, of course, many levels of research users among health policy-makers and decision-takers. To date, initiatives to build the capacity of research users to interpret evidence have not included actual legislators themselves; instead, they target technical officers and advisers on the assumption that they will then advise the elected representatives appropriately (*5*). We will describe an effort to help legislators themselves understand the elements of the evidence they need to make decisions (*6*).

AN EXPERIENCE IN BOTSWANA. The Botswana parliament has 61 members, 57 of them elected and 4 appointed by the head of state. The minimum educational requirement is 7 years of schooling; before the 2014 elections, all members had at least school-leaving certificates (12 years), and many had university degrees. Hoping to make parliamentary debates more grounded in evidence, the minister responsible for the national HIV response in Botswana asked CIET Trust, an international research and training group, to train members of parliament (MPs) on the use of research evidence (*6*).

Research assistants contacted MPs by telephone or in person to ask about their needs and interest in training about health-related research evidence. They managed to interview roughly half of the MPs; none refused, but some were unavailable within the allotted time frame. Almost all of those interviewed said they needed better access to reliable evidence and wanted training in how to interpret and evaluate evidence. They cited jargon and unexplained technical language and statistical terms as key blocks to their understanding and use of evidence. They complained of not enough research assistants and lack of those who are available.

> ***"After the training, [it was] noted that parliamentary debate ... was more sophisticated and focused on evidence."***

Training organized under the auspices of the National AIDS Coordinating Agency was then offered to MPs from all parties on two mornings, with a similar session a year later at the request of the MPs. The training focused on three key areas: the value of counterfactual evidence (having a control or comparison group); how biases can distort findings and reports; and the need for evidence about the impact on a population of policies, rather than change in individual risk.

For presentation of evidence, the training emphasized the number needed to treat (NNT) to prevent one adverse outcome, and the unit cost per case saved, in preference to parameters of individual impact like relative risk or simply cost of activities. One session explained research terminology; items

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Botswana parliamentarians discussing evidence.

PHOTO: MOSES MAGADZA

legislators asked about most were in a RCT: control or comparison group, statistical significance, number needed to treat, and bias. A hands-on session asked participants to review the value of examples of health research evidence for health policy-making. The scenarios summarized from published articles ranged from small studies with no control group, to large, well-performed RCTs (see the photo).

Of 54 elected representatives, 36 attended one or both of the training sessions, including seven ministers, the deputy speaker, the leader of the opposition, and the chair of the parliamentary committee on health and HIV and AIDS. The lively and engaged group reported that concepts like NNT and cost per case saved had immediate resonance for them.

Training legislators about evidence will not transform health policy-making overnight. Having access to good evidence and the ability to interpret it are not enough to ensure that policies are evidence-based. Many other considerations come into play; studies have documented hindrances to evidence-informed health policy-making in both low- and high-income countries (*7*, *8*), and systematic reviews identified many barriers to health policy-makers' using evidence, such as competing influences (*9*, *10*).

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Training legislators to interpret and evaluate research evidence is uncharted territory. The feedback from the Botswana legislators was very favorable; they asked for further sessions to cover the topics in more detail and for the training to be offered to other decision-makers. After the training, one of the authors (M.M.), a senior parliamentarian, noted that parliamentary debate—for example, around the updated national HIV policy—was more sophisticated and focused on evidence. But it is early yet, and we have not attempted any formal measurement of the longer-term impact of the training. Thus far, we have demonstrated the feasibility and acceptability of this sort of training and have increased our understanding of what elected representatives want to learn and what they can cope with.

If elected representatives had a good understanding of the evidence they need to support rational decision-making, they could start to demand different kinds of evidence. For example, they might start to require those who submit budgets, or researchers receiving government funding, to produce evidence about population benefits in addition to individual benefits, about the number needed to treat to prevent one adverse outcome, and about the unit costs per case saved of different programs. They might push to see the results of systematic reviews and research syntheses. Or they might at least demand studies with acceptable counterfactual evidence. They could become active and informed parties in setting the research agenda.

COULD IT WORK ELSEWHERE? A first step for researchers to make this a reality is to get beyond the idea that legislators have neither the background nor the interest to learn about how to interrogate and use evidence to support decision-making.

Several factors contributed to success in Botswana and could be relevant to similar training elsewhere. Seeking the views of the MPs ahead of the training made them partners in the effort and guided the level and content of the sessions. To increase relevance of the learning materials, we used examples from Botswana or the southern Africa region. Linking the training to a particular topic—in this case, it was HIV prevention policies—increased its immediate relevance. Other factors contributed in Botswana and might be less easy to replicate elsewhere. Botswana has a small and functional parliament. In another setting, major conflicts within the legislature could make multiparty training such as this very difficult to implement. We also had a powerful champion in the Minister for Presidential Affairs and Public Administration, who had received master's training in evidence-based planning, as part of a program to build the capacities of researchers in southern Africa (*11*). Identifying a similar key supporter could help when attempting to introduce training for MPs in other parliaments. ■

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ACKNOWLEDGMENTS

The training of Botswana legislators was part of the African Development AIDS Prevention Trials capacity (ADAPT) program, a CIET Trust program carried out with support from the Global Health Research Initiative—a research funding partnership composed of the Canadian Institutes of Health Research; Department of Foreign Affairs, Trade and Development Canada (DFATD), and the International Development Research Centre (IDRC); supported by a grant from IDRC, Ottawa, Canada—and with the financial support of the Government of Canada provided through DFATD.

10.1126/science.1256911

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ECOLOGY

Microbes ride the current

Biogeographical diversity in bacterial populations can arise without need for natural selection

By **Stephen Giovannoni**[1] *and* **Diana Nemergut**[2,3]

How do differences in marine bacterial populations arise in the ocean? On page 1346 of this issue, Hellweger *et al.* (*1*) investigate this question with a model based on ocean currents, parameterized with data from the most ubiquitous and abundant ocean bacterium, *Pelagibacter*. The model assumes that mutations are neutral—that is, they cause no change in the fitness of organisms, so that selection cannot act on them. The results show that neutral processes are enough to generate biogeographical patterns in marine bacteria without any adaptive evolution taking place.

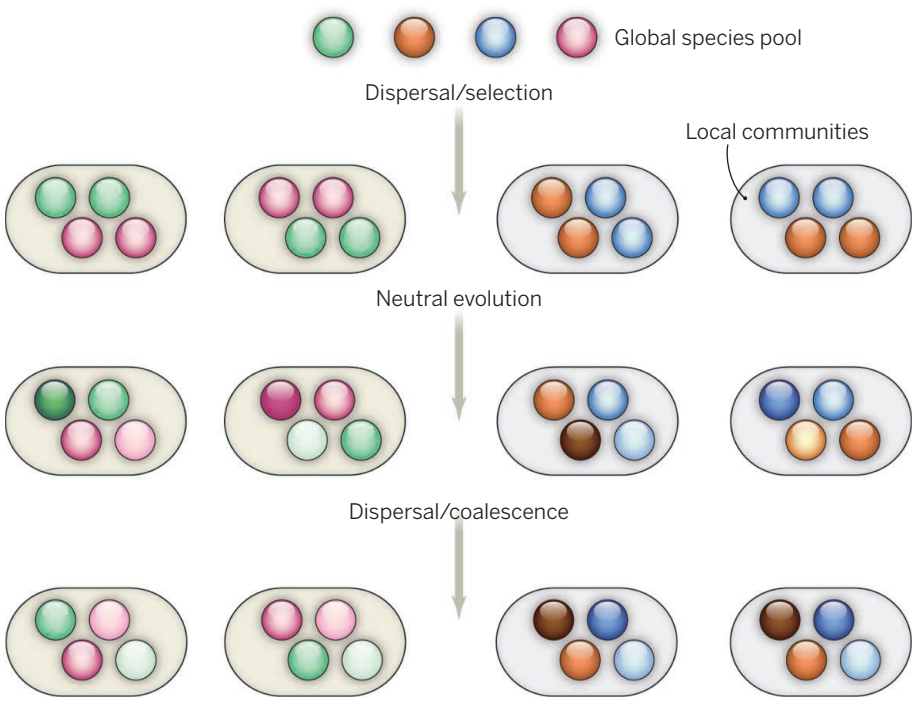
The evolutionary and ecological forces driving biodiversity traditionally have been considered separately (*2*). But microbes evolve quickly, and figuring out why microbes are where they are and how they interact requires ecological and evolutionary perspectives to come together (*3*). Hellweger *et al.* do just that, combining analogous processes from population genetics (mutation and gene flow via recombination) and community assembly (dispersal via ocean currents) into a unified model that predicts the joint outcome of evolutionary and ecological processes.

In their analysis, neutral variation is generated through DNA substitutions that do not alter fitness, but the concept applies across levels of biological organization from nucleotides to ecosystems. For example, the gene content of genomes can change without a shift in function through a neutral process called nonorthologous displacement (*4*). Likewise, Hubbell has suggested that taxa at the same trophic level in an ecosystem can be ecologically equivalent (*5*). To understand the true importance of microbial diversity, we must unravel the relevance of neutral processes across biological levels of organization.

Natural selection does, of course, happen dynamically in massive microbial populations (*6*, *7*), but Hellweger's results show that we cannot attribute all geographical variation in microbial plankton populations to natural selection. Microbial life is small, numerous, and easily transported, leading some to suggest that no bacterium, archaean, or eukaryotic microbe is dispersal-limited. If one takes this idea to its logical conclusion, then in every ecosystem the abundant microorganisms are the fittest taxa from a global seed bank, and selection determines microbial community composition and function ("everything is everywhere, but the environment selects") (*8*, *9*). Given a large enough sample, sufficient disequilibrium due to transport, and strong selection, this situation may indeed arise. However, Hellweger *et al.*'s model shows that neutral evolution can outpace dispersal, helping to create biogeographic patterns that are not driven by natural selection.

Biogeographical variation in microorganisms is well documented and has been attributed, in part, to dispersal limitation (*10*). For example, Reno *et al.* observed no evidence of gene flow between geographically isolated hot spring populations of the thermophile *Sulfolobus islandicus,* suggesting that dispersal barriers are sufficient to make these populations genomically distinct and evolutionarily independent (*11*). Studying soil fungi, Talbot *et al.* also reported a high degree of endemism, consistent with the interpretation that geographical distance plays a role in structuring communities (*12*). However, these studies were based on observations of contemporary patterns in biodiversity and could not rule out the possibility that an unmeasured environmental variable structures the community. Hellweger *et al.* provide a mechanism for how such geographical patterns could arise through neutral processes, even in a well-mixed environment like the ocean.

How do Hellweger *et al.*'s projections compare with empirical observations? *Pelagibacter*, the organisms used in their model, have been prime subjects for biogeographical studies because of their prevalence in marine metagenomes. Two closely related SAR11 ecotypes, IA1 and IA3, segregate strongly with temperature (which is highly correlated with latitude) (*13*), and genome sequences amplified from single cells of an expanded set of taxa displayed separations among tropical, temperate, and polar regions (*14*). There is, however, one important



No selection needed. Hellweger *et al.* show that stochastic processes associated with mutation, demographics, and dispersal can lead to variation between different marine sites in the absence of selection. In the diagram, small circles with different colors are different microbial taxa; different shades of the same color represent neutral variation, and different colored ovals represent marine environments with different conditions.

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caveat to all these studies: Compared with plants and animals, microbial turnover is rapid, and selection can cause very rapid shifts in community structure. Indeed, the two *Pelagibacter* ecotypes associated with latitudinal biogeography in previous reports are both endemic to Woods Hole, where they vary rhythmically in seasonal cycles (*15*). Clearly, in this instance the environment selects. Knowing more about when it does—and when it does not—will require more of what Hellweger *et al.* have done: mixing field data with modeling to test predictions about the role of population size, mutation rates, and growth rates in dispersal limitation.

Things can get truly interesting when natural selection and neutral processes interact. In marine ecosystems, plankton populations are not at equilibrium; they are at the mercy of ocean currents, as in Hellweger *et al.*'s models. Cells that are constantly transported in dynamic systems probably never stop evolving. Models like those of Hellweger *et al.*, but with added dimensions of selection, could provide the foundation for a comprehensive analytical framework that simultaneously considers the continuous processes of transport, mixing, and selection that shape plankton diversity.

As Hellweger *et al.*'s work shows, some variation in community structure may not be predictable based on natural selection, leading to questions about the potential relevance of neutral processes for ecosystem function. Thus, their analysis not only fills an important gap in the intersection of evolutionary and ecological theory but also can provide meaningful avenues to simplify microbial complexity to its essence: the patterns of function that matter for how ecosystems work, and how that may change in the future. ■

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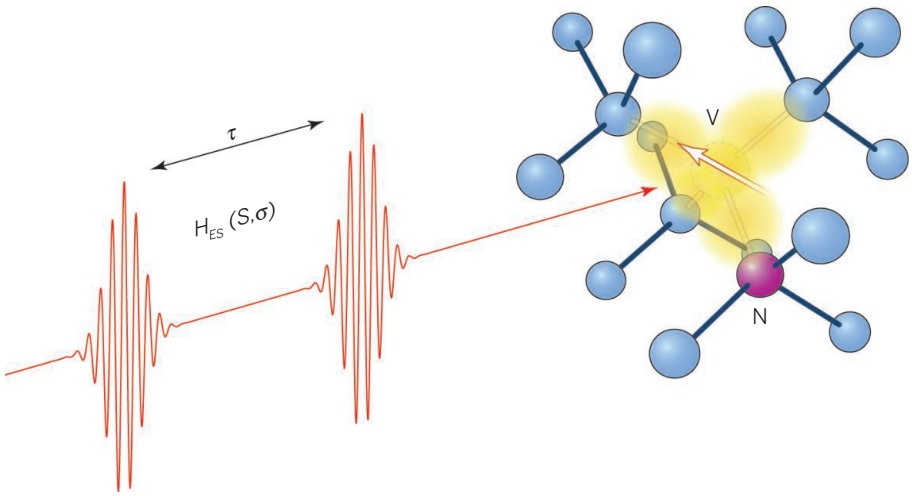

Control and manipulate. Bassett *et al.* (*1*) use a pair of subpicosecond resonant pulses to manipulate the orbital state of a single NV center, illustrated by occupation of dangling bonds (yellow) around the nitrogen (N) and vacancy (V) that form the defect. The ultrafast pulses switch the defect between the ground and excited orbital states, affecting the $S = 1$ spin (orange). By measuring the resulting spin evolution during time τ between the pulses, the excited-state Hamiltonian H_{ES} can be reconstructed, including both the spin (S) and doubly degenerate orbital (σ) degrees of freedom.

PHYSICS

Diamond dynamics under control

Ultrafast spectroscopy is used to control and manipulate quantum states in diamond

By **Lilian Childress**

Ultrafast pulses from mode-locked lasers have become a preeminent tool for characterizing and controlling systems as diverse as biologically active molecules and semiconductor quantum dots. By varying the time between successive pulses, a vast range of temporal dynamics can be investigated, revealing dissipation mechanisms and even coherent processes. On page 1333 of this issue, Bassett *et al.* (*1*) realize ultrafast control over the optical transitions of a nitrogen-vacancy (NV) center, an atomic-like defect in diamond that has recently received considerable attention in the context of solid-state quantum devices. Overcoming technical challenges associated with addressing a single emitter at cryogenic temperatures, their work probes dynamics on nano-, pico-, and even femtosecond time scales, creating a precise map of the NV center's excited state. Moreover, they use the same tools to realize fast, all-optical control over a long-lived quantum bit (qubit) formed by the defect's spin degrees of freedom.

The enabling technology developed by Bassett *et al.* is a time-domain quantum tomography (TDQT) technique that is used to reveal the structure of the defect's excited state (see the figure). Two resonant subpicosecond pulses are used to transfer the NV center into and then out of its excited state with precision timing. During the pulses, the spin state $S = 1$ of the defect remains coherent. By tomographically measuring the spin state after the second pulse [using established fluorescence detection techniques (*2*)], the temporal evolution of the spin within the excited state can be reconstructed. Moreover, the polarization of the picosecond pulses determines which of two excited-state orbitals is populated, completing the set of controls needed to fully determine the excited-state structure. By varying the NV center's initial spin state, the pulse pair polarization and timing, and the spin detection axes, Bassett *et al.* can measure the magnitude and phase of each possible term in the symmetry-constrained excited-state Hamiltonian.

This precision tomography protocol builds on many decades of work to tease out the structure of the diamond NV center.

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Theoretical models (*3*, *4*) combined with continuous-wave spectroscopy (*5*) have already given extensive insight into its excited state. The new TDQT results improve on the precision of earlier measurements while also adding two new features: experimental verification of the phase of each parameter in the excited-state Hamiltonian, and measurement of the dissipation mechanisms at play. In addition, Bassett *et al.* use ultrafast Ramsey spectroscopy to directly probe the coherence of the optical transition. This technique provides a glimpse of orbital dynamics over time scales spanning six orders of magnitude, as well as a direct measurement of dephasing on the optical transition.

The work of Bassett *et al.* carries important implications for quantum devices based on diamond defects. On one hand, a detailed understanding of electronic structure is critical for applications in quantum information science, where each physical system encoding a qubit must be calibrated (including local inhomogeneities) and all decoherence mechanisms must be carefully accounted for. Indeed, understanding the NV center's excited-state structure has been critical to breakthrough experiments, such as the recently demonstrated teleportation between distant spins in diamond (*6*). On the other hand, ultrafast manipulation can also be used in quantum gates. As Bassett *et al.* show, fast resonant transfer to and from the excited state can induce nontrivial rotations of the NV-center spin. For a qubit encoded in two Zeeman sublevels, this enables all-optical qubit control with a speed comparable to the fastest microwave gates.

"The spin manipulation and excited-state tomography developed by Bassett* et al. *represent new additions to the ultrafast toolbox."

It is worth noting that the spin gates of Bassett *et al.* differ from more familiar optical spin manipulation based on Raman transitions, in which two spin states are driven off resonance with a common excited state to induce rotations between them. Intense, highly detuned pulses can realize extremely fast Raman gates in, for example, the hyperfine levels of trapped ions (*7*) or spins in semiconductor quantum dots (*8*). In these systems, a large fine-structure or spin-orbit splitting makes it possible to drive specified excited states far from resonance to minimize spontaneous emission losses. By contrast, the NV center has relatively small fine-structure splittings on the order of gigahertz. A highly detuned Raman pulse would therefore excite transitions to multiple levels, causing destructive interference in the spin rotation. Raman transitions have been used to demonstrate all-optical gates in the NV center, but they work best for low-intensity, near-resonance excitation (*9*). The qubit control demonstrated by Bassett *et al.* presents a new tactic, ultimately limited by spontaneous emission losses but much faster than currently achieved NV Raman gates. One could argue that this resonant approach bears a closer resemblance to techniques used in double quantum dots, where an electronic spin Hamiltonian is rapidly manipulated by changing orbital states via electrode voltages (*10*).

The spin manipulation and excited-state tomography developed by Bassett *et al.* represent new additions to the ultrafast toolbox. In focusing on a single spin, their work complements existing ultrafast techniques, most notably multidimensional coherent spectroscopy (*11*), that directly probe optical excitation and emission correlations to reveal energy transfer processes. Indeed, these new TDQT techniques arrive hot on the heels of the first multidimensional spectrum of NV centers (*12*), which measured the temporal dynamics of the defect's vibronic modes. Continued development of ultrafast spectroscopy may help to elucidate other processes, including the poorly understood role of vibronic modes in optical spin preparation and detection. However, the NV center, currently the best-studied among a vast range of atomic-like defects, might just turn out to be a test case. Ultimately, Bassett *et al.*'s method may have the greatest impact in other atomlike systems, such as defects in silicon carbide (*13*), where it could provide insight into unexplored energy landscapes. ■

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10.1126/science.1257840

IMMUNOLOGY

Innate lymphoid cells sweeten the pot

Immune cells prompt gut epithelial cells to produce carbohydrate nutrients for resident bacteria

By **Lora V. Hooper**

Our intestines are brimming with a dense community of microorganisms that number in the trillions. This microbiota includes bacteria that metabolize dietary carbohydrates that would otherwise evade digestion. Although most gut bacteria are beneficial, problems can arise if the community changes dramatically or if bacteria invade deeper tissues. The intestine has therefore evolved strategies to control this ecosystem. One such strategy involves the synthesis of cell surface carbohydrate chains that can be used as a food source by the microbiota. On page 1310 of this issue, Goto *et al.* (*1*) show that specific immune cells trigger the synthesis of these carbohydrate chains in the epithelial lining of the intestine.

Nearly all mammalian cells decorate their surfaces with complex sugar chains that are attached to proteins or lipids embedded in the surface membrane. These carbohydrates perform basic functions such as mediating cell-cell and cell-matrix interactions. Importantly, their structural motifs can be globally remodeled by changes in the expression of the glycosyltransferases that catalyze their synthesis.

Gut bacteria induce such a remodeling of the carbohydrate landscape in the intestinal epithelium. Germ-free mice, which are microbiologically sterile, produce epithelial surface carbohydrate chains that tend to terminate with sialic acid. However, after germ-free mice are colonized with bacteria, their epithelial cells increase expression of fucosyltransferase 2 (FUT2) (*2*). Consequently, their epithelial carbohydrate chains change so that they tend to terminate with fucose, reflecting the carbohydrate structures found in mice that are colonized throughout their lives (*3*).

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Why alter epithelial carbohydrates in the presence of bacteria? Certain mutualistic microbiota species have a distinctive ability to forage host-produced carbohydrate chains (*4*). For example, *Bacteroides thetaiotaomicron*, a common gut inhabitant, expresses a variety of glycoside hydrolases including fucosidases (*5*). These enzymes not only allow bacterial processing of complex dietary carbohydrates, but also enable hydrolysis of host-produced carbohydrate chains. Host-derived fucose and other monosaccharides can then be used for carbon and energy by the bacteria. This foraging strategy becomes key when simple carbohydrates from the host diet are limited or when the foraging species must compete with other microorganisms for limited nutrients (*3*, *4*).

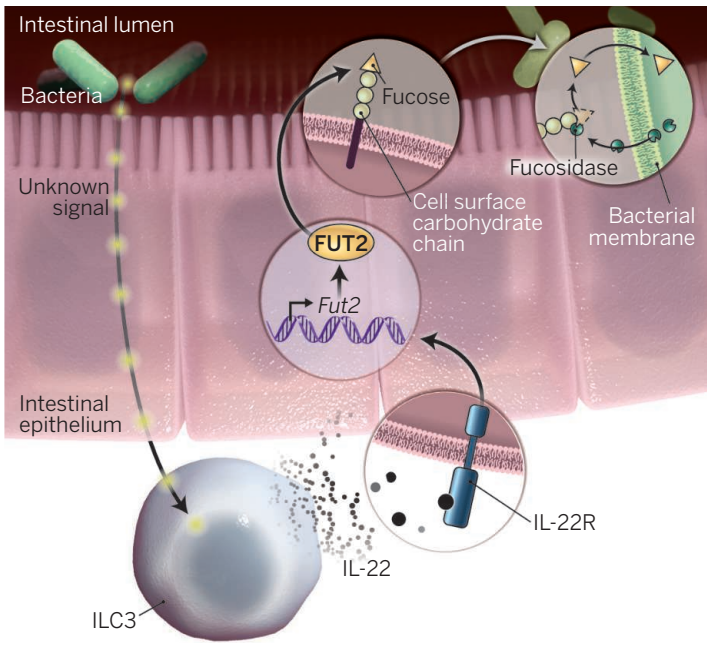
In contrast to mutualistic bacteria, most gut pathogens lack the enzymatic machinery required to forage complex carbohydrates. Thus, host production of certain carbohydrate structures likely supplies a selective advantage to the bacteria that can metabolize them. This could be adaptive for the host by allowing selective recruitment and retention of bacteria that can also metabolize complex carbohydrates in the diet for the mutual benefit of bacteria and host (*6*).

How bacteria induce gut epithelial fucosylation has been a mystery. Epithelial cells can detect bacterial molecules through cell surface receptors, and thus it seemed possible that this simple interaction could elicit FUT2 expression. However, Goto *et al.* found that bacteria trigger epithelial fucosylation through an unexpectedly circuitous route involving innate lymphoid cells (ILCs), immune cells that circulate in the tissues beneath the intestinal epithelium.

Like all immune cells, ILCs combat infections (*7*). They share a common developmental origin with T cells but lack specific antigen receptors. Goto *et al.* noted that in mice genetically engineered to lack a particular ILC subset called "type 3" ILC (or ILC3), FUT2 expression decreased, accompanied by a striking reduction in epithelial fucosylation in the gut. Antibody-mediated depletion of ILC3 from wild-type mice produced a similar result, further indicating that ILC3 are essential for epithelial display of fucosylated carbohydrates.

How are bacterial signals transmitted from ILC3 to epithelial cells? A defining feature of ILC3 is secretion of the cytokine interleukin-22 (IL-22), which binds to IL-22 receptors on epithelial cells (*8*). Although intestinal bacteria did not alter ILC3 numbers, they stimulated IL-22 production by ILC3. Mice engineered to lack IL-22 furthermore had reduced FUT2 expression and lowered epithelial fucosylation, suggesting that it is the IL-22 produced by ILC3 that stimulates the fucosylation pathway. Indeed, when IL-22 production was restored in ILC3-deficient mice, epithelial fucosylation returned to normal. Thus, bacteria stimulate an ILC3–IL-22–epithelial cell relay circuit that induces fucosylated carbohydrate synthesis in epithelial cells (see the figure).

To complicate matters, lymphotoxin, another cytokine produced by ILC3, was also required for epithelial fucosylation. But unlike IL-22, lymphotoxin did not require bacteria for its expression. It is not clear why both cytokines are required for epithelial fucosylation, but Goto *et al.* speculate that lymphotoxin may make epithelial cells competent to receive IL-22 signals, perhaps by inducing expression of the IL-22 receptor.



Gut ecosystem management. Upon sensing bacteria through an unclear mechanism, type 3 innate lymphoid cells secrete IL-22, which stimulates a receptor (IL-22R) on intestinal epithelial cells to increase expression of the fucosyltransferase FUT2. This results in global remodeling of epithelial carbohydrate structures such that they terminate with fucose. The terminal fucose moieties can be cleaved by secreted bacterial fucosidases and used by the bacteria as a source of carbon and energy. The ability to forage host-derived carbohydrates may confer a survival advantage to bacteria in the competitive environment of the intestine.

Another well-studied strategy for managing the gut ecosystem involves the production of bactericidal proteins by the intestinal epithelium. Interestingly, ILC3 also direct epithelial expression of regenerating islet-derived protein 3 γ (RegIIIγ) (*9*), a bactericidal protein that restricts bacterial access to the intestinal epithelial surface and thus limits invasion of host tissue (*10*). This suggests that ILC3 control both a "carrot" (fucosylated carbohydrates) and a "stick" (RegIIIγ), which work in concert to influence the composition and location of intestinal bacteria. This could enable preferential recruitment of mutualistic bacteria to niches close—but not too close—to the gut surface, perhaps restricting them to the mucus matrix that overlies the intestinal epithelium. In addition to providing the host with metabolic benefits, this could ensure benevolent occupation of niches that would otherwise be accessible to pathogens. Indeed, Goto *et al.* show that the gut pathogen *Salmonella typhimurium* more readily invades the tissues of FUT2-deficient mice than of wild-type mice, suggesting that epithelial fucosylation restricts pathogen infiltration.

The Goto *et al.* study provides a deeper understanding of the biological functions of ILC by showing that these cells are essential for intestinal microbiota management. A broader question is whether ILCs promote mutualistic bacteria-host relationships in humans. If so, the findings of Goto *et al.* could lead to new insights into human disorders, such as inflammatory bowel disease, where there is a breakdown in these relationships. ■

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10.1126/science.1259808

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DISASTER MANAGEMENT

A global strategy for protecting vulnerable coastal populations

Short-term emergency response and long-run investments are needed

By **Edward B. Barbier**

The 2014 Working Group II report of the Intergovernmental Panel on Climate Change (IPCC) warns that low-lying coastal areas are increasingly exposed to risks from sea-level rise, flooding, and extreme storm events (*1*). Low-lying coasts of developing countries in particular face two types of vulnerability: (i) a lack of capacity to respond quickly and effectively to natural disasters and (ii) declining protection for people and property as coastal habitats disappear. A science-based global strategy for protecting coastal populations should address both sources of vulnerability, through investments in short-run emergency response and long-term coastal adaptation.

POLICY

Recent events in the Philippines are a reminder of this vulnerability. Typhoon Haiyan, which struck the Philippines in November 2013, led to widespread destruction, economic damages, population displacement and casualties (see the photo). Such extreme events are sudden shocks to communities and economies, which often take years to recover (*2*). The remoteness of the affected region in the Philippines, the scale of devastation, and the limited capacity for emergency relief have further hindered recovery. When Typhoon Rammasun landed in July 2014, it brought additional death and devastation to areas still recovering from Haiyan.

Risks are growing in many coastal regions, as population expansion, economic development, and urbanization degrade coastal ecosystems that protect against storm surge, saline intrusion, and erosion (*1*, *3*–*5*). Improving the global emergency response is necessary to help affected areas recover more rapidly from extreme storm events. Reducing long-run vulnerability requires managing, protecting, and restoring protective coastal systems, including marsh, mangroves, seagrass beds, and barrier islands (*1*).

DISASTER TASK FORCE. In the aftermath of a coastal disaster, saving lives and providing immediate assistance to survivors are paramount. The effectiveness and speed of response depend on telecommunications, transport, and relief coordination. An affected developing country, such as the Philippines after Typhoon Haiyan, is often too incapacitated to provide vital logistical support to international relief efforts.

There is a need for a global emergency task force that is well-equipped and capable of restoring telecommunications and transport and coordinating support. Such a task force must be the "commandos" of any international relief operation. It should be the first to arrive in a disaster zone, with the priority of repairing and establishing basic telecommunications. The task force should also set up the transport facilities necessary for relief operations. Finally, the task force should identify; coordinate; and where possible, facilitate the delivery of relief supplies needed in the disaster area. Scientific research could enhance the effectiveness of such a task force by identifying vulnerable coastal populations and regions, forecasting the scope and scale of future threats, and improving early-warning systems for imminent coastal hazards.

Because of the technical and engineering capacity required of the task force, it could require around 600 personnel and $1.3 billion in equipment and other inventory (*6*, *7*). With the additional rapid response function capability, the task force may need $2 billion in initial funding and $400 million annual operating costs (*8*).

A global task force could operate under the UN Office for the Coordination of Human Affairs (OCHA) (see the table), through the Central Emergency Response Fund (CERF). Already, OCHA oversees three funds for disaster assistance: the CERF, the Common Humanitarian Funds (CHFs), and Emergency Response Funds (ERFs). In 2012, these funds allocated $920 million for emergency disaster relief, ~7.2% of global humanitarian funding (*8*). The CERF received $426 million in 2012, which would have to double to finance the new task force with $400 million annually or just over 3% of current global humanitarian funding (see the table). UN member countries and the private sector currently funding the CERF should expand their financing for this purpose.

Alternatively, developing countries could organize regional task forces, e.g., for Latin America and the Caribbean, Asia, and Africa. Funding could be arranged via regional banks, such as the African Development Bank, the Asian Development Bank, and the Inter-American Development Bank.

LONG-TERM ADAPTATION. Long-term adaptation is also required to address the increasing vulnerability of low-lying coasts to natural disasters. Coastal population densities are nearly three times that of inland

Typhoon Haiyan—the Philippines deadliest recorded natural disaster. A house damaged by Typhoon Haiyan on the eastern island of Leyte.

PHOTO: PHILIPPE LOPEZ/AFP/GETTY IMAGES

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areas, and they are increasing exponentially (*1*). About 40 million urban residents globally are exposed to a 1-in-100-year extreme coastal flooding event, and by 2070, it will be 150 million people (*9*). The most vulnerable populations are in the poorest countries and are least able to afford disaster risks and damages. In developing countries, about 14% of the population and 21% of the urban dwellers live in vulnerable low-elevation coastal zones (*10*).

A long-term strategy for coastal adaptation should have two objectives: (i) protecting coastlines and populations from risks posed by damaging storms and (ii) restoring valuable coastal systems—such as salt marshes, oyster and coral reefs, mangroves, seagrass beds, and beaches—and other landforms that act as natural barriers (*1*, *3*–*5*). A study for the United States has shown that, if these habitats are left intact, the exposure of vulnerable populations and residential property to coastal hazards can be reduced by half (*3*). Comprehensive plans to reduce coastal vulnerability should also include key infrastructure investments—such as seawalls, dikes, barrages, and diversions—and improved institutional and coastal community response capability.

In Samoa, long-term coastal adaptation included developing district infrastructure management plans supported by a national strategy, participatory consultation with local villages, and improved training and capacity-building for plan implementation (*11*). After repeated experience with devastating cyclones, Bangladesh developed an integrated coastal management response that includes the construction of multistoried cyclone shelters, improvement of forecasting and warning capacity, establishing a coastal volunteer network, and mangrove reforestation (*1*).

Another example of long-term adaptation is Louisiana's 2012 Coastal Master Plan (*12*). The plan will build between 1500 and 2070 km^2 of new land, much of it restored marsh, over the next 50 years to provide storm protection and other ecosystem benefits. In 2010, dedicated dredging on the Barataria Basin Landbridge in Louisiana created 490 ha of intertidal marsh and nourished an additional 640 ha, at a total cost of $36.3 million. In addition to expanding these restoration efforts, the plan will construct levees and other protective structures to reduce substantially the risk from a 100-year storm. Overall, the plan lowers the risk of flood damage to Louisiana's coast by $5.3 billion to $18 billion annually (*12*).

Such long-term adaptation strategies and investments are needed globally. Although developed countries do not need international assistance for a strategy, developing economies—especially in tropical Latin America, Asia, and Africa—may lack the capacity and funds to develop and implement a long-term plan. Assistance could be provided to local and national authorities through a collaborative and coordinated effort instigated by the World Bank, the UN Development Programme (UNDP), and the UN Environment Programme (UNEP). These multilateral institutions have the necessary expertise to instigate and to assist with such long-term planning efforts, including targeting investments to vulnerable populations in coastal zones.

To support these efforts, there needs to be a greater commitment to adaptation science targeted to coastal areas (*13*, *14*). This research should focus on further understanding the role of ecological engineering, restoration, and protecting coastal habitats in shielding coastal populations and assets from storm events (*3*–*5*); the development of more resilient socio-ecological systems (*2*); informing the extensive preparations and coastal planning needed to manage climate risks, avoid damages, and improve emergency preparedness; and assessing the costs and benefits of coastal adaptation (*13*). In addition, scientific research needs to be proactive in mitigating future risks through guiding coastal management decisions, identifying vulnerabilities, improving foresight, and developing options (*14*).

Such long-term planning efforts and research in developing countries should be supported through international climate change adaptation funds. This financing could be done on a cost-sharing basis with relevant governments and local jurisdictions in coastal zones. Currently, around $2.9 billion in climate change adaptation funds have been pledged (*15*). If one-fifth (>$575 million) went toward a program of support of long-term global adaptation in vulnerable coastal zones, then this could be the basis for supporting strategies like the Louisiana Coastal Master Plan in multiple regions of the developing world.

The IPCC report (*1*) equates vulnerability to climate change risks with lack of preparedness. The two-tier global strategy for reducing the vulnerability of low-lying coasts would go a long way to reducing the vulnerability of coastal populations, given the increased risks from sea-level rise, flooding, and extreme storm events. But such a strategy also needs to be supported by scientific research that improves the effectiveness of a global emergency task force, identifies vulnerable coastal zones, and develops potential mitigation options to reduce the risks posed by future coastal hazards. ■

Short-term emergency response

- **Emergency Task Force for Coastal Disasters**
  Restore quickly telecommunications and coordinate support in any coastal disaster zone worldwide
- **Governance**
  Operate under the UN Office for the Coordination of Human Affairs (OCHA) or as regional task forces (e.g., Africa, Asia, and Latin America)
- **Financing**
  Require $2 billlion initial funding and $400 million annual operation costs, or an additional 3.1% of global humanitarian aid (*7*)

Long-term coastal adaptation

- **Coastal Zone Investment Plans**
  Develop and implement comprehensive coastal protection and restoration strategy over several decades
- **Governance**
  Operate under national and local jurisdiction with assistance from the World Bank, UNDP, and UNEP
- **Financing**
  Require cost-sharing and $575 million assistance, or 20% of global climate change adaptation funds (*15*)

A global strategy for protecting vulnerable coastal populations

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10.1126/science.1254629

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BOOKS *et al.*

COGNITIVE SYSTEMS

What is in a word

By **Laurie E. Cutting**

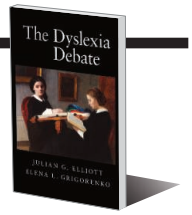
While many of us take for granted our ability to read, this is not the case for many individuals in society, with approximately one-quarter or more of U.S. students typically performing at Below Basic levels on the National Assessment of Educational Progress (2013). The causes for such poor performance are likely multifaceted, including downstream effects from environmental influences, such as lack of appropriate schooling, as well as biological difficulty in learning how to read. While *The Dyslexia Debate* would appear on the surface to cover only a segment of individuals with reading difficulty (dyslexia), the book really tackles broader issues of those who struggle to read, regardless of origin or how we actually label it.

The Dyslexia Debate provides an up-to-date and comprehensive synthesis of what is known about the cognitive and neurobiological origins of reading difficulty. The book also highlights that, despite considerable scientific progress in this area, the field is still in its infancy in terms of understanding why so many individuals struggle to learn to read, what should be done to help, and how to best operationalize reading difficulty in terms of definition and label(s).

At the outset, Julian Elliott (at the School of Education, Durham University) and Elena Grigorenko (at the School of Public Health, Yale University) review all of the different definitions of dyslexia, from its earliest conceptualization to current definitions across various organizations and countries. Although this section is perhaps somewhat lengthier than necessary, it is extremely valuable in revealing why there is so much debate about the exact definition of dyslexia and thus, in turn, why there is debate in how to label those who struggle with reading. Some use the terms "dyslexia" and "reading difficulty" synonymously; others consider difficulty in sounding out words and dyslexia as synonymous; and yet others use dyslexia entirely differently, as a distinct disorder from other reading difficulties. The review also provides an important context, as there is one fundamental constant across all definitions: substantial struggle with reading has a biological basis and origin. While environment can modify this biological predisposition, in what manner, under what conditions, and how biological and environmental variability and their interactions result in different reading outcomes are largely unknown.

Herein lies one of the central themes throughout the book: the tools that we currently have to dissect out biological and environmental contributions or to disentangle the specifics of reading difficulty, and even more so the specific case of dyslexia, are not sufficient. While Elliott and Grigorenko are able to point to some clear generalities in terms of cognitive characteristics (phonological processing weaknesses) and neurobiological signatures (left hemisphere anomalies), the picture becomes much more complex when examined further. Processes may exist either in addition to or as a distinct subtype of reading difficulty, such as visual attention and lower-level deficits contributing to phonological processing weaknesses. Additionally, while historically it has been suggested that reading difficulty is linked to corresponding strengths, fully unpacking this information is needed.

The debate over how we should label reading difficulties, and whether dyslexia is a term that should remain in use, is addressed head on toward the end of the book. There, Elliott and Grigorenko challenge us to think about what we can say and do using the current state of the science and raise the thought-provoking idea that dyslexia is not in and of itself a helpful term. Their opinions are largely centered on the fact that no specific cut points exist and therefore definitions are variable; they suggest that we should thus retire the term "dyslexia" and use "reading disability" to capture anyone who struggles with reading at the word level until a later time when we can understand better the relevant distinctions.



The Dyslexia Debate
Julian G. Elliott and Elena L. Grigorenko
Cambridge University Press, 2014. 290 pp.

Should we subsume all reading difficulty under one label because we currently can only detect reading on a continuum and can't disentangle environment and biology? Although this is enticing in its simplicity, I respectfully disagree: Science needs to evolve so that we can make distinctions between various types of reading difficulties, and the dyslexia label, along with the broader reading disability term, pushes us to keep propelling science forward. This issue of fuzzy boundaries occurs in multiple developmental disorders—and many other medical disorders for that matter [some argue that most medical disorders are not discrete; for an example, see (*1*)].

Thus, Elliott and Grigorenko, perhaps unknowingly, provoke us to think not only about dyslexia per se but about the struggle across multiple developmental disorders that present on a continuum. Throughout medical history, there are instances in which disorders have been defined in one manner, but then the definitions are refined and more-precise boundaries are set as the science evolves.

One then circles back to what Elliott and Grigorenko close with: that the issue is not so much in using the label of dyslexia or not but in understanding how to best define the boundaries and cut points. Right now we don't have the tools to make these very clear demarcations. But, as they state, just because we don't yet have the tools to unravel the complex interactions between genes, brain, and environment, it does not mean that the problem doesn't exist. This, ultimately, is the heart of the issue for defining, diagnosing, and most importantly helping those overcome reading difficulty. *The Dyslexia Debate* provides us with a thought-provoking way to consider these issues and challenges us to figure out the best ways to tackle them while we wait for the evolution of science to provide the greatest clarity.

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10.1126/science.1258446

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PROBABILITY

What are the chances?

By **Eve Limbrick-Oldfield**

We have all experienced coincidences or rare events that we find difficult to believe. Throughout history, people have tried to find an explanation for the occurrence of such events, and these have often taken a supernatural form. For example, people have provided explanations that rely on superstitious beliefs, the intervention of a higher power (such as a god), or an unknown force such as extrasensory perception. It may be a part of human nature to explain rare events in such a way. However, David Hand (at Imperial College London), in *The Improbability Principle*, presents a rational set of rules that, once understood, can explain why we all experience such events. Instead of relying on supernatural explanations, he argues that the laws of nature suggest that we should not be surprised by rare events. In fact, he tells us, even unlikely events happen all the time.

The majority of the book is concerned with describing a set of five rules that together form the Improbability Principle. Each rule is explained using a multitude of real-world examples. The author draws from diverse topics, including parapsychology (the scientific study of paranormal phenomena), financial markets, and gambling. Such examples are embedded within accessible lessons on the rules of probability.

The law of inevitability describes the simple fact that something must happen. If you hit a golf ball, it must land somewhere. The possible landing locations consist of a huge list of improbable events (at least before you hit the ball), but one of these has to happen.

In the 1990s, a group of people took advantage of this law to try to win the Virginia State Lottery. The jackpot was abnormally large at $27 million, and the consortium calculated that there were just over 7 million possible sequences of numbers that could win. Therefore, if they could buy 7 million tickets, all with unique sequences, they could make a profit of $20 million. The probability of a particular ticket winning was still low, but one of the tickets had to win.

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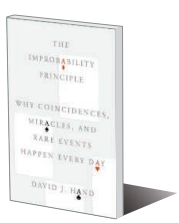
The law of truly large numbers describes the fact that there are often an extraordinarily large number of opportunities for rare events to happen. The probability of one person being struck by lightning within a year is 1 in 300,000. But with Earth's population reaching 7 billion, lightning strikes don't seem so surprising.

The law of the probability lever says that a small change in circumstances can have a huge impact on the expected probabilities of events. A tragic example of this law contributed to the wrongful imprisonment of a woman who had lost two children to sudden infant death syndrome (SIDS). The juries were told that the probability of losing two children to SIDS was 1 in 73 million and that a double homicide was much more probable. However, the probability of losing a second child to SIDS increases if you have already lost one child, and this was not factored into the original probability calculation. The conviction was overturned because of medical evidence, but the judges also criticized the statistical evidence as "grossly misleading" (http://innocent.org.uk/cases/sallyclark/#incompetence).

The Improbability Principle
Why Coincidences, Miracles, and Rare Events Happen Every Day
David J. Hand
Scientific American/Farrar, Straus and Giroux, 2014.
283 pp.



The law of selection manifests itself in a variety of ways, all centering on how probabilities can be modified by the manner in which you select the events. For example, publication bias can lead to an overrepresentation of positive results from clinical trials. Another example of this law, hindsight bias, can suggest that we should have been able to predict an event based on evidence presented after the fact.

Speed cameras provided Hand with an excellent example of the law of selection in action. These are placed to prevent future accidents. Locations are selected based on the number of road deaths that have occurred in the preceding year, and then the number of deaths over the next year are measured. At this point, it would be tempting to attribute any reduction in deaths solely to the introduction of speed cameras. But we can't do this, due to the manner in which we selected the locations—only including those with very high death rates. Some of the original deaths were chance events, unrelated to any potential local hazards. There will be a reduction in the following year independent of cameras or any other intervention. It is equivalent to rolling 100 die, then selecting only those that landed on a six to roll again. You would expect only some of these die to land on a six again. A proper experiment would have to randomly select the speed camera locations so that these random fluctuations can be separated from the effect attributable to speed cameras.

The final rule, the law of near enough, describes how our assessment of coincidences can actually increase the perceived likelihood of their occurrence. Things don't have to be an exact match for us to regard them as identical, and thus we expand the number of potential matches that we interpret as rare events.

Throughout the book are many examples of the consequences of being unaware of aspects of the Improbability Principle, and so the final chapter is concerned with how we might use these laws to inform our lives. There are certainly principles that I will take away and remember, but I can't help but believe that I will still be amazed and think about fate the next time I am presented with a "coincidence" or rare event.

10.1126/science.1256007

PHOTO: STEVE MASON/THINKSTOCK

Downloaded from www.sciencemag.org on September 12, 2014

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LETTERS

Edited by **Jennifer Sills**

Retraction

IN OUR REPORT "X-ray fluorescence spectrometry of asteroid Itokawa by Hayabusa" (*1*), we analyzed the major elemental ratios of asteroid 25143 Itokawa with the x-ray spectrometer (XRS) onboard the Hayabusa spacecraft. We used an improper analytical procedure, which resulted in erroneous identification of x-ray fluorescence peaks and statistically insignificant conclusions. Therefore, we retract the paper.

Our XRS team confirmed that our original analysis was inaccurate in parallel with both the steering committee of the Institute of Space and Astronautical Science, Japan Aerospace Exploration Agency, and an investigation team appointed by the institute.

Figure 1 of our Report shows the x-ray spectrum, which is fitted with some x-ray fluorescence peaks and continuum components. The figure shows the smoothed data. The smoother x-ray spectrum was used in the model fitting (statistical analysis). We should have used the raw data and examined it in detail before proceeding to the modeling.

In our data analysis, x-ray photon energy calibration (relation of detector channel number to x-ray photon energy) was conducted using the observed data, because the instrument had no onboard calibration source, such as ^{55}Fe radioactive nuclide. The observed raw data was smoothed to identify the expected x-ray fluorescence peaks effectively. In the smoothed profile, we detected two apparent intense peaks. We erroneously identified the peaks as the anticipated x-ray fluorescence of Mg and Si.

The energy calibration formula we obtained differed from the formula we had obtained in our pre-flight ground testing; we attributed the difference to a drift of the XRS instrument parameters due to temperature variation of the instrument. However, recent detailed investigation of the past data shows that it is more appropriate to adopt a different formula for the energy calibration. Furthermore, the peak we identified as the x-ray fluorescence of Mg is likely to be an artificial peak from the instrument. Thus, the x-ray photon energy axis of Figure 1 was incorrect.

Our reinvestigation revealed another error. In the model fitting to obtain the major elemental abundance ratio of the target asteroid Itokawa, we analyzed the smoothed data, not the raw data, which resulted in inflated statistical significance. In a properly done elemental analysis, the signal-noise ratio is too low to support our conclusion of chondritic elemental abundance.

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A woman tends to her chickens in Uganda.

PHOTO: ANDREW AITCHISON/ALAMY

Livestock crucial in hunger equation

THE EDITORIAL "Zero hunger" by M. S. Swaminathan (1 August, p. 491) rightfully points out the importance of family farms and the need to shift the focus of tackling global hunger from food security to nutrition security. However, his description of a family farm limited to crops to support nutritional security is incomplete.

Family farms or farms operated by small holders represent most of the agriculture in developing countries. In addition to crops, livestock play a substantial role in improved nutrition, food, and economic security and resilience on small farms (*1*). Globally, meat, milk, and eggs currently provide nearly 13% of the energy and 28% of the protein consumed (*2*). Furthermore, the "livestock revolution"—the massive recent increase in meat consumption in developing countries (*3*)—is being driven by higher demand, which is expected to more than double in developing countries by 2050 and to expand almost fourfold in sub-Saharan Africa (*4*). In addition to high levels of energy and protein, animal source foods provide essential amino acids and many micronutrients and vitamins, all of which contribute to the high-quality diet that is critical for maternal health and children's growth and learning (*5, 6*).

Most food-insecure people live in South Asia and Africa, where livestock make huge net contributions to human nutrition by converting nonedible feed such as grass, crop residues, and waste products into valuable animal protein. Livestock's contribution goes beyond food; animals provide draft power for cropping, which helps farmers increase the amount of land under cultivation, and manure to restore soil fertility (*7*). Moreover, for most of the rural poor, livestock represent resilience and are often the only form of currency available to trade for needed food, school fees, and medical costs.

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Ancient trade between India and Indonesia

IN HIS NEWS FEATURE "Sailing Sinbad's seas" (27 June, p. 1440), A. Lawler describes the development of the Indian Ocean trade from the 5th century B.C.E. to the 8th century C.E. The map (p. 1441) indicates that Indonesia and Island Southeast Asia joined these networks in the 8th century C.E. Archaeological data from the islands of Bali, Java, and Sumatra show that sea trade in this region began 900 years earlier.

Excavations at the late prehistoric sites of Sembiran and Pacung on the northern coast of Bali (*1, 2*) have produced high counts of fine Indian pottery with specific parallels from the 2nd and 1st centuries B.C.E. in India, Sri Lanka, as far west as the Red Sea in Egypt (*3, 4*), and in Southeast Asia, including peninsular Thailand (*5*), the Indonesian island of Java (*6*), and Vietnam (*7*).

Simultaneous contacts with India and mainland Southeast Asia some 2000 years ago have been documented since the first excavations at Sembiran and Pacung, and 2nd century B.C.E. to 1st century C.E. dates were later obtained from Pacung burials (*8, 9*). (My team's recent excavations seem consistent with these results.) These dates match the 2nd century B.C.E. to 3rd century C.E. dating of burial layers containing fine Indian pottery at the site of Batujaya in northwestern Java (*6*).

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ERRATA

Erratum for the Report: "Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome" by B. Chalhoub *et al.*, *Science* **345**, 1260782 (2014). Published online 12 September 2014; 10.1126/science. 1260782

Erratum for the Report: "Engineering Modified Bt Toxins to Counter Insect Resistance" by M. Soberón *et al.*, *Science* **345**, 1256132 (2014). Published online 15 August 2014: 10.1126/science.1256132

Global health

What works

Existing tools can deliver big payoffs

By **Barbara Jasny, Leslie Roberts, Martin Enserink,** *and* **Orla Smith**

Science holds the promise of revolutionary new ways to fight the diseases of the world's poor. But breakthroughs in the lab aren't always necessary. The past 15 years, when aid for global health tripled to more than $31 billon, have shown that tools in hand today can help achieve dramatic improvements, but only if they are used effectively. Bednets can't prevent malaria if people don't use them. Cholera epidemics are relatively simple to stop, but people need access to clean water and sanitation. Vaccines could eradicate measles, but there are still children who don't get them. Ebola, too, can be contained, if patients are isolated soon enough, their contacts monitored, and the dead buried safely and quickly.

Now that the surge in funding has slowed and the United Nations is preparing new objectives to succeed its Millennium Development Goals, it is critical to target spending where it can be most effective. The most powerful tools address not just individual health but whole communities. This special issue of *Science* focuses on diverse areas where public health can be improved by making better use of the resources we have in our grasp, ranging from tailoring engineering projects to meet the needs of material- and infrastructure-limited regions, to building a surveillance network for the detection of drug resistance, to empowering women. Education is key for the general public, for decision-makers, and for the next generation of researchers, medical students, and public health professionals.

For this survey of global health, *Science* has joined forces with *Science Translational Medicine*, which examines vaccine development, strategies against emerging infections, progress in point-of-care diagnostics, and ways to promote mental health and neonatal health. We look at what works, what doesn't, and what's needed to move forward.

This special issue was also edited by Caroline Ash, Brad Wible, Gilbert Chin, and John Travis.

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Somali mothers wait to have their babies examined before receiving a five-in-one vaccine against several potentially fatal diseases.

PHOTO: © BEN CURTIS/AP

After the windfall

Plateauing budgets for global health sharpen the focus on what really works

By **Martin Enserink**

The good times are over in global health funding. After a decadelong surge, spending has plateaued—and the effects are being felt around the world.

Investments in health for poor and middle-income countries began growing in the 1990s, then exploded after the turn of the century, as the graph below shows. Rich countries stepped up their donations, with much of the funding channeled through new public-private partnerships like the Global Fund to Fight AIDS, Tuberculosis and Malaria and GAVI, the Vaccine Alliance. Billions earned in the software industry were transferred to developing nations after Bill and Melinda Gates started their charitable foundation in 2000. The bounty was spent buying and distributing drugs, vaccines, and bed nets; fighting malnutrition; and shoring up flagging health systems. Roughly one-tenth went to research and development, a bonanza for scientists.

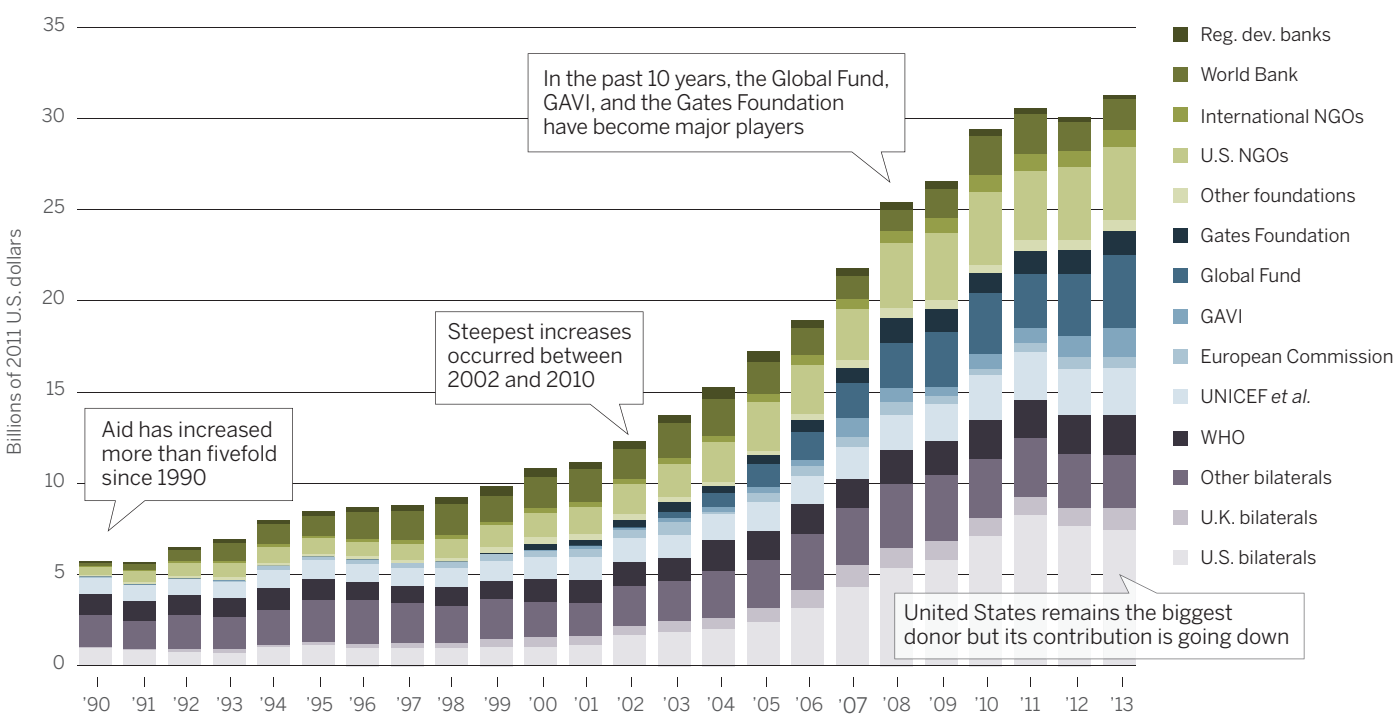
But since 2008, the global financial crisis has led rich countries to tighten their belts. The global partnerships now have trouble raising money, and developing countries—some of which enjoy robust economic growth—are expected to shoulder more of the cost. And, increasingly, people are asking for hard data about the return on investment. Just showing that pills or bed nets were delivered is no longer enough: Donors want evidence of their impact.

What are the results of the surge of the 2000s? Can we scientifically measure the impact of the billions invested? And now that the explosion has ended, what happens to poor countries that still have massive burdens of disease as well as rapidly growing populations? In this special news section, *Science* tracks the impact of the funding explosion in global health—and what happens now that it's over.

Meanwhile, here's another figure to keep things in perspective: The $31.3 billion spent on global health in 2013 is less than 1% of what rich countries spent on their own health last year. By any measure, the surge has ended too soon. ■

The end of the surge

Aid for global health, by channel



Source: Institute for Health Metrics and Evaluation

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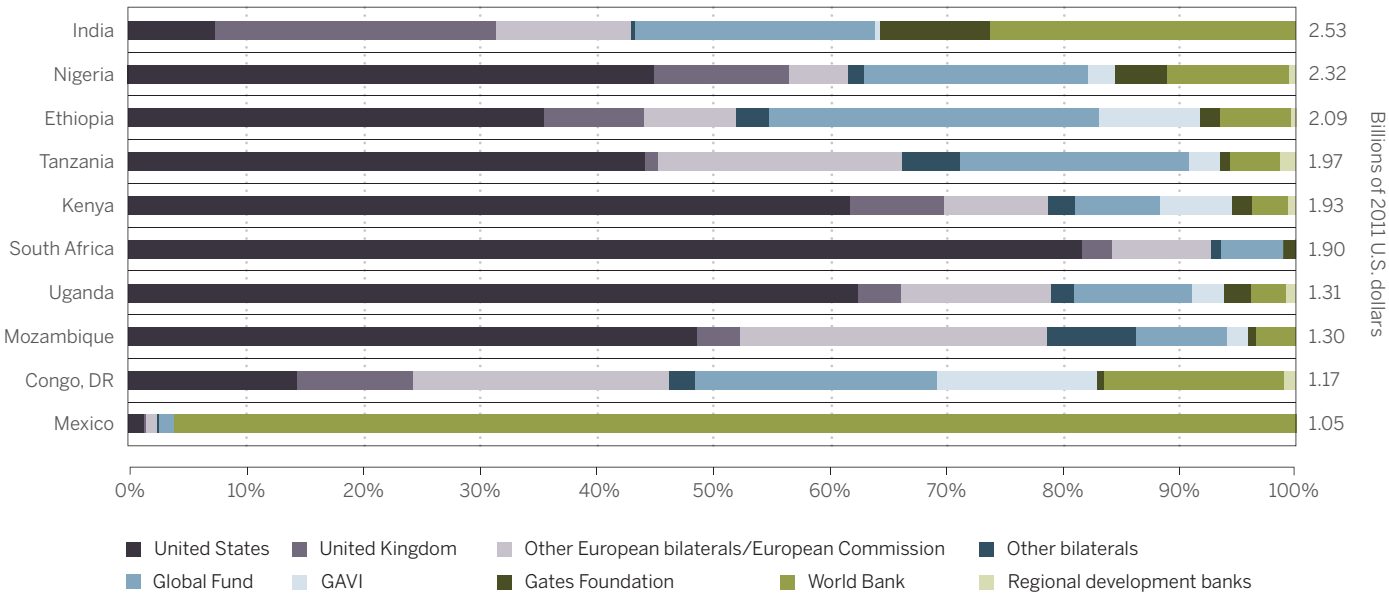
The top 10 beneficiaries

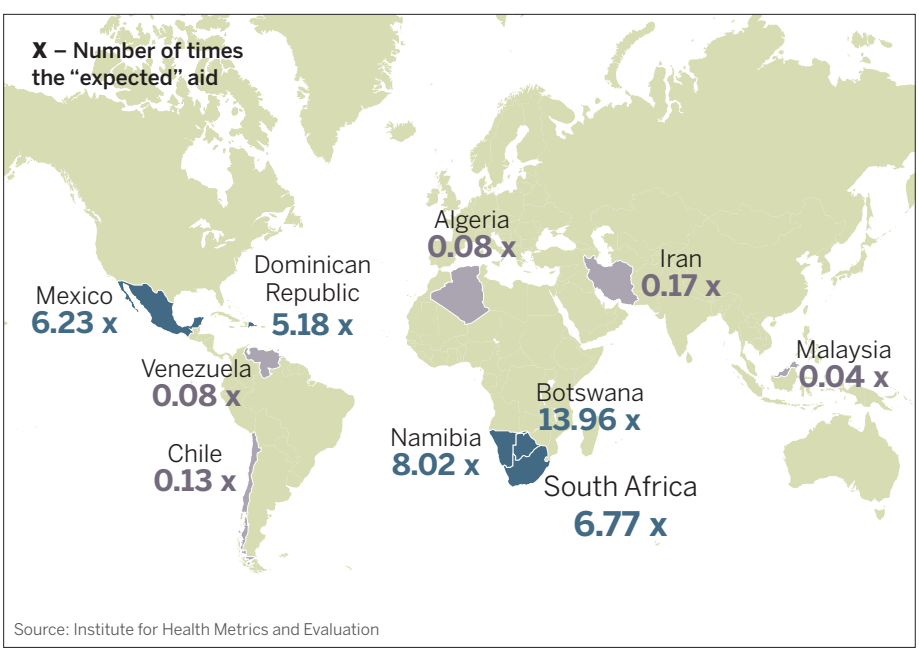
Of 140 low- and middle-income countries receiving public health aid in the years 2009 to 2011, populous India received the most: more than $2.5 billion. Of the remaining top 10 countries, eight are in Africa, and for all but one, the United States was the single biggest donor. Mexico received roughly $1 billion in assistance from the World Bank, thanks to a special project to strengthen the country's health and health insurance systems.



Source: Institute for Health Metrics and Evaluation

Winners and losers

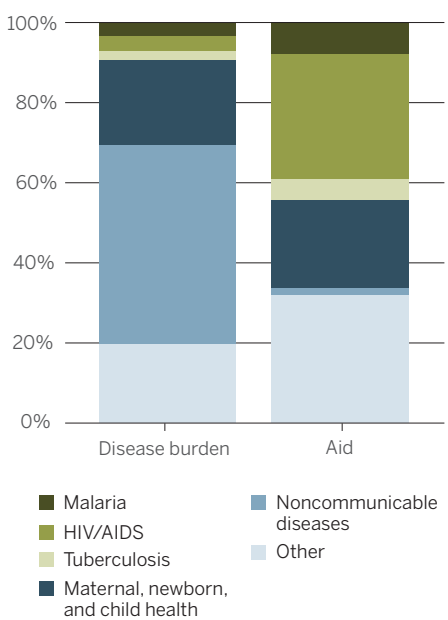
This map shows the biggest winners and losers in the global health bonanza, based on how many times the "expected" aid—based on disease burden and gross domestic product—they received in 2010. Botswana, Namibia, and South Africa, which all received major funding for HIV/AIDS, led the pack. Five countries—including Iran and Venezuela, which have strained relations with the United States—received less than one-fifth of the aid one would expect them to get. Many political, historical, and economic factors influence how much aid countries receive, says IHME researcher Joseph Dieleman; year-to-year variation also plays a role.



Source: Institute for Health Metrics and Evaluation

Skewed funding

The diseases that cause the highest burden—expressed in disability-adjusted life years, or DALYs—don't get most of the international largesse. In 2010, HIV/AIDS received the biggest chunk; little aid went to noncommunicable diseases like diabetes, whose burden is large and growing.



Source: Institute for Health Metrics and Evaluation

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A hard look at global health measures

Researchers seek convincing evidence that large-scale projects save lives

By **Jon Cohen**

Since 2002, rich countries have poured more than $10 billion into malaria control. The money has helped pay for planeloads of bed nets treated with insecticides, hundreds of millions of doses of a powerful combination therapy, widespread indoor spraying of homes, and prophylactic treatment of pregnant women, an especially vulnerable group. The generous, large-scale programs have saved the lives of hundreds of thousands of people, most of them African children.

Or have they? It may sound strange, but some analysts say we don't really know. Yes, the World Health Organization estimates that between 2000 and 2012, malaria cases have dropped by 25% worldwide and deaths have been cut by 42%. But in April, researchers at the widely respected Center for Global Development (CGD) in Washington, D.C., triggered a fierce debate among malaria experts when they wrote in a blog post that they couldn't find a single study with convincing data that showed how a large-scale intervention directly led to lower numbers of cases or deaths. (CGD and the Disease Control Priorities Network wanted an example for the third edition of *Millions Saved*, a book that documents proven successes in global health.)

The CGD researchers don't doubt that malaria interventions can work. Controlled clinical trials among several thousands of people have shown with statistical

PHOTO: © KHAM/REUTERS/CORBIS

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Hats off to Vietnam's helmet law

By **Martin Enserink**

A decade ago, it wasn't unusual to see a Vietnamese family of five cheerfully braving the frenzied traffic of Hanoi on a single motorcycle—with nobody wearing a helmet. No longer. A stringent law passed in 2007 has made helmets compulsory—and has proven that such laws, which require political will more than money, can have a huge impact on public health. Research suggests that the law's passage saved more than 1500 lives the first year and reduced serious head injuries by almost 2500.

Traffic injuries aren't the first thing most people think about upon hearing "global health," but road accidents kill an estimated 1.24 million people worldwide annually, about the same number as tuberculosis and twice as many as malaria. That's why the World Health Organization (WHO) has made road safety a priority. In developing nations in particular, there are huge opportunities to drive down the death toll.

In Vietnam, the economic boom of the 1990s led tens of millions to trade in their bicycles for motorcycles, causing injuries to soar. (Cars are still relatively rare.) In 2010, Vietnam had 24.7 traffic deaths per 100,000 people, compared with 11.4 in the United States and 3.0 in Sweden. The country has had helmet laws on the books since 1995, but at first helmets were compulsory only on major roads, and enforcement was weak. Many Vietnamese resisted what they called rice cookers, saying they were uncomfortable in the tropical heat—or an assault on their hairdos.

In 2007, a new law required riders to wear helmets on all roads at all times. Fines were set at $6 to $12, a whopping 30% of the average monthly income. Foreign governments and nongovernmental organizations pitched in with money for education campaigns and free helmets. When the law took effect on 15 December 2007, helmet use jumped from less than 40% to almost 100% literally overnight.

It wasn't the end of all problems. Many riders initially didn't strap their helmets, which was then made an offense as well. Many of the helmets on the market are flimsy, and the government is still struggling to enforce quality standards. But a study published in 2010 by researchers at WHO's country office in Hanoi found 16% and 18% reductions in the risk of serious head injuries and deaths, respectively, in 2008.

Researchers at the Center for Global Development (CGD) plan to include the Vietnam law in the next edition of *Millions Saved*, a book on proven successes in global health—but strictly speaking, the evidence doesn't meet CGD's standards. The center prefers to see a randomized study or at least a "quasi-experimental" study design (see main story). That didn't happen in Vietnam. CGD decided to include the case anyway because it's difficult to see what else—besides the law—could have caused the drop. CGD's Miriam Temin hopes Vietnam's success will inspire other countries. ■

A "Helmets for Kids" ceremony, sponsored by Bloomberg Philanthropies, at a Hanoi school in 2012.

significance that each can reduce cases and deaths. But efficacy in a carefully managed, tightly monitored study does not equal effectiveness in the messiness of the real world. Confusing matters further still, weather patterns, an economic upswing, or improved housing can also have a big impact on disease.

The CGD researchers are part of a growing movement that seeks harder data about the number of lives actually saved by the billions poured into health in poor and middle-income countries. Such evidence is critical, proponents argue. After a decadelong explosion, funding for global health has leveled off (see p. 1258); governments and charities need to know the impact of their dollars to justify their investments and to change programs that don't work well enough or not at all.

The new field of what is called impact evaluation is rapidly gathering steam. Large global health donors and developing world governments have widely accepted that they need better evidence of what works, and several new institutes are devoted to gathering it. Publications that use impact evaluation methods have skyrocketed. It's becoming increasingly difficult for the development assistance world to take credit for changes that might have occurred without their interventions—and to ignore the possibility that the money might have spared more people from disease if spent elsewhere.

"Agencies have come to realize that impact evaluation is the only way you can meaningfully talk about results," says Howard White, who heads the International Initiative for Impact Evaluation (3ie), a nonprofit launched in 2008. "They want to be able to go back to their funders or boards and say, 'We've lifted 18 million out of poverty.' "

But debates are raging about what constitutes convincing evidence of effectiveness. Randomistas, as some derisively call them, will only seriously consider supersized versions of the randomized, controlled studies used to evaluate the efficacy of drugs and vaccines. Others, like the researchers at CGD who select case studies for *Millions Saved*, considered other evidence as well. (The sidebars about

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specific successes and disappointments in Vietnam, Zambia, South Africa, and Peru in this special news section are based on draft case studies in the upcoming book.)

A few prominent critics, meanwhile, say the new focus on evidence is going way too far. They worry that it diverts money and attention from the actual battle against disease. And rigorous attempts to measure impact can cause unease among major donors, the groups they fund to roll out programs, and disease advocates, says Ruth Levine, a development economist at the William and Flora Hewlett Foundation in Menlo Park, California, who edited the first edition of *Millions Saved*. "The support for global health rests on a collective hope that money is turning into lives saved, and anything that punctures that belief is really very threatening," Levine says.

UNTIL 2000, hardly any impact evaluations were done in global health, or for that matter in development aid in general. "If you asked anyone what their impact was—and I don't care whether it was diabetes, hypertension, HIV—the answer would have been, 'We're spending X amount of dollars,'" says Mark Dybul, who heads the Global Fund to Fight AIDS, Tuberculosis and Malaria, which was formed in 2002.

The Global Fund and the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), which started in 2003 and was later headed by Dybul, together have spent more than $60 billion on HIV/AIDS, and both have received flak for not taking a close enough look at their own impact. For instance, they have long used the number of patients given antiretroviral drugs as a major yardstick of success. But people don't always take their pills, or they may drop out of treatment. So the precise public health impact, as well as the cost-effectiveness of specific programs, remained unclear.

Even if public health does improve after the rollout of a program, there may be no causal relationship. What's needed for a thorough evaluation, says epidemiologist Nancy Padian, who has appointments at both the Berkeley and San Francisco campuses of the University of California (UC), is a way to assess what would have happened if the intervention had not occurred. This is known in the lingo of impact evaluations as a counterfactual, and it's akin to a placebo control in a drug trial. "It's all about having the most robust counterfactual you can have," says Padian, who was a lead scientific adviser for PEPFAR.

A landmark demonstration of the value of this approach involved a social welfare program launched in Mexico in 1997, called PROGRESA, in which families received cash

PHOTO: © MARIANA BAZO/WORLD BANK

It's a wash: Hands-on hygiene in Peru

By **Kai Kupferschmidt**

Few public health interventions promise an easier payoff than getting people to wash their hands with soap. It's easy, and studies suggest it could prevent up to two-fifths of all diarrheal disease and one-fourth of pneumonia, the two biggest childhood killers in poor countries, saving up to 1 million lives every year. With hand-washing, there's none of the controversy surrounding condoms and birth control. Yet despite decades of effort and millions of dollars, only between 3% and 34% of people in poor countries regularly wash their hands. Simple routines, it turns out, are not always easy to instill.

In 2008, the Peruvian government, with support from the World Bank and the Bill & Melinda Gates Foundation, launched a media campaign called "clean hands, healthy children" to improve hygiene in 40 randomly selected districts across Peru. Radio spots, brochures, and posters, targeted at millions of mothers and children, stressed the importance of washing hands after going to the toilet or changing a baby and before cooking and eating. In 44 more districts, the media campaign was launched together with further interventions: Primary schools added hand-washing lessons, and teachers and local leaders educated mothers. More than 80,000 simple hand-washing stations designed by the company Duraplast and fashioned out of old plastic bottles—one filled with water and one with soap—were distributed to households. The campaign even introduced a cartoon superhero, Super Jaboncin, who fights germs using water and soap.

But the results were far from superpowered. A 2012 evaluation found that the media campaign alone did not change behavior at all. In communities with the more intensive interventions, hand-washing before food preparation increased from 10% to 17%. But researchers did not find a decrease in either diarrhea or pneumonia. (A campaign in Vietnam had similar results.)

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Mass hand-washing campaigns in Peru did not cut disease as anticipated.

That is not surprising, says Valerie Curtis, who directs the Hygiene Centre at the London School of Hygiene & Tropical Medicine. "You have to get a fairly large behavior change to pick up a significant health effect," she says. And that is a lot harder than it sounds.

Partly, it's a question of timing, says Jacqueline Devine, who was involved in the project at the World Bank. People are more likely to adopt new habits during major life changes, so, for instance, targeting women expecting their first child might be more effective than trying to influence all mothers and children, she says.

The bigger problem is that long-term health considerations do not drive behavior, Curtis says. What does are things like love, fear, and wanting to be accepted and admired—to "look sexy," Curtis says. "Most of what we do is not a rational response to a perceived threat." Public health messages that engage disgust, like "soap it off—or eat it later," might have a better chance of sparking behavior change, she says. ■

for keeping their kids in school and using preventive health services. PROGRESA's main architect, Mexican Deputy Finance Minister Santiago Levy, was worried that the next government might shut the initiative down unless hard evidence showed that it worked to improve kids' health. Levy enlisted an evaluation team led by UC Berkeley health economist Paul Gertler.

Gertler proposed taking advantage of the fact that Mexico could not afford to roll out PROGRESA nationwide all at once. He suggested a lottery to determine which communities could participate in the program first. Other villages would start 2 years later; they became the counterfactual. The comparison showed that the cash transfer led to a significant drop in illness and hospital visits among children, and adults benefited, too. The study was "a phenomenal breakthrough," Levine says, and PROGRESA survived.

RANDOMIZED, CONTROLLED STUDIES create their own counterfactual by randomly assigning participants to intervention or control groups; a mainstay of clinical research, they have rapidly multiplied in global health (see graphic). One leader is the Abdul Latif Jameel Poverty Action Lab (J-PAL), founded in 2003 at the Massachusetts Institute of Technology to do such studies in health and other development projects. J-PAL has since done follow-up evaluations of PROGRESA (renamed Oportunidades) and studied the impacts of hand-washing promotion on diarrhea in Peru, double-fortified salt on anemia in India, and deworming on school attendance in Kenya. 3ie, founded 5 years later, was established to fund evaluations and serve as an online repository of high-quality studies.

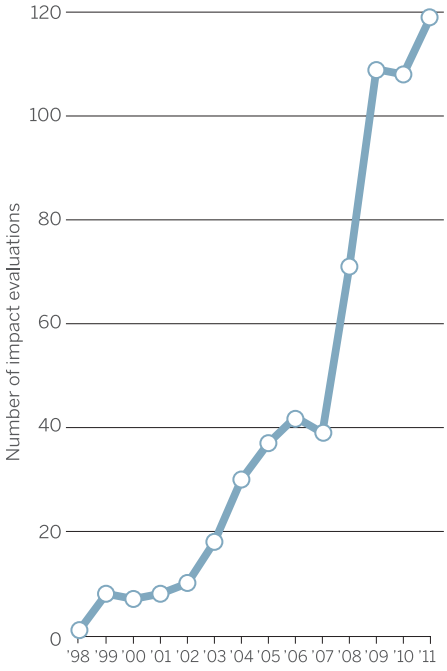
While randomized, controlled trials may be the ideal for measuring impact, they aren't always feasible. It's also widely considered unethical to withhold a proven intervention—some of them life-saving—from one group of people simply to test how well a large-scale rollout works. So scientists have developed several less rigorous methods. Eligibility criteria, for example, have a built-in counterfactual: If an intervention applies to people only under 14, kids who just turned 15 become good comparators. Sophisticated techniques can also "match" the group receiving an intervention to an artificial counterfactual created by statisticians. "There is a suite of methods," Padian says, some of which aren't used in standard drug or vaccine trials but are considered convincing to many, including the CGD editors of *Millions Saved*.

The Global Fund and PEPFAR have embraced the idea of more rigorously measuring impact. "It's becoming a top priority," says Deborah Birx, who heads PEPFAR. Today, both programs want to track how many people on HIV treatment have fully suppressed the virus for prolonged periods, which means they're actually healthier. To accomplish this, PEPFAR is distributing computer tablets to clinics and asking them to record—and report in real time—HIV levels in patients on treatment. Dybul says he's also "really adamant right now" about finding out how specific clinics are doing, instead of focusing on national data. That's a great step forward, says Stefano Bertozzi, dean of the School of Public Health at UC Berkeley. "If you know you have clinics in one country that go from 25% to 90% of patients being virally suppressed, as a manager, you have incredible information to know what's working and what isn't," Bertozzi says.

But the rising popularity of impact evaluations has triggered plenty of debates, which some have slagged as "wonk wars." A single impact study often doesn't mean much because the results may not apply elsewhere, says Harvard University economist Lant Pritchett, a prominent critic of impact evaluations and a nonresident fellow at CGD. The randomistas tend to overlook the "key failing" in developing countries, he says: Organizations don't

But does it work?

The rise of impact evaluations in global health and development



Sources: 3ie and William Savedoff

Hard cash boosts child health in South Africa

By **Jon Cohen**

Money may not buy love or happiness, but if you give a poor family a small sum each month for each of their children, it leads to measurable improvements in their health, education, and well-being. That's the lesson from an ambitious program in South Africa, the Child Support Grant (CSG), which directly reduces poverty with cash transfers.

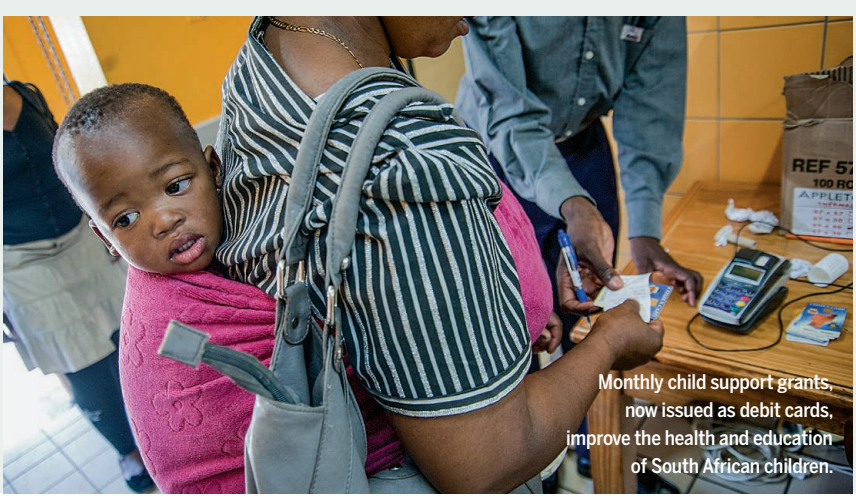
In South Africa, any family that can demonstrate financial need is entitled to a monthly stipend of about $30 per child, no strings attached. In 1998, when the CSG started, the cutoff was 6 years old; today it is 17, and the program reaches 11 million children. Families can apply to receive monthly payouts for each child, which costs the government more than $3 billion each year.

In 2008, the Cape Town–based Economic Policy Research Institute (EPRI) set out to see how well the program worked, surveying 2500 people in five provinces. Rather than simply comparing families that received help with those that did not, the study took advantage of CSG's rocky start. At first, many eligible families did not receive the CSG, as a documentary aired on South African TV in 2001 revealed. The exposé spotlighted widespread serious illness in poor, rural children whose caregivers, mainly grandmothers, had not registered for the CSG, often because registration sites were far from their homes or they found the required paperwork overwhelming. In response to the public outcry, the government dispatched vans to the area to sign up eligible families en masse. "That almost created a natural experiment so years later, we could compare the youth in towns where the mobile registration had stopped and signed up people to other youth who were passed over and weren't getting the grant," says economist Michael Samson, EPRI's director of research.

He and his colleagues considered the cash per child the "unit dose" and asked how children fared in households that had received different doses. In May 2012, the South African government and the United Nations Children's Fund, which funded the EPRI study, published a report detailing the results. Children in families that received higher doses had improved growth, decreases in illness, better grades and attendance at school, and were less likely to take risks with sex, drugs, and alcohol when they reached adolescence. The report asserts that the program is "one of the most comprehensive social protection systems in the developing world."

Samson says there are "massive scarcities of opportunities" for South Africa's poor. "A cash transfer effectively allows the household to invest in breaking intergenerational poverty," he says, noting that unconditional cash transfers have become popular throughout Africa. And in South Africa, he says, the CSG is the main source of income for more than 20% of households.

Samson says many families that are eligible for CSGs still are not receiving them, leading some to urge the government to drop the means test and provide cash transfers to every household with children. "It's the best way to eliminate the exclusion of the most marginalized," he says. He points out that two-thirds of South African families are eligible, and says for wealthier families this would be a welcome tax rebate. "Either way, you win." ■



Monthly child support grants, now issued as debit cards, improve the health and education of South African children.

PHOTO: © MADELENE CRONJÉ/MG

work. "The policemen don't police, the teachers don't teach, and the doctors don't doctor," he says. "We know for sure that varies orders of magnitude across countries of the world." How then can you assume that a successful intervention in South Africa will translate to Colombia?

The drive to use "easily measured indicators" to claim success and impress donors also worries Michel Kazatchkine, Dybul's predecessor at the Global Fund's helm. Kazatchkine would like to move beyond quantitative indicators to more qualitative ones, like changes in laws or social policies. "Numbers of lives saved is a very American concept," he says. "The European audience would wish to know about something a little more conceptual than just a number. Have we changed the system and addressed the roots and the causal determinants and insured that the people, in addition to having their lives saved, live a proper life?"

CGD'S EFFORT to weigh malaria interventions stirred new controversy. Malaria control didn't make it into the first two editions of *Millions Saved*, in 2004 and 2007, which documented triumphs from global ones like smallpox eradication to little-known efforts to combat diarrheal disease in Egypt or trachoma in Morocco. Although malaria has plummeted in many countries, the CGD researchers said none of the existing evaluations met their criteria: a study of a large-scale intervention of at least 2 years duration that demonstrated a clear, causal link to a drop in disease or death. They also wanted to see evidence that the intervention had an acceptable cost based on the number of cases averted or lives saved.

"We know from a bunch of small-scale studies that bed nets can protect you from mosquitoes biting you," says Amanda Glassman, who heads global health policy at CGD. "That's not what we're interested in evaluating." In the real world, nets aren't always used, for instance because they're uncomfortable on hot nights or people think there are few mosquitoes around.

The blog posting led to fierce rebuttals from both the U.S. President's Malaria Initiative (PMI) and the Roll Back Malaria Partnership. Erin Eckert, an epidemiologist at PMI, says impact evaluations are critical. But when it comes to the type of national level programs that CGD is evaluating, she says a "rigorous academic definition of impact evaluation is not always necessary or appropriate." As Eckert and a colleague wrote in a riposte to CGD's blog, "The malaria field is full of examples of solid evaluations of interventions and the impact of scaling up those interventions on malaria burden."

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Across Africa's meningitis belt, people have lined up for MenAfriVac.

PHOTO: © MONIQUE BERLIER/PATH

A new vaccine vanquishes meningitis A in Africa

By **Kai Kupferschmidt**

For more than a century, the *Neisseria meningitidis* bacterium has swept across large swaths of Africa every few years. Striking during the dry season, it causes meningitis that kills 5% to 10% of those infected and leaves many others deaf or disabled. Although effective—and expensive—vaccines exist against the meningitis strains that plague Europe and the United States, no good one was available to protect Africans against serotype A, the most common strain in Africa. That has changed—and the result, says Brian Greenwood, an epidemiologist at the London School of Hygiene & Tropical Medicine, is "probably the most dramatic success I have ever seen."

In 2000, the World Health Organization convened global health experts who came up with an idea: Make a safe and effective vaccine specifically for Africa for an African price. And make it fast. With $70 million from the Bill & Melinda Gates Foundation, the Meningitis Vaccine Project, a public-private partnership headed by infectious disease specialist Marc LaForce, got started. The basics were agreed upon quickly: To induce long-lasting immunity, the vaccine would have to be a conjugate, a meningococcus A polysaccharide joined to a tetanus protein to elicit a stronger immune response. And it would have to cost less than 50 cents a dose, a price the Serum Institute of India Ltd. agreed to deliver even before development began.

MenAfriVac is that vaccine. By 2009, trials in Senegal, Mali, and other countries had shown the vaccine to be safe and effective, and in December 2010, Burkina Faso, at the time the hardest hit country in Africa's meningitis belt, became the first to roll out the vaccine. Within just 10 days, about 70% of the target population—anyone between 1 and 29 years old—had received it. No cases of meningitis A were recorded the next year. In Chad, three regions introduced the vaccine in December 2011 in the midst of an epidemic; during the first half of the following year, those regions recorded just 57 cases of meningitis, none of them caused by *N. meningitidis* A, Greenwood and his colleagues reported in January in *The Lancet*. Across the rest of the country, the case number was 18 times higher, 44 per 100,000. "In every single country where the vaccine has been introduced, group A *Neisseria* meningitis disease has fallen to zero," LaForce says.

What made it such a success? For one, people in West Africa desperately want the vaccine—almost every family there knows the devastating impact of meningitis firsthand, says Seth Berkley, who heads GAVI, the Vaccine Alliance, which has budgeted $370 million to introduce the vaccine across the continent. Another reason: Unlike earlier vaccines, MenAfriVac also gets rid of the bacterium in asymptomatic people, further increasing herd immunity. By the end of this year, Berkley estimates 200 million people will be vaccinated, with the remaining 100 million slated for 2015.

How long the vaccine's protection will last is still unclear, however, and scientists worry that other strains of the bacterium could take over the continent. Meanwhile, the Serum Institute of India has already set its sights on the next goal: an affordable vaccine that will protect against meningitis strains C, Y, W, and X as well. LaForce says that vaccine will enter field trials in 2015. ■

The CGD researchers eventually met with their critics, including Eckert, to sort through the literature, and they agreed that one large-scale intervention in Zambia had enough evidence that it worked, and thus deserved inclusion in the 2015 edition of *Millions Saved*. The study, by a team that included researchers from Harvard's School of Public Health and the PATH Malaria Control and Evaluation Partnership in Africa, enrolled 81,600 farmers, half of whom received insecticide-treated bed nets, whereas the other half didn't. There was a nearly 50% drop in self-reported malaria among farmers with the nets.

The debate seems set to continue. "What we've found doing a massive trawl of the literature is that the quality of evidence for well-regarded and well-funded interventions is still pretty poor," says Miriam Temin, the coordinating editor of the new edition of *Millions Saved*. It remains difficult for many to accept, she says, that just understanding the effect of a drug, a vaccine, or any other intervention on a human body isn't enough. "We think of the body as something with unknown processes," Temin says. "Wouldn't it be interesting if we thought of communities that way?" ■

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As cholera goes, so goes Haiti

The West's poorest nation confronts a new disease

By **Sam Kean**, *in Mirebalais, Haiti*

A few meters away from where one of the worst cholera outbreaks in history started, several Haitian teenagers are enjoying a bath. Behind them looms the razor-wired U.N. compound from which cholera-laden sewage leaked into the nearby Meye stream in October 2010. But the teens splashing in the Meye today pay no attention, intent on washing clothes and scrubbing down a motorcycle. When asked if they fear cholera, they say no, because they're not dirty people. Besides, what other choice for water do they have?

It's the same scene all across Haiti. Every river, storm drain, and irrigation canal teems with people washing, drinking, defecating, and discarding trash. Unfortunately, the water's temperature and salinity provide a perfect incubator for cholera bacteria, and a disease never recorded before in Haiti has now attacked 700,000 people and killed 8500.

Last year, Haitian health officials unveiled plans to eradicate cholera within 10 years. But given Haiti's chronic problems, many observers are skeptical. "This cholera organism is well established in the Haitian environment and is likely there to stay," says Daniele Lantagne, an environmental engineer at Tufts University in Medford, Massachusetts, who does relief work in Haiti and investigated the United Nations' role in the outbreak.

The source of this pessimism isn't so much Haiti's health care system. Many clinics there are primitive, yes—small and hot, with rusty IV stands and goats, chickens, and dogs wandering through the grounds. But the system has seen success in some areas recently, like limiting the spread of HIV and lymphatic filariasis.

The pessimism springs instead from Haiti's continual lack of infrastructure. Really, cholera is simple to prevent. It spreads through contact with infected feces, "so you just need some form of separating poop from water and food," says Louise Ivers, a physician with the Boston-based Partners In Health (PIH), a medical aid group. But almost every effort to improve access to latrines, sewers, and safe drinking water in the past century has failed.

Between 1995 and 2012, foreign governments and private groups provided $15 billion in aid to Haiti, including $815 million for health efforts and $313 million for water and sanitation projects. And yet, because of various chronic problems—including poverty, corruption, and government instability (Haiti had 13 governments between 1986 and 2002 alone)—most of the country still lacks basic infrastructure. The battle against cholera, then, is a microcosm of the larger, systematic problems that have made Haiti the poorest and most intractable nation in the Western Hemisphere.

THE MAGNITUDE-7.0 EARTHQUAKE that devastated Haiti in January 2010 killed 220,000 people and caused $7.8 billion in damage, more than Haiti's gross domestic product. The deaths also gutted government agencies, leaving them understaffed when cholera struck.

Ironically, U.N. peacekeepers at the compound near Mirebalais introduced the disease. Some came from Nepal, where cholera is endemic, and although they showed no symptoms of cholera, genetic fingerprinting has matched the strain of cholera in Haiti to the strain prevalent in Southeast Asia. Witnesses also saw leaky sewage pipes within the compound. Haitian cholera victims and their families have recently filed a class-action suit against the United Nations in U.S. court, seeking compensation.

Once introduced, the disease exploded because of the lack of safe drinking water and toilets. In Caribbean nations overall, 80% of people have access to decent sanitation. The 2010 cholera outbreak also spread to the Dominican Republic, Haiti's wealthier neighbor, but it suffered just 31,000 cases and 471 deaths, due to superior infrastructure. Nearby Cuba suffered 700 cases and three deaths.

PHOTO: © SWOAN PARKER/REUTERS/CORBIS

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Waterways like irrigation canals and trash-filled sewers helped cholera overrun Haiti in a matter of weeks.

In Haiti, though, access to some kind of toilets hovers near 20% and had been declining for years even before the earthquake struck. Within major cities, many people dispose of waste with "flying toilets," plastic bags they hurl outdoors. In rural areas, where 10% of people have adequate sanitation, people often defecate in waterways like the Meye, and cholera—which causes explosive diarrhea—drove more people to use the river as a toilet. The Meye flows across Haiti's central plateau and empties into the country's largest river, the Artibonite. So cholera quickly spread from the interior to the coastal city of St. Marc, and from there it overran the country.

In city after city that October, sleepy health clinics were inundated with hundreds of victims per day, many of them carried in. (Victims can lose 20 liters of water daily, leaving them too dehydrated to walk.) Overwhelmed doctors—some of whom, Lantagne says, literally had no idea what cholera was—began stacking patients two to a bed, then on the floor. In some clinics, watery feces puddled on the ground.

Given the earthquake-cholera double whammy, many Haitian people feared the world was ending. Some stopped eating to avoid contaminated food and tied cloths around their stomachs to quell hunger pangs. One local health minister reportedly fled her clinic, too scared to enter. Even some voodoo priests—who normally insist on treating patients themselves—admitted they were powerless and directed people to proper doctors.

Aid groups like PIH immediately erected specialized cholera clinics with bleach hand- and shoe-washing stations; the smell of bleach still pervades the clinics. Digicel, Haiti's ubiquitous cell phone company, also chipped in by bulldozing hills to make space for clinics. For most of 2011 and 2012, cholera levels remained high, up to 14,000 cases per week. But in 2013 the numbers dropped, and Haitian clinics now record about 290 cases per week; perhaps hundreds of others contract cholera but don't seek treatment. While welcome, that drop introduced its own dilemmas. As the crisis waned, PIH and its affiliates said that raising money became harder. And while most cholera clinics now sit idle—rows of empty cots beneath huge tents—PIH cannot shut them down. Cholera levels tend to cycle, so the caseload could spike again during Haiti's winter rainy season. Plus, even 290 cases per week makes Haiti's the worst cholera outbreak in the world, Ivers notes.

WITH THE CRISIS SUBSIDING, the Haitian health ministry is trying to head off a recurrence. Jean-Renold Rejouit, the health commissioner for Haiti's central plateau, says that in the past, his department could only respond to disasters, not prevent them. "We were acting as firemen," he says. "We never had the chance to get ahead." To finally get ahead, the government is seeking assistance for a 10-year, $2.2 billion plan to eliminate cholera, including $1.6 billion to improve water and sanitation. Foreign governments and charities have ponied up $222 million so far.

Some experts question the government's approach, however. They see clean water and better sanitation as inextricably linked, but the Haitian government has starkly different ideas about addressing each. For water projects, which support whole communities, the government welcomes the help of aid groups. PIH recently managed a $2500 project to rebuild a concrete water station for one village and connect it, through several kilometers of pipes, to a natural spring and chlorination facility. Although the station is not exactly easy to access—the path to the station runs up steep slopes with no shade and plenty of boulders to stumble over—it cuts down the commute for safe water by 30 minutes for local families. That's not insignificant when they make several trips each day, often with small children in tow or baskets of laundry atop their heads.

But the government does not want aid groups building latrines, which usually benefit just one family. Rather, it wants people to build their own latrines, to promote personal responsibility.

Liz Campa, a water and sanitation expert with Zanmi Lasante, a sister organization of PIH, agrees. "Communities in Haiti have the capacity to improve their environment," she says. "Let them do it." She adds that latrines cost much less than water stations. Even fancy latrines with metal sheeting and concrete bases run about $100, and the price drops if people use wood and palm fronds and dig the 3-meter pits themselves. Some native Haitians concur.

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Jean Magloire, a health activist, faults many Haitians for squandering time and money playing dominoes and the Haitian lottery instead of saving up for or digging latrines themselves.

Ivers, though, feels conflicted. She supports the mantra of responsibility. But when she relayed that line at a community meeting, she says people laughed: "They asked me, 'How do we even get started?' " Half of all Haitians live on less than $1 per day, and goods, even food, are surprisingly expensive. Saving even $100 seems daunting.

Campa says PIH will make exceptions and build latrines for widows, people with AIDS, and other vulnerable groups. But for the most part it respects the government's wishes and focuses instead on clinics, hygiene campaigns, and delivering cholera vaccines.

PLANS TO IMPROVE WATER and sanitation face other hurdles. Few aid groups—PIH being an exception—focus on much beyond relieving immediate needs. And while aid money is still flowing to Haiti, funds have a history of disappearing there. After the earthquake, the United States gave Haiti $2.25 billion in aid. But no one knows how $1.5 billion of that was spent, says Vij Ramachandran, a fellow at the Center for Global Development in Washington, D.C. Given Haiti's history of corruption, she adds, at least some was probably stolen.

"Unless aid is invested in building local institutions and strengthening the government," Ramachandran says, "you will not see any real change."

Cultural habits can also slow efforts to improve public health. Women traditionally gather drinking water in Haiti, but because it's women's work, such chores often take low priority. At one fountain near Mirebalais, despite the quickly setting sun, women waited around with a dozen empty jugs while teenage boys washed their motorcycles. Bad hygiene habits also persist. Many Haitians say that cholera convinced them to start washing their hands after defecating and stop drinking irrigation water in the fields. But others admit they still don't bother with such niceties.

Rejouit, the health commissioner, insists that Haiti can eradicate cholera. Other doctors remain pessimistic, especially near St. Marc and other hard-hit districts. Still others say they are hopeful, but they realize that hope is about all Haiti has ever had. One of them, Patrick Ulysse, a health coordinator for PIH, nodded at the prospect of a cholera-free Haiti. "I'm optimistic," he said. He then paused and half-smiled: "I have to be." ■

PHOTO: THE GEORGE INSTITUTE FOR GLOBAL HEALTH AT PEKING UNIVERSITY HEALTH SCIENCE CENTER

China tries to kick its salt habit

A country with one of the world's saltiest cuisines confronts its hypertension problem

By **Mara Hvistendahl**, *in Shanghai, China*

China has long had a love affair with salt. Since about 2200 B.C.E., when the country first produced it, salt has been an important preservative for vegetables and meats. Entire regional cuisines are described simply as "salty," and individual dishes have monikers like "salt-and-pepper pork." In Tibet, locals drink a salty yak butter tea in place of water.

All told, the average rural Chinese citizen consumes 12 grams of salt daily, according to the 2010 Global Burden of Disease study; the average American takes in 9 grams, while the daily maximum recommended by the World Health Organization (WHO) is 5 grams. "High salt intake is part of Chinese food culture," says Wu Yangfeng, a cardiovascular specialist at the Peking University Clinical Research Institute who heads the George Institute for Global Health, China, in Beijing. It is also an acute health problem—but one that researchers believe can be tackled.

Salt is a major contributor to an alarming rise in hypertension in China's rapidly aging population. Some 54% of Chinese adults aged 45 and older now have hypertension, according to the China Health and Retirement Longitudinal Study, among the highest rates in the world. Hypertension is a risk factor for stroke—the leading cause of death in rural China in 2010—and other cardiovascular diseases.

As the country develops, Chinese are also eating more meat and engaging in less physical activity, which drives the rise in hypertension and chronic disease. Those lifestyle factors are hard to combat, especially in a people tasting modern life for the first time. Meanwhile China's overburdened health care system is ill-equipped to treat hypertension directly, given the legions of patients. An estimated 40 million Chinese aged 60 and over with the condition haven't been diagnosed, and of those who have been, only a small proportion get their blood pressure under control. But researchers say reducing China's salt consumption is feasible—and could have a major impact.

In fact, the country is an ideal place to try an intervention, says Bruce Neal, an epidemiologist with the George Institute

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An interviewer with the George Institute for Global Health speaks to a Tibetan villager about eating habits and health problems (*left*); the Chinese diet is one of the world's saltiest (*above*).

and the University of Sydney in Australia. In developed countries, most salt is consumed through processed food or in restaurants; efforts to fight hypertension have focused on pressuring the food industry to lower salt content—often unsuccessfully. In China, salt is primarily added to meals during home cooking or at the table, so prevention messages can target the individual. And the salt industry is controlled by a state monopoly, meaning that there are only a few distributors. "That's really important if we want to intervene," Neal says.

There is still debate about whether it's useful to lower salt intake below 6 grams a day (*Science*, 24 May 2013, p. 908), but most scientists agree that bringing down China's very high levels is a relatively cheap, effective public health intervention. Based on disease models from elsewhere, Wu and colleagues estimate that reducing the average Chinese person's intake by a mere gram a day could save 125,000 lives a year.

But whether such a behavioral intervention is feasible on a large scale—and to what degree it would actually reduce cardiovascular disease—hasn't been carefully studied. A study by the George Institute published in 2007 found that introducing a low-sodium salt substitute lowered blood pressure in 608 high-risk adults in northern China, but it didn't look at outcomes like stroke. A study in Taiwan did measure such health effects, but it involved just a few thousand men living in one retirement home and didn't control well for errors, Neal says.

Neal and Wu are the lead investigators of a huge randomized controlled trial to test the health impact of salt reduction among a broader group of people who live and cook at home. The China Salt Substitute and Stroke Study has recruited 21,000 participants with a history of hypertension or stroke in more than 600 villages in northern China and Tibet. Since early July, participants in some villages have received a salt substitute in which sodium chloride is partially replaced with potassium chloride, which has been shown to lower blood pressure; these people also receive regular advice on lowering salt intake. Patients in control villages use normal table salt and receive advice only at the start of the study. Over the next 5 years, investigators will record stroke and other cardiovascular events, while urine samples will reveal changes in sodium and potassium intake.

The study comes at a time when hypertension and cardiovascular disease are already firmly on the Chinese government's agenda. A spate of health care reforms introduced in 2009 (*Science*, 1 February 2013, p. 505) includes free blood pressure checks and partially subsidized drugs for hypertension patients. The country has set a target of reducing salt consumption to 9 grams per person by 2015. If the study shows that using the salt substitute can bring down disease, Wu and Neal plan to lobby government leaders to introduce a substitute nationwide and subsidize its manufacture. (Now, producing the new substance can cost up to twice as much as regular salt.) In their vision, shops would offer a subsidized salt substitute alongside regular salt.

Of course, people would actually have to buy and use the substitute. Neal says that an alternative strategy would be to convince the salt industry to gradually reduce sodium content in China's entire salt supply—a goal that may be possible because of the salt monopoly.

Other countries should pay attention, says Feng He, an epidemiologist at Queen Mary University of London's Wolfson Institute of Preventive Medicine. WHO estimates that 80% of cardiovascular deaths occur in low- and middle-income countries; in many of them, hypertension patients can't afford treatment, so prevention is paramount. Salt substitutes may prove particularly useful elsewhere in Central and East Asia, where sodium intake is among the highest in the world—and where, like in China, consumers add much of it to their diets themselves. It may be a tradition that's well worth breaking. ■

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Zambia fights to sustain its malaria success

A model for malaria interventions is at risk as funding flattens and the disease smolders on

By **Kai Kupferschmidt**

In the life-and-death battle against malaria, success is easy to measure for Philip Thuma, a doctor in Macha, in the southern region of Zambia. Here, on the gentle rolling landscape of this rural outback, where the poorest of the poor live off the maize they grow, the parasitic disease used to have a devastating effect. Little more than a decade ago, at the small district hospital that Thuma runs, up to 1500 children were admitted with malaria every year. About 60 of the young patients died. Now, there are about 30 admissions a year, and two or three deaths, Thuma says.

Such gains are impressive for a country where malaria has long been endemic. Soon after the rains start around October, *Anopheles gambiae*, *Anopheles arabiensis*, and *Anopheles funestus*—the three mosquito species that transmit malaria here—start breeding. Cases multiply, reaching a peak in April and May. The swampy parts in the north of the country are particularly hard hit.

Between 2006 and 2011, however, the World Bank, the U.S. President's Malaria Initiative, the Global Fund to Fight AIDS, Tuberculosis and Malaria, and other donors directed more than $300 million into the country to combat the disease, making it a testbed for prevention and treatment. "One of the goals of early investment in fighting malaria in Zambia was to establish a proof of concept," says Kent Campbell, director of the malaria control program at PATH, an international nonprofit organization—a case study in how malaria illnesses and deaths "could be quickly and dramatically decreased."

In 2003, Zambia was the first African country to adopt artemisinin combination therapy to treat malaria. From 2006 to 2011, more than 24 million bed nets treated with insecticide were distributed. Indoor spraying with a long-lasting insecticide covered more than 6 million homes in the same time. In some areas, whole communities were screened for malaria and anyone who tested positive, even if they were not feeling sick, was treated.

The efforts paid off. In a recent analysis, Nancy Fullman of the Institute for Health Metrics and Evaluation at the University of Washington, Seattle, and colleagues found that malaria cases had declined by about 7% per year between 2000 and 2013, reducing deaths by about 60% over that period. "This is great news for the country," she says.

Now, as donor commitments flatten, public health officials in Zambia and those aiding them are focused on consolidating their success. Worrisomely, the gains are uneven,

PHOTO: © JOHN STANMEYER/VII/CORBIS

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A worker sprays the inside of a hut with an insecticide in Lusaka province in Zambia. Indoor spraying is one of the main interventions used to curb the spread of malaria in the country.

with the southern part of the country faring better than the north. "There are parts of the country where malaria transmission has not changed at all or increased over the last few years, despite all the efforts," says William Moss, an epidemiologist at the Johns Hopkins Malaria Research Institute in Baltimore, Maryland. And the overall progress shows signs of eroding. Malaria cases nationwide, after dropping from nearly 5 million cases in 2006 to about 3 million in 2009, rose again to about 4.5 million in 2011, for reasons health officials still struggle to explain.

Key to maintaining Zambia's status as a poster child for progress against malaria, most agree, are continued commitment by donors and the national government, renewed efforts in the north, quick reaction to new malaria cases in the south, and attention to neighboring nations. If that isn't done, experts warn, the disease could come back in full force.

THUMA HAS BEEN IN ZAMBIA long enough to see malaria all but disappear—and then come roaring back. In 1976, when the son of medical missionaries came to the southern African country to work as a doctor, malaria was not a big problem. In each district there was a health inspector who coordinated campaigns to combat malaria, for example spraying houses with insecticides every year.

Then, at the end of the 1970s, the price of copper, an important export for Zambia, dropped and the Zambian economy tanked. Investments in malaria control were scaled back. "The whole infrastructure sort of fell apart," Thuma says. On top of that, in the 1980s, resistance to chloroquine, the main medicine against malaria at the time, emerged. "By 1990 to 1995 there was malaria all over the country again," Thuma says. "It was terrible."

Other countries have had a similar experience. A 2012 study evaluated 75 instances since 1930 when malaria reemerged in a country after it had been beaten back to varying extents. Civil wars, natural disasters, and resistance against chloroquine or the insecticide DDT fueled some of the setbacks. But Richard Feachem, director of the global health group at the University of California, San Francisco, says that in the end, "The reason was almost always the same: The budget was cut right back, the malaria team were partially or entirely laid off."

Donor money for malaria interventions is still flowing into Zambia, and the country also devoted $24 million from its own budget to malaria in 2013 and $27 million this year—sums unheard of in sub-Saharan Africa, according to Campbell. So why are some of its hard-fought gains eroding?

Even a short financial blip can disrupt a country's gains: In 2010, Campbell says, money to replace bed nets—which begin to lose efficacy after 3 years—temporarily ran short, and that immediately led to a resurgence of malaria. It may also be that natural variation played a bigger role in Zambia's earlier gains than many have thought. A drought in southern Zambia a decade ago killed off large numbers of *A. funestus* mosquitoes, Moss notes. "They haven't really recovered and now there is *A. arabiensis*, a less efficient vector."

In the north, on the other hand, *A. funestus* and *A. gambiae* are still spreading the disease, and they have now developed resistance to pyrethroid insecticides. A new indoor spraying campaign with actellic, a more expensive chemical that still kills the insects, is supposed to start soon and may help to beat back malaria, Moss says. But more research is also needed to understand why the north hasn't enjoyed the same successes as the south, says Ubydul Haque, of the Emerging Pathogens Institute at the University of Florida in Gainesville.

To sustain the gains in the south, public health officials are deploying a strategy called reactive case detection. If any new malaria case is diagnosed, a group of physicians is sent to the home of the patient, and anyone living within a radius of 140 meters is tested for malaria and treated if they are found to be infected. "It's almost like outbreak detection" for a fast-spreading virus, Moss says. The long-range goal of such efforts is to get transmission of malaria down to zero in this part of Zambia.

Even if that effort succeeds, no one should rest easy, Feachem says. "Donors tend to go where there is the most disease and with malaria that is a mistake," he warns. After halting transmission, preventing reintroduction should become the new focus, he adds. Zambia borders eight other nations, more than any other African country. To keep malaria from reestablishing itself, health officials will need to identify groups of people most likely to bring the parasite back into the country, in order to focus screening and, if needed, treatment on them.

In the long run, however, neighbors will need to mirror Zambia's gains. "The success of Zambia can only be sustained by success of bordering countries," Feachem says. Then, perhaps, Thuma can have some confidence that Zambia's roller-coaster history with malaria won't repeat itself. ■

More than 24 million bed nets treated with insecticide were distributed in Zambia between 2006 to 2011.

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OPINION

Halving premature death

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Death in old age is inevitable, but death before old age is not. Except where HIV or political disturbances predominated, mortality rates have been decreasing for decades, helped by sanitation, health care, and social changes. Even in low-income countries, at current death rates, three-quarters of newborn infants would survive to age 50, and half would survive to age 70. If disease control keeps progressing and economic development proceeds, then within the next few decades—except where disasters or new epidemics supervene—under-50 mortality should fall to less than half of today's 15% global risk, and under-70 mortality should be less than one in six.

World under-5 mortality decreased from 14% of all live births in 1970 to 5% in 2010. It could reach 2% by 2030, with continued attention to female education, perinatal care, vaccination, adequate nutrition, curative treatment, and other key child survival determinants. World under-50 mortality halved from 28% in 1970 to 15% in 2010 (5% under-5 plus 10% later), but varies 10-fold between countries, with further inequalities within countries. Its main causes, apart from maternal and perinatal mortality, include communicable diseases, undernutrition, and fatal injuries (suicide, homicide, and, particularly, accidents). They also include alcohol, a major cause of injuries, and tobacco.

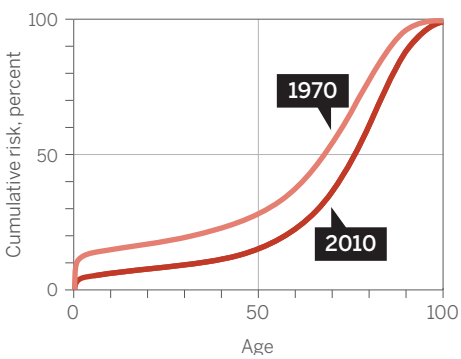
Noncommunicable diseases (NCDs), such as cancer, stroke, heart disease, and emphysema, cause a quarter of all deaths before age 50, plus four-fifths of those at ages 50 to 69. The most important external factor is smoking, which still causes about a quarter of all cancer deaths in the European Union and a third of all cancer deaths in the United States, plus comparable numbers of deaths from other diseases. Smoking also causes many deaths in China, India, Russia, and worldwide (totaling about 5 million deaths a year, and rising). Fortunately, quitting works; smokers who stop before age 40 (and preferably well before 40) avoid over 90% of the risk. Each year, governments make US$300 billion directly or indirectly from tobacco, so at constant prices, a one-third reduction would lose governments $100 billion. But if tax increases double the current prices and thereby reduce smoking by one-third, this would save just as many lives and gain governments $100 billion a year.

The World Health Organization's recent resolution on NCDs calls for reducing smoking by a third by 2025, decreasing hazardous alcohol drinking, and halting the increase in obesity. It also supports the wide use of generic drugs such as statins and antihypertensives to prevent NCD recurrence.

If 2010 mortality rates continue unchanged (which they will not, as death rates are already falling), then in 2030 there would be about 20 million deaths before age 50, and 20 million more at ages 50 to 69. For many countries, halving their 2010 under-50 mortality rate by 2030 would be a feasible target; worldwide, it would avoid 10 million of the 20 million deaths projected above for 2030. Adoption of this target would reinforce current successful efforts to reduce maternal and child mortality and death from HIV, tuberculosis, malaria, and other communicable diseases, but would also require serious and successful efforts to substantially reduce accidents and NCD mortality.

World mortality, 1970 and 2010

Decreasing risk of premature death



"For many countries, halving their 2010 under-50 mortality rate by 2030 would be a feasible target..."

Changes in mortality at ages 50 to 69 are dominated by NCD mortality, where some important control measures may take decades to have their full effects. Although reductions of about one-quarter or one-third could well be seen by 2030, halving global mortality in this age range will take longer. But even if the ultimate goal is to substantially reduce under-70 mortality, halving under-50 mortality would be a more immediate target and could be achieved worldwide by 2030 (or in the 2030s). With additional gains in later middle age, some 40% of all premature deaths would be avoided.

Public health and medicine do not offer eternal life, but do offer a more comfortable life and an increasingly good chance of avoiding premature death. Still, however, almost all of us will die before 100.

– **Richard Peto, Alan D. Lopez, and Ole F. Norheim**

10.1126/science.1259971

Published by AAAS

Downloaded from www.sciencemag.org on September 12, 2014

CREDIT: UNITED NATIONS POPULATION DIVISION LIFE TABLES/HTTP://ESA.UN.ORG/WPP

PERSPECTIVE

Putting women and girls at the center of development

Melinda French Gates

The development field needs to be more serious about gender inequities and women's empowerment. By ignoring gender inequities, many development projects fail to achieve their objective. And when development organizations do not focus on women's empowerment, they neglect the fact that empowered women have the potential to transform their societies. I also review the Gates Foundation's record on gender and propose some approaches to improve it.

I have formal training in computer science and business—not in international development. But in the decade and a half since my husband and I created the Bill & Melinda Gates Foundation, I have received a practical education in the field. Given our backgrounds in the computer industry, we started with a bias toward technological solutions. Our optimism about technology has not changed, but we have gradually gained a greater appreciation for the social and cultural factors that influence how individuals, communities, and countries develop.

One of the most important, and most complex, of these factors is gender. In recent years, the development field has increasingly focused on gender equality and women's empowerment—and for good reason. No society can achieve its potential with half of its population marginalized and disempowered.

The work our partners have done in this area has influenced me greatly. My goal is to be clear and specific about what it means to me and for our foundation to put women and girls at the center of global development. Put simply, we cannot achieve our goals unless we systematically address gender inequalities and meet the specific needs of women and girls in the countries where we work.

We have not always been as intentional in this area as we might have been (Fig. 1, top) or as we intend to be in the future. As a result, we have lost opportunities to maximize our impact across all of the areas in which we work.

Why gender inequality matters

Research clearly shows that inequality between the sexes limits development for everyone (*1*). A recent report found that if African smallholder women farmers had equal access to land, labor, information, technology, fertilizer, and water—and equal opportunity to use those resources effectively—agricultural production across the continent would increase by 20% (*2*). People are going hungry while we try to figure out how to address gender inequality and empower women.

Not that it is easy to figure out. It is hard enough to close gaps in access and make sure that women are participating in development programs on an equal basis. For example, many women are so busy with household responsibilities that they do not have time to take part, despite the best efforts of development organizations (*1*, *3*). It is even more difficult to fully understand the underlying social and cultural norms that prevent women from realizing the full benefit of that participation and then find ways to shift those norms.

For example, we are partners with the Grameen Foundation on a 4-year-old project in Ghana called MOTECH, which uses mobile phones to provide health information to pregnant women.

Grameen switched their effort from text messages to voice messages, realizing that text messages would not reach women who could not read—a serious problem in communities where girls' educational opportunities are limited.

But even if a household has a cellphone, the women in the household may not have access to it. The husband typically maintains control over the family phone. Grameen started asking women what days and times they were most likely to have access to the phone so that the calls would be more likely to reach them.

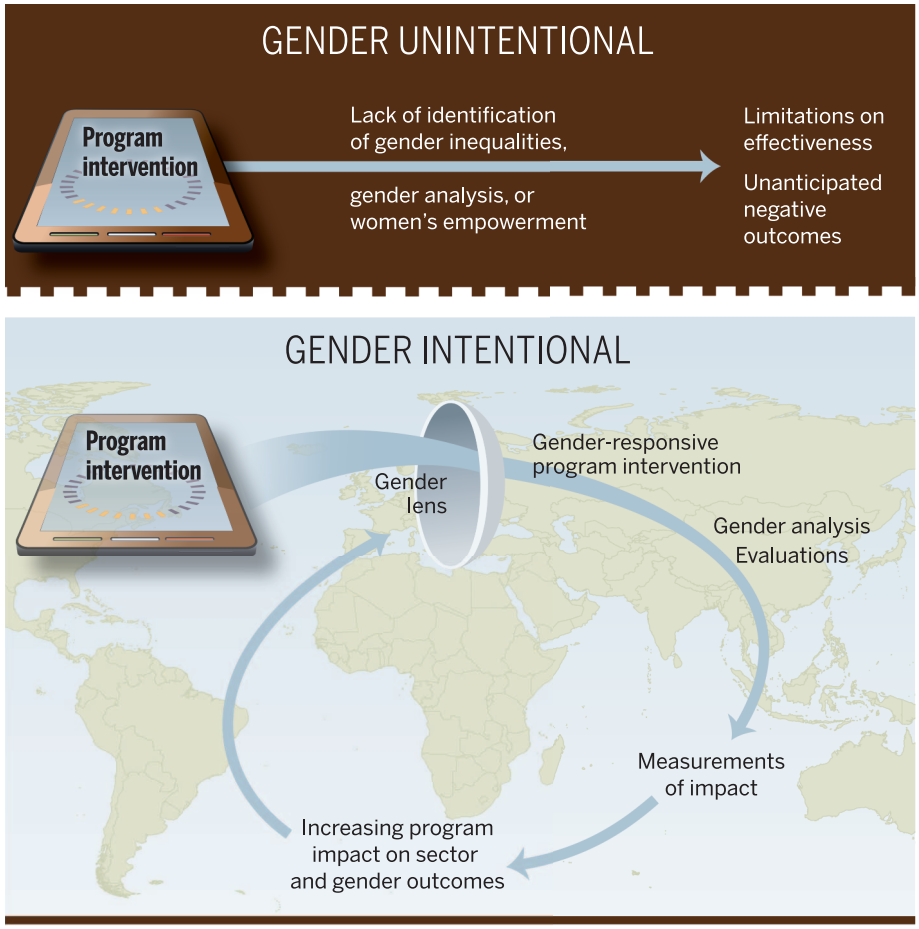


Fig. 1. Gender Unintentional. The upper scenario depicts interventions (for example, a family planning program) to improve outcomes (for example, modern contraceptive prevalence rate) without explicit consideration of gender inequalities. Gender inequality and women's empowerment (GEWE) interventions (for example, formation of self-help groups) are absent, and the impact of programs on GEWE outcomes is not measured. Thus, the impact of gender inequalities on outcomes is unknown, and could limit program effectiveness. Furthermore, there could be unanticipated negative outcomes (for example, women who seek contraceptives may suffer violent reactions by their husbands). **Gender Intentional.** Here, gender inequalities are systematically identified, and context-relevant gender interventions are designed to close gender gaps. Thus, gender inequalities are not exacerbated and the program is more likely to have positive effects.

Bill & Melinda Gates Foundation, Seattle, WA, USA.

Grameen then realized that the effectiveness of their health messages varied a great deal depending on how they were conveyed and who was conveying them. Grameen conducted research to determine which voices would be most persuasive to women (*4*). They learned that women in rural Ghana preferred getting advice from an older woman, but they sometimes asked that the voice be a man's because they wanted their husbands' support, and men were more likely to listen to advice from another man.

Despite Grameen's fine-tuning of the project, the biggest challenge of all remained: Some women lack the power—the agency and voice—to make important decisions about their own lives and act on them free of retribution or fear (*5*). Even if a woman receives a message about the benefits of giving birth at the local health facility instead of at home, she might not have the social permission or support to go to the facility or pay for the care.

Grameen has continued to refine its program so that the health messages reach not just pregnant women but also husbands, mothers-in-law, and grandmothers, all of whom exert influence over decisions about the family's health care. Ultimately, even the most ingenious mobile-communications strategies must be accompanied by an approach to helping women surmount obstacles within their own household and community.

In agricultural development, our goal is to help smallholder farmers increase their productivity, which will lead to better nutrition and greater income. However, we have found that one consequence of increasing productivity is that come harvest time, some men simply take the crops that women had been working so hard to cultivate, then spend the profits. When yields are small, women may be free to sell any surplus left over after feeding the family; it is generally seen as a household's "pocket change." But when the surplus is large enough, husbands often take over.

One of the crops we invest in is a legume called pigeon pea, known as the "poor man's meat" in parts of Africa because it is an important source of nutrients. Our partners' work with pigeon pea has been a big success. Yields are going up, and hundreds of thousands of hectares are now planted with an improved variety. However, one partner, Cooperative for Assistance and Relief Everywhere (CARE), estimates that only 3 in 10 women get to keep the proceeds from the high-yielding pigeon pea they tended for months, and this type of effect can be seen in much of our work in agriculture.

To ensure that women can control the profits from their labor (and therefore stay motivated to grow pigeon pea and other crops), our partners are developing complementary interventions to help address these additional challenges. For example, engaging husbands and wives in structured conversations about household expenditures and responsibilities seems to lead to more collaborative ways of splitting up the proceeds from women's labor, ultimately benefiting all members of the household.

There is no easy way to remake social norms so that they are more equitable. Change requires innovative thinking to help women and girls overcome the structural barriers to equality. This work will necessarily include engaging men and boys because their attitudes about the role of their mothers, daughters, sisters, and wives will have an enormous impact on improving opportunities for both sexes.

Women and girls as engines of development

Targeting women and girls as beneficiaries of development programs is only one part of putting them at the center of development. The other part is recognizing their role as agents of change.

"we cannot achieve our goals unless we systematically address gender inequalities and meet the specific needs of women and girls in the countries where we work"

Women tend to invest more of their earnings than men do in their family's well-being—as much as 10 times more (*1*, *6*–*8*). This fact is critical to the way our foundation thinks about the role of women and girls in development. The upshot is that a woman or a girl with some measure of power is busy improving her community in myriad ways. So, it makes sense to invest in development outcomes by investing in the women who are driving them every day.

Empowerment is not tangible in the same way as the choice to deliver a baby at a facility or in the home. Therefore, it is not always clear how to invest in empowerment, or how to measure it. However, the research in this area identifies some key elements of empowerment, including education, control over resources, decision-making authority, and physical safety. Girls' access to education, the most studied variable, is especially powerful. Each extra year of education is associated with a 10 to 20% increase in income (*9*, *10*).

There are strong associations between women's empowerment and specific health and development outcomes (Fig. 1, bottom). For example, women's control over resources is associated with better outcomes in family planning; maternal, newborn, and child health; nutrition; and agricultural development (*1*, *8*, *11*–*13*).

To cite one instance, a recent review of a CARE program in Bangladesh shows that health and nutrition programs were substantially more effective at reducing stunting in children when households also participated in activities that contributed to women's empowerment (*14*). CARE created self-help groups of women and adolescent girls in order to help increase their decision-making power; reduce gender-based violence; raise awareness of their educational entitlements; and build their leadership, advocacy, and literacy skills.

The evidence also suggests that the process may work in the other direction, too—that investing in key health and development outcomes can contribute to women's empowerment (*1*, *8*). Unfortunately, the evidence needs to be strengthened. It could be improved if donors were more willing to fund evaluations of these complex models, something our foundation will increasingly take on.

The Gates Foundation and gender

At our foundation, we will not use the complexity of resolving gender inequality as an excuse for failing to think and act more intentionally about putting women and girls at the center of what we do.

We will systematically increase our focus on women's specific needs and preferences and on addressing gender inequalities and empowering women as a way to help our partners succeed and us achieve our mission of helping all people to lead healthy, productive lives.

Part of this focus will involve analyzing many of our grants and strategies through a gender lens, to make sure that gender inequalities are not getting in the way of the results we hope to achieve. Another part will involve greater accountability for how our strategies and grants contribute to women's empowerment over the long term. If we believe that women themselves are agents of development, then we must invest in their agency and evaluate the results.

Our foundation can do more to support our grantees to gather and use the right data and perform rigorous evaluations. Currently, many of our grants do not measure gender inequalities or women's empowerment or disaggregate data by sex. If we gathered this information more systematically, we would understand more about how to design programs to benefit both women and men, enabling us to maximize our impact (Fig. 1, feedback loop).

It may take additional financial investments to help our teams make these adjustments and to support our partners in their work in this area. We are prepared to make the investments necessary to improve our approach to thinking about gender issues because we believe the payoff greatly outweighs the increase in expenditures.

In 2015, our foundation will focus on asking key questions and considering evidence about gender inequality and women's empowerment, with an eye toward increasing our impact. Toward that end, we will be launching this fall a multimillion-dollar Grand Challenge on putting women and girls at the center of development. Our Grand

Challenges program, which started in 2004, poses big, open-ended challenges in health and development and provides funding for innovators from multiple fields and all over the world to develop creative solutions. This will be our first Grand Challenge on gender inequity and women's empowerment, and it is intended to mark the beginning of our concerted push in putting women and girls at the center of our work.

I come to work every day because I believe that our foundation has a role to play, along with our thousands of partners, in making the world a more equitable place. A necessary element of that role is helping women and girls realize their own power to advance the well-being of their families, their communities, and their societies.

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10.1126/science.1258882

PERSPECTIVE

The state of global health in 2014

Jaime Sepúlveda[1]* and Christopher Murray[2]

The global health landscape looks more promising than ever, although progress has been uneven. Here, we describe the current global burden of disease throughout the life cycle, highlighting regional differences in the unfinished agenda of communicable diseases and reproductive, maternal, and child health and the additive burden of emerging noncommunicable diseases and injuries. Understanding this changing landscape is an essential starting point for effective allocation of both domestic and international resources for health.

The turn of this century coincided with a reinvigorated energy to improve the state of global health. New institutions (such as the GAVI Alliance and the Global Fund to Fight AIDS, Tuberculosis, and Malaria), more and better funding (such as the Bill and Melinda Gates Foundation), and renovated political will [such as United Nations Millennium Development Goals (MDGs)] have already made an impact by reducing mortality among children and mothers and accelerating declines in HIV/AIDS, tuberculosis, and malaria in low-income countries. Despite the welcome news about rapid declines in childhood mortality almost everywhere, there are persisting health inequalities, emerging conditions, and important regional variations in causes of death that require closer scrutiny.

This paper focuses on the state of global health from the perspective of the population and not of the governance or financial architecture. We use the results of the annual assessment of the Global Burden of Disease (GBD) Study (*1*), which provides a methodologically consistent assessment of levels and trends in prevalence and mortality by cause for 188 countries since 1990. The recent GBD 2013 Study is based on the work of a scientific collaborative with more than 1050 investigators from 106 countries (*2*). Our intention is to present data in a high-level descriptive way. In short, we illustrate the following:

1) There is an unfinished agenda regarding communicable diseases and reproductive, maternal, and child health, mostly concentrated in sub-Saharan Africa and South and Southeast Asia.

2) The well-recognized epidemiological transition (from high burden of communicable diseases to noncommunicable diseases, injuries, and violence) is well under way in low- and middle-income countries, with increasing probability of death from these factors in some age groups.

3) There is a mismatch between needs and development assistance for health (DAH), which warrants a broader discussion, especially in geographical areas where resources can be most catalytic.

The burden of disease across the life cycle

As context, it is important to remember current population dynamics. The world has reached a population of 7.2 billion, with 138.8 million births and 54.9 million deaths taking place in 2013. Fertility rates are declining steadily almost everywhere, with the exception of sub-Saharan Africa, where rates are high and declining more slowly. By 2030, it is estimated that there will be 8.3 billion people on this planet, with 13% over the age of 65 years—the fastest-growing age group (*3*). Life expectancies at birth in 2013 show great inequalities, from 45.6 for males born in Lesotho to 86.4 years for females born in Japan (*1*).

The probability of dying is not constant in life; it has variations in the human life cycle, with higher risks in the extremes of the age spectrum: the first few days after birth and after 70 years of age (*4*). In addition to age, there are huge disparities in the probability of dying because of differences in sex, country, and cause (Fig. 1). Risk factors—such as high blood pressure, smoking, alcohol abuse, inadequate nutrition, and poor diet—are associated with specific causes of death and exhibit regional variation (*5*). It is critical not only to understand when and where important health outcomes occur but also to identify the underlying risk factors.

Newborns and children under the age of 5

MDG 4 aims to reduce by two-thirds the under-5 mortality rate (U5MR) between 1990 and 2015. By 2013, global U5MR had decreased by 48%; only 27 of 138 developing countries are likely to achieve the MDG target. Last year, 6.3 million children died before their fifth birthday; 41.6% (2.6 million) of those deaths occurred within the first 28 days of life (*6*).

Today, the 10 countries with the highest child mortality are all in sub-Saharan Africa. This region not only has the highest burden of

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communicable, neonatal, and nutritional diseases, it also has the highest burden due to noncommunicable diseases in young children (e.g., sickle cell disorders, congenital anomalies, epilepsy, and asthma). A child born in western sub-Saharan Africa is almost 30 times more likely to die by the age of 5 than a child born in Western Europe (U5MR of 114.3 versus 3.9 per 1000 live births) (*6*). Although malaria is a substantial cause of death (9.3%), lower respiratory infections and diarrhea are also key contributors, accounting for 14.4 and 8.3% of total childhood deaths in 2013, respectively. Many of these lives could be saved with affordable interventions, such as exclusive breastfeeding up to 6 months, oral rehydration salts, vaccination, and insecticide-treated bed nets. Additionally, because income growth and maternal education are key contributing factors to the reduction in child mortality (*6*), continued investments in these areas, as well as family planning and contraceptive programs, are critical.

Over the past decade, the proportion of neonatal deaths has increased as a share of total under-5 deaths in many countries (*6*). The leading cause of neonatal death is preterm birth, or being born before 37 weeks of pregnancy are complete. Each year, an estimated 15 million babies are born too soon (*7*). In 2013, nearly 12% (742,381) of under-5 deaths were due to preterm birth complications, with the highest years of potential life lost (YLL) among neonates in South Asia and sub-Saharan Africa. It has been estimated that up to three-quarters of babies born too soon without access to intensive care units could be saved with affordable interventions, such as early and exclusive breastfeeding and skin-to-skin contact to improve thermal regulation (*7*). Scale-up of these newborn care interventions and strategies to improve access to quality antenatal care and obstetric services are needed.

Maternal health

Pregnant women comprise a special group within the early adulthood life-cycle period. Maternal mortality accounts for 9.1% of all deaths among 15- to 49-year-old women. MDG 5 has mothers as an explicit priority, with the target goal of a 75% reduction in maternal mortality ratio (MMR; the number of women who die during pregnancy and childbirth per 100,000 live births). The global MMR has only decreased by 26.2% since 1990, with 376,034 maternal deaths in 1990 and 292,982 in 2013. Clearly, maternal mortality has not shown sufficient progress. This means that only 16 countries are expected to achieve the MDG 5 goal; seven of these are developing countries (*8*).

Regional differences in maternal mortality are striking. In 2013, less than 2000 maternal deaths occurred in developed regions (MMR of 12.1), in contrast to developing regions, which accounted for the vast majority (291,171 deaths; MMR of 232.8). Sub-Saharan Africa accounted for almost half of all maternal deaths, and two countries accounted for more than one-third: India (71,792) and Nigeria (36,698) (*8*). Inequalities in access to quality care are an important driver of these striking disparities. For instance, in developing regions, only half of pregnant women receive the World Health Organization (WHO)–recommended minimum of four antenatal care visits. Of the 135 million births in 2011, an estimated 46 million women delivered alone or without adequate care (*9*).

Early adulthood: 15 to 49 years

The general pattern of the epidemiological transition from communicable disease toward noncommunicable disease has occurred in many regions. Since 1990, the number of deaths resulting from communicable, maternal, neonatal, and nutritional causes has decreased by more than a quarter, whereas deaths from noncommunicable diseases and injuries increased by about 41.8 and 10.7%, respectively (*1*, *2*). The exception is the unfinished agenda of maternal and child health, as illustrated above, and infectious diseases in many parts of Africa and Asia. Among 15- to 49-year-old adults in developing settings, HIV/AIDS and tuberculosis are major contributors to YLL. In 2013, more than 1.4 million 15- to 49-year-old individuals died from HIV/AIDS and tuberculosis worldwide; about two-thirds of these deaths occurred in sub-Saharan Africa (*10*).

Although there is a shift toward a larger fraction of deaths in early adulthood from noncommunicable diseases and injuries, it is critical to note that the age-standardized YLL rates for these conditions are higher in low-income countries as compared with middle-income and

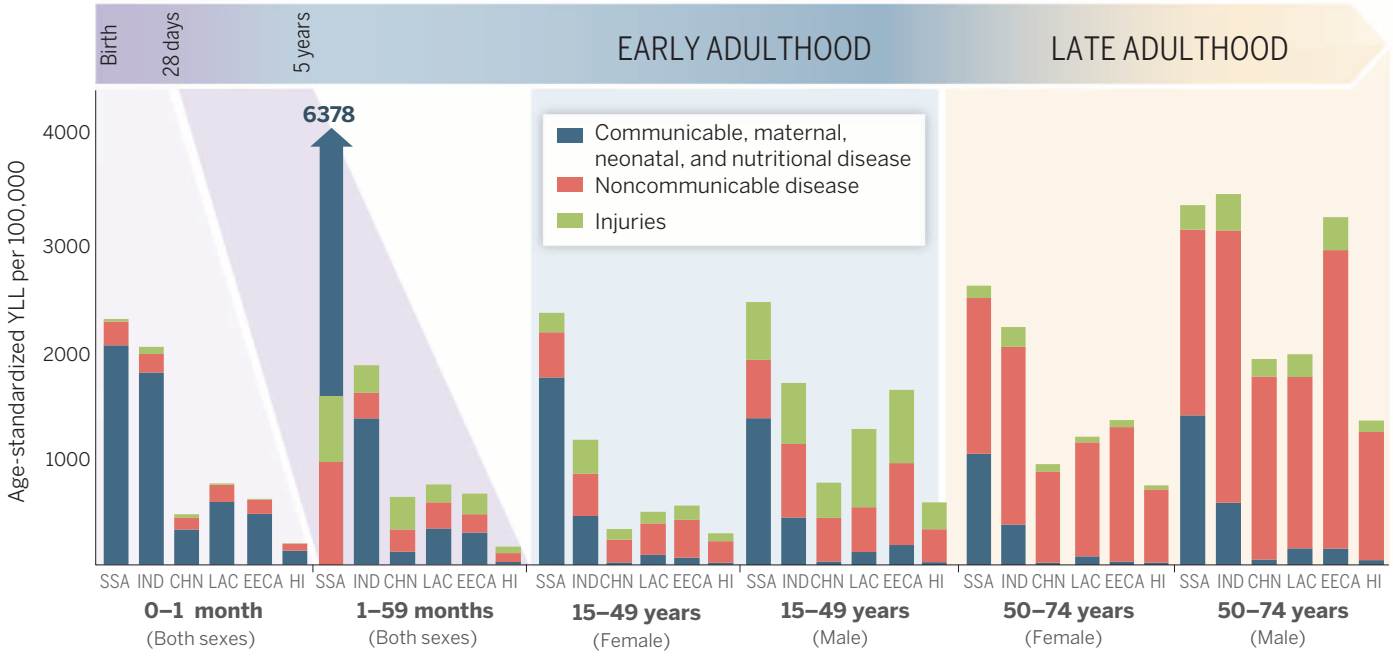

Fig. 1. The burden of disease across various life stages and regions due to specific cause categories, depicted as age-standardized YLL per 100,000. These six geographical regions cover most but not all of the world's population, and their selection is intended to show important regional variations: sub-Saharan Africa (SSA); India (IND); China (CHN); Latin America and Caribbean (LAC); Central Europe, Eastern Europe, and Central Asia (EECA); and high income (HI). The burden of communicable, maternal, neonatal, and nutritional diseases in SSA exceeds the YLL scale, and ages 5 to 15 and 75+ years are not shown.

Age-standardized YLL due to interpersonal violence (per 100,000)

Males aged 15-49 in 2013

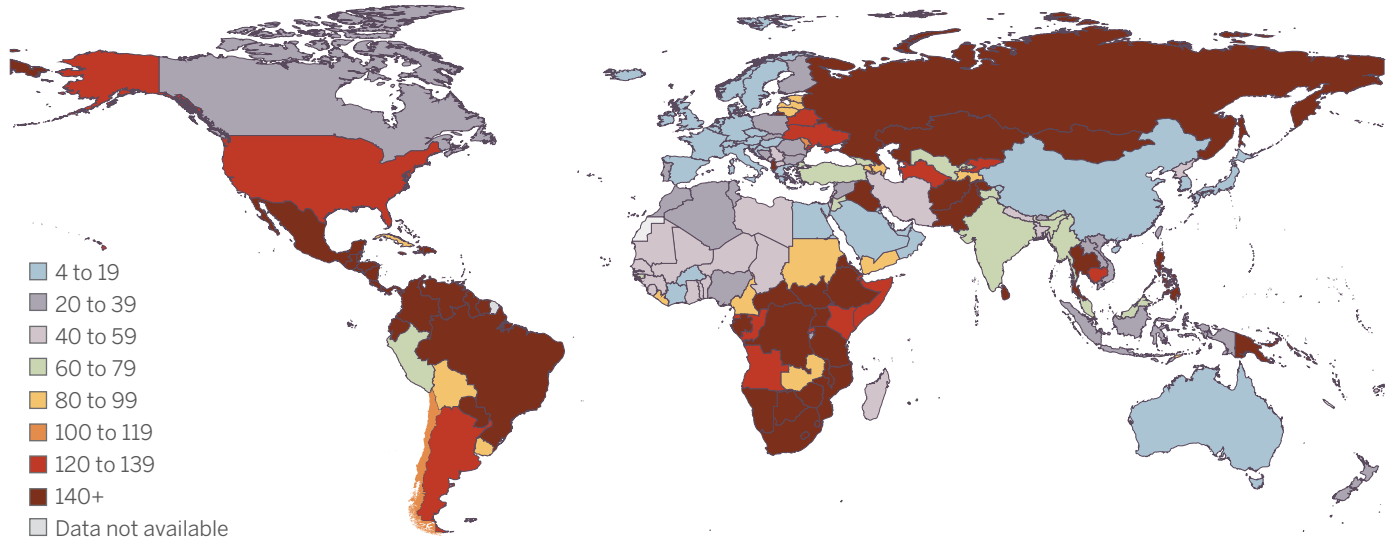

Fig. 2. Age-standardized YLL due to interpersonal violence (per 100,000) among males aged 15 to 49 in 2013.

Age-standardized YLL due to diabetes mellitus and prevalence of obesity

Individuals 20 years and older

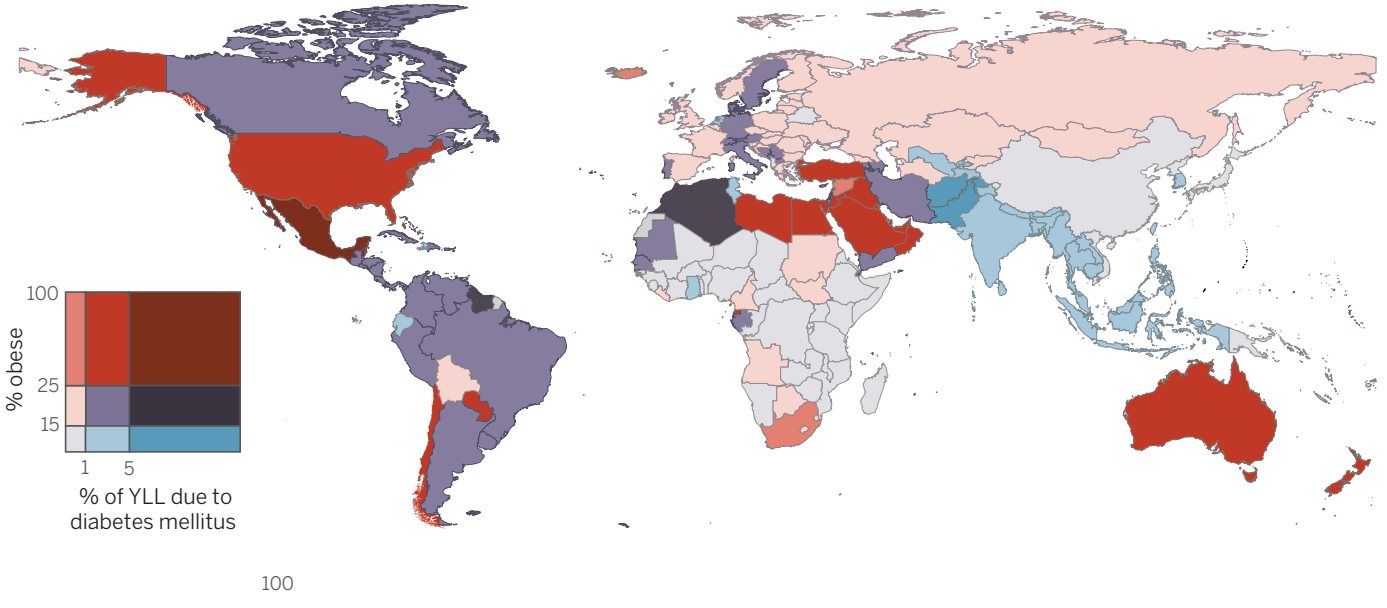

Fig. 3. The percent of age-standardized YLL due to diabetes mellitus and prevalence of obesity among individuals aged 20 years and older. Obesity among adults is defined as a body mass index >30 kg/m^2. The legend is not drawn to scale.

high-income countries. The pattern of this shift toward noncommunicable diseases and injuries has marked regional variations. For example, injuries were the second leading cause of YLL among males aged 15 to 49 years across five geography clusters we examined and the leading cause in Latin America. Among women aged 15 to 49 years, injuries were second to noncommunicable diseases, except in sub-Saharan Africa and India, where the burdens of communicable diseases and maternal mortality are high. Upon analyzing 2013 data by more detailed cause and country, we observed specific regional foci. Road traffic deaths were highly concentrated in West and Central Africa, the Middle East, and Southeast Asia. Deaths resulting from interpersonal violence were highest in Central and South America, South Africa, and Eastern

Table 1. Disease burden in YLL as compared with DAH. Country income levels are classified by the World Bank on the basis of estimates of gross national income, whereas DAH data are from 2010 (*13*).

	Low income		Lower middle income		Upper middle income	
	YLL	DAH	YLL	DAH	YLL	DAH
HIV/AIDS	7.6%	41.6%	3.7%	32.0%	4.8%	41.1%
Malaria	11.2%	14.3%	4.8%	9.6%	0.0%	2.2%
Tuberculosis	3.1%	3.3%	3.5%	6.6%	1.0%	7.0%
Maternal, newborn, and child health	37.8%	17.1%	32.1%	23.7%	8.1%	7.0%
Noncommunicable diseases	20.7%	0.2%	34.0%	1.0%	65.3%	2.9%
Other	19.7%	23.5%	21.9%	27.1%	20.8%	39.8%

Europe (Fig. 2). Contributing to these regional concentrations, alcohol use was the leading risk factor for violence and injuries in Eastern Europe, most of Latin America, and southern sub-Saharan Africa (*5*).

Late adulthood: 50 to 74 years

From 1970 to 2010, life expectancy has increased globally from 56.4 to 67.5 and 61.2 to 73.3 years for males and females, respectively (*11*). With this capacity to live longer and the prolonged exposure to various risk factors, the fraction of deaths from noncommunicable diseases is on the rise. Age-specific rates, however, are tending to decline. In 2013, more than two-thirds of global deaths were attributable to noncommunicable diseases: 8 million deaths were due to cancer; 17.3 million due to cardiovascular and circulatory diseases; 1.3 million due to diabetes mellitus.

After disaggregating the data in the 50-to-74 age group across the six geographic clusters, we again observed regional variation for these noncommunicable conditions. Overall, YLL rates are lower in high-income countries as compared with low- and middle-income countries. Cancer and cardiovascular disease were most prominent in Central Asia and Eastern and Central Europe, although several countries in sub-Saharan Africa, Oceania, and the Middle East also had high burdens of cardiovascular disease. Diabetes among older adults was most concentrated in Oceania, Latin America, North Africa, and the Middle East. These regional hot spots align with findings that high blood pressure is a key risk factor in Central and Eastern Europe, whereas high body mass index is important in Latin America, Oceania, North Africa, and the Middle East (*5*). As the epidemic of obesity continues to spread across the globe, the burden resulting from associated health outcomes (e.g., diabetes) will also continue to rise (Fig. 3). Of the 671 million obese individuals worldwide, 62% live in developing countries (*12*).

Setting priorities in global health

An understanding of the broad trends in local and global health is an important input into the assessment of health priorities. For instance, on a country-by-country basis, it can serve as a critical starting point for national debates on domestic resource allocation and policymaking. However, many other factors should feed into this discus!sion, such as the availability of effective and affordable technologies, priorities for the most disadvantaged, and the tradeoffs between DAH and national resources (*13*). Ideally, health investments (both domestic and external) should be focused on explicit priorities, defined by local authorities and based on epidemiological and economic evidence.

To stimulate reflection on priorities for DAH, it is useful to compare where DAH goes compared to the burden of disease in countries classified by economic status (Table 1). In 2013, DAH reached $31.3 billion, a 5.5-fold increase from 1990 (*14*). Most DAH has gone to infectious diseases and maternal mortality in low- and middle-income countries. However, although noncommunicable diseases and injuries account for a substantial burden of disease, targeted DAH to these conditions is low.

The allocation of DAH may be the best use of resources to improve health of the poor; however, it may also be influenced by underrecognition of the epidemiological transition in many regions. Some global risks are becoming highly prevalent and will have consequences for generations to come. For instance, nearly a quarter of children and adolescents in developed countries are overweight or obese; in developing countries, this proportion has increased from 8.2 to 13% since 1980 (*12*). Because overweight and obesity are clear risk factors for cardiovascular disease and diabetes, this rising global epidemic demands collective action. More research and development of affordable strategies and improved scale-up of proven interventions are needed to tackle risk exposure or adoption. The promotion of healthier lifestyles and stricter fiscal policies for tobacco, sugar, and alcohol are measures that could be more widely implemented.

Descriptive epidemiology is not the only input into setting global and national priorities, but it is an essential starting point. Recognizing regional themes that play out on top of a more universal pattern of the epidemiological transition is important for understanding variation in the epidemiology of disease and for informing the delivery of effective health interventions. Moreover, reliable and updated metrics on the local, regional, and global burden of disease are unique instruments for galvanizing change through evidence-based political commitment. As we transition to the post-MDG era and the new goals to be agreed upon by the United Nations General Assembly (now defined as Sustainable Development Goals), it will be important to understand regional health patterns and to implement policies for sustainable change across the life cycle.

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ACKNOWLEDGMENTS

We thank N. Santos (UC San Francisco) for exceptional assistance in the development of this paper and S. Biryukov (IHME) for analysis of the Global Burden of Disease Study 2013 data.

10.1126/science.1257099

PERSPECTIVE

Getting essential health products to their end users: Subsidize, but how much?

Pascaline Dupas

Although coverage rates and health outcomes are improving, many poor people around the world still do not benefit from essential health products. An estimated two-thirds of child deaths could be prevented with increased coverage of products such as vaccines, point-of-use water treatment, iron fortification, and insecticide-treated bednets. What limits the flow of products from the producer's laboratory bench to the end users, and what can be done about it? Recent empirical research suggests a crucial role for heavy subsidies.

The great majority of households in the developing world cannot afford essential health products at their retail price. This suggests that such products have to be subsidized if coverage is to expand rapidly. There are a number of theoretical reasons why such subsidies are warranted. First, many of the diseases affecting the developing world are communicable, and therefore both prevention and prompt treatment have social benefits (they reduce transmission risk) in addition to private benefits. Second, subsidizing experimentation with products and services can help households learn how valuable these products are.

However, there has been substantial debate about how to implement subsidies. Controversy has been particularly spirited on the question of cost sharing: What share of the cost should be borne by the end user? If this share is too low, some subsidy takers will generate no health gains, either because they do not need the subsidized product or would not use it. Conversely, if the share is too high, some people who would use the product and become healthier do not take it up because they cannot afford it. These conditions are known as overinclusion and overexclusion, respectively. The pricing debate grew out of the prevailing thought that, for most relevant products, subsidies to reduce overexclusion would necessarily create a lot of overinclusion. With bednets, for example, it was thought that when given away for free, many are wasted on people who do not value them enough as health products and instead misuse them—for example, as fishing nets or wedding veils.

Very large subsidies are necessary to increase usage in the short run

A number of recent experimental studies randomized the price at which households or communities could access a preventive health product. Their results suggest that while overexclusion is indeed a concern in the absence of subsidies, the risk of overinclusion when subsidies are introduced is much lower than previously thought.

Demand for many products appears to drop rapidly as the price increases (*1*). In Ghana, take-up of water filters worth $14 (U.S.) fell from 89 to 21% when the price increased from $1.40 to $4.20 (*2*) (Fig. 1). In Kenya, take-up of bednets worth $7 was below 20% for any price above $2.50 (*3*) (Fig. 1). Providing health information about the product typically did not reduce price sensitivity (*4*–*6*).

Because demand falls quickly as the price increases, only heavy subsidies yield high demand. But does this subsidized demand translate into high usage? The second key finding of randomized pricing studies is that it does, because many of those enticed to acquire a product only at low prices actually use it. In Ghana, close to 80% of heavily subsidized water filter owners were using their filter 1 month after they received the subsidy (*2*). In Kenya, 90% of free bednet beneficiaries were sleeping under the subsidized net 1 year later (*3*). Thus, whereas overexclusion is important in the absence of large subsidies, overinclusion under heavy subsidies appears limited (Fig. 1). For each product, the gap between the two ends of the dashed line is a measure of overexclusion at the highest price; the gap between solid and hollow markers at any price point is a measure of overinclusion.

Households do value heavily subsidized products

The third finding from recent pricing studies is that people need not have paid for something to value it. Households are as likely to use their bednet when they get it for free as when they have to pay for it (*4*, *7*–*9*). People are as likely to use their water filter if they paid a lower price for it (*2*). This is surprising for two reasons. First, standard economic theory suggests that charging nontrivial user fees should help target products to those who need them the most—and thus those most likely to put them to appropriate use. But this reasoning breaks down when people face credit constraints and their ability to pay is lower than their willingness to pay. When those who could use a product the most are too poor to pay cash for it, higher prices screen out the poor, not just those with lower health returns from using the product (*9*). Second, it is often thought that the act of paying itself makes people value a

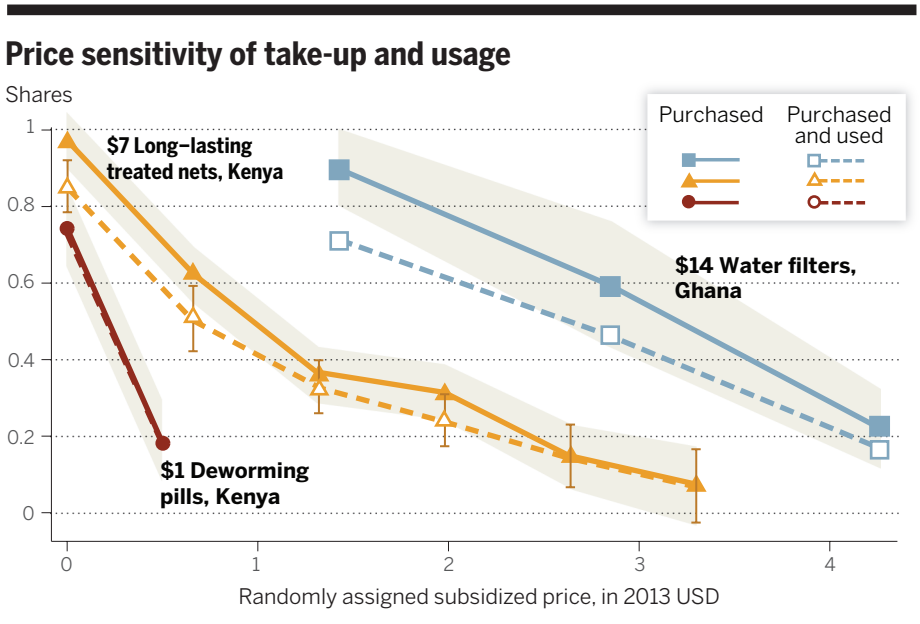

Fig. 1. Price sensitivity of take-up and usage. Usage of water filters was measured after 1 month (*2*). Usage of bednets was measured after 1 year (*3*). Usage of deworming pills is identical to take-up because the pills were placed in children's mouths by teachers (*5*). Shaded areas correspond to 95% confidence intervals for purchase rates. For purchase and usage of bednets, 95% confidence intervals are indicated with capped bars.

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product or service more; the feeling of "guilt" from having spent money on something that is left unused would make people more diligent users. However, experimental studies designed to test the importance of this psychological phenomenon suggest that this consideration is not important when it comes to essential health (*2*, *7*, *10*). The cost of poor health seems a good enough incentive to put products to good use.

When overinclusion becomes a concern

The finding that the risk of overinclusion is limited does not apply to medical treatment. Overtreatment with antimalarials is common, and so is overtreatment with antibiotics, with resulting drug resistance concerns. Heavy subsidies can exacerbate this problem. A pricing experiment with artemisinin combination therapy, a drug to treat malaria, found that 64% of adults ingesting the drug when offered at the heavily subsidized price proposed by Kenya under the Affordable Medicine Facility for malaria did not have malaria (*11*). Overtreatment is driven in great part by the lack of access to reliable diagnostic services and the nonenforceability of prescription-only schemes. The next generation of studies should attempt to solve the problems of how to improve access to accurate diagnostic services in rural areas of the developing world, and how to change the incentives of informal drug outlets—the ubiquitous source of medical treatment in these areas.

Are subsidies good in the long run?

Even for preventive products, where overinclusion is less problematic, it still does not follow that high subsidies are good policies. An important concern that has been raised is that heavy subsidies, by keeping prices artificially low, may create expectations that prices should remain low. This would lower households' willingness to accept price increases and limit take-up once subsidies are lifted, creating a dependency trap. The evidence to date mitigates this concern. A pricing experiment in Kenya found that households randomly sampled to receive a free or highly subsidized bednet were significantly more likely to pay for a similar bednet a year later, despite already owning one (*3*). It appears that the subsidized bednet trial allowed households to learn the benefits of owning one, which increased their willingness to pay for another one in the future. Moreover, there was no effect of having received a free bednet on the willingness to pay for a water treatment product, which suggests that respondents had no expectation of free health product trials becoming the new norm.

Quality concerns

Low demand sometimes reflects poor quality of service. Health providers often operate as a local monopoly, be it the sole prenatal clinic or the sole drug shop, and may not be subject to regular quality controls. This leaves room for agency problems such as corruption and shirking that can undermine the effectiveness of subsidy programs.

Villagers looking at new mosquito nets donated in an effort to fight malaria in Matongo, Zambia, in April 2008. [Photo: Kim Ludbrook/EPA]

One might worry that health workers could be corrupt, delivering subsidized products to the ineligible for a bribe or extorting illegal payments from the eligible. Overexclusion of targeted households because of such corruption is assumed to be deep and wide, but a recent audit study of bednet distribution programs by nurses at prenatal clinics in Ghana, Kenya, and Uganda found limited corruption among prenatal nurses and, as a result, very high coverage rates among targeted beneficiaries (*12*). Corruption may be rife in other distribution programs, however. More research is needed on this issue.

Health workers may shirk, by not showing up to work or by making no effort. Empirical studies from a number of countries have shown alarmingly high levels of provider absenteeism (*13*), as well as low quality for health services in both the private and public sectors (*14*). These can limit the effectiveness of subsidies at reducing overexclusion. Although effort levels were high in the aforementioned bednet distribution programs (*12*), provider absence has been shown to affect take-up of other free services such as immunization and HIV prevention (*15*, *16*).

Concerns over low provider effort have spurred recent research on the effectiveness of incentive schemes for health providers (*17*–*19*), an important topic beyond the scope of this perspective.

Incentives for end users

Sometimes demand remains recalcitrantly low even when the product is free, of good quality, and widely available. This may call for demand-side incentives, which can be thought of as negative prices. In a randomized controlled study in India, where reliable immunization camps were set up in villages, some parents were given a kilogram of lentils per immunization, along with a set of plates per fully immunized child. This incentive scheme led to an increase in full immunization rates from 18% in the control group to 39% in the incentivized group; the cost per shot (including the cost of the incentives) ended up being lower in the incentivized villages, thanks to economies of scale (*15*). Such "conditional" transfers, in cash or in kind, are increasingly common. Many of these programs, primarily antipoverty programs, give mothers cash if they or their children attend regular health checkups. The evidence to date suggests that such cash incentives are effective at increasing take-up of health services and are sometimes effective at improving health outcomes (*20*–*22*). Of course, the health impacts of these incentive schemes depend on the quality of the services and products offered.

Conclusion

A low-hanging fruit for improving health outcomes in the developing world is to increase the coverage of already existing and proven health products and services. Recent field experiments by economists find that for products with large social benefits, free distribution is the most cost-effective strategy for increasing coverage of essential health products and services. It is not always enough, however. In India, child immunization rates remain low despite full subsidies. Additional incentives can make an important difference in this case.

But is spending on individual subsidies and incentives the best use of typically limited government and international funding for public health? The biggest strides in the history of the reduction of child mortality in the United States came from infrastructural investments—in particular, investments in safe water (*23*). Malaria was eradicated from the southern United States and southern Europe, obviating the need to identify optimal pricing policies for individual malaria prevention and treatment products. Institutional pushes are needed for such infrastructural investments to take place in the two dozen countries that account for three-quarters of today's child mortality burden. Until these investments are made, micro-level subsidies and incentives appear necessary.

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ACKNOWLEDGMENTS

Supported by NIH grant P01HD061315-01 and NSF grant 1254167.

10.1126/science.1256973

PERSPECTIVE

Models of education in medicine, public health, and engineering

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Discussion on global health in both the academic and the public domain has focused largely on research, capacity building, and service delivery. Although these efforts along with financial commitments from public and private partners have contributed to a broader appreciation and understanding of global health challenges, the reflection of global health in academic training has largely been lacking. However, integrative models are beginning to appear.

The current approach to global health practice has largely been "on-the-job training" that does not provide a fundamental and sound theoretical basis for the challenges faced by global health practitioners. As a result, although practical experiences help, lack of sound foundations affects the ability to make a lasting impact. In general, there is a growing need to integrate the demands and the local perspective into mainstream education for diverse disciplines in both developing and developed countries (*1*).

Disciplinary education in three key areas of global health—namely, medicine, public health, and engineering—are also evolving to reflect the needs for training in an increasingly globalized world (*2*). We believe that our engineers, clinicians, and public health professionals will be better off in solving the great problems of our time by appreciating the challenges of both resource-rich and resource-constrained environments.

New models of medical education are focusing on integrating systems-level understanding of human physiology and also on integrating medical knowledge with that of nursing and public health. Public health education, on the other hand, which historically has been the domain of medical professionals or epidemiologists, is now increasingly accommodating students and scholars who come from a diversity of backgrounds, including anthropology, economics, sociology, and engineering. Similarly, engineering education is also incorporating more real-world challenges that not only focus on the core concepts but the applications of the concepts in complex environments. Using our personal experiences in developing and implementing new models of education, combined with the vision of innovation in disciplinary education from our respective institutions, our goal in this Perspective is to discuss how these emerging models of education may change the way we train global health practitioners and hence meet the technical capacity needs of the rapidly growing discipline.

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New educational models in medical education

Health and health care are about understanding the individual—their biology, their behaviors, and their predisposition to health or disease. Although enormous gains in health care can be attributed to the traditional model of medical education designed by Flexner over 100 years ago, the challenges of the 21st century will not be met if we do not rethink the way in which we train clinicians and expect them to practice.

It can be argued that globally, we are challenged by a similar set of problems seen through different lenses: Resource-poor countries are challenged to design systems that can provide access and quality, whereas resource-rich countries are challenged to design affordable systems that can sustain access and quality. What is the role of a medical student or a clinician in this environment? How can modern medical education train medical professionals to address these challenges comprehensively, while continuing to promote cutting-edge research, innovation, and quality care?

The opportunity to innovate and drive convergence to a common health and health care platform is the challenge for the 21st century. Achieving this requires educational programs that encourage a new type of medical graduate: one who understands systems beyond the individual organ; understands disease and cure in context; and has developed the knowledge, skills, and tools to innovate, seeing the global context as a resource for understanding and ideas rather than as simply "developing countries in need."

The need for rethinking medical education globally has been echoed in several high-level reports, including the 2010 Lancet Commission on Health Professional Training for the 21st Century (*3*). The 2010 report argues for a transformational change in health-professional education that focuses on a global-perspective, systems-thinking, transprofessional education that is competence-based, and close alignment of the

education system with the health care delivery system. This requires a fundamental shift in the way we structure the education of health professionals across disciplines and specialties. An interdisciplinary approach that incorporates key concepts of population health, provides a perspective of multiple specialties, and provides room for technological innovation and field-based experiences is a departure from the existing models of medical education that are tightly packed and leave little flexibility for experiences outside the confines of highly structured curricula.

Existing institutions often create barriers to transformational thinking, and the required shifts can be difficult if not impossible to achieve. Schools of medicine, nursing, and public health are often physically, theoretically, and operationally distant; students and faculty have reduced opportunity to interact and to share common problems from differing perspectives. Team-based training within a global perspective is often absent or limited, and the power and importance of this training is underrecognized.

The Aga Khan University and the University of Toronto are testing new models for courses that bring together students from various disciplines across geographic boundaries using blended learning strategies. The goal is to create a new capacity of scholars who are on one hand rigorously trained, and on the other aware of the tools of other disciplines, so that they are able to provide the right care at the right time.

The current trial links 4th-year undergraduate science and arts students at the University of Toronto with advanced nursing and medical students in Tanzania and Uganda and education students in Pakistan in a common course that relies on case-based problems built around a shared resource (Science of Early Child Development, www.scienceofecd.com). Such "global" models of courses focus around areas of common importance, such as child development, pediatric disorders, and health systems design.

Nearly all specialties are engaged, in some form, in dealing with global health issues. Yet, this engagement often seems disconnected from learning opportunities for residents or fellows. Elective opportunities do exist within many programs, but these tend to be based on individual needs or desires, are typically underresourced, and do not focus on core global-health learning objectives. There is great potential to be gained by more formalized course content during residency and linkage, where possible, to bilateral exchange. These exchange opportunities are often highly valued by residents but are frequently more ad hoc in their organization and experience. Although "clinical medicine" is often the focus of this exchange, there are equally important learning opportunities related to the health systems within which clinical medicine is delivered.

Resource-poor countries that are beginning to invest more heavily in medical education have the opportunity to "skip" the past century's models and to explore innovative and potentially transformational models that can be built from scratch and have impact within their country but also add new knowledge to the global challenge (*4*). Greater community-based emphasis that better aligns training to needs, the building of educational and health systems that integrate public and population health and health care, and education and models for training that use and leverage the existing public health system are all areas of important potential innovation. Use of mobile technologies has allowed materials to be archived in an affordable way, increased access to new information, and provided students with an opportunity to understand current mobile and e-health paradigms and challenges. If properly designed, implemented, and evaluated, these models can and will affect thinking globally.

The Aga Khan Development Network (AKDN; www.akdn.org) is working to achieve such an integrated model of medical education in East Africa. As a private not-for-profit development organization, the AKDN is building an integrated health system in East Africa and linking this to a Faculty of Health Sciences that is committed to a transformational education model. A unified Faculty of Health Sciences across disciplines will focus on competence-based and transprofessional training. In the first 2 years, medicine (6-year program) and nursing (4-year program) students will start with a general arts and sciences core curriculum focused on establishing a common language of understanding and critical thinking. Subsequent professional training years build on principles of population health, human development, ethics, professional practice, and health systems function. From the first year, students will have interdisciplinary learning opportunities within community settings that will integrate these concepts through direct care, program development, and research projects. Building international relationships that promote global learning will be an important strategy within the curriculum.

"The opportunity to innovate and drive convergence to a common health and health care platform is the challenge for the 21st century."

Models in global public health education

Global public health is a relatively new discipline, dealing with entire populations and aiming to promote health and interventions that could improve the quality of life of society as a whole. Historically, global public health professionals were largely trained as clinicians with expertise in infectious diseases. In addition, in many countries health professionals are trained within a disease and curative approach, which leads to poor adaptation in the face of new and complex challenges that may be rooted in a complex web of medical, socioeconomic, religious, and cultural issues. This has been true in such diverse global problems as AIDS and obesity. The recognition of the existence of social determinants of health are often beyond the scope of current training provided to clinicians. Public health training for clinicians also comes at a cost, both to the trainee and the system. The current models of training adds additional years, which takes the clinicians away from their ability to practice. However, emergence of new challenges has provided us with the realization of our collective interdependence and the need for multidisciplinary approaches.

The emergence and intensity of global health concerns have increased at a rapid pace. In this new context, it is necessary that professionals be capable and trained to work in evidence-based policy development and ready to take prevention actions and interventions adapted to the concrete local realities. We envision future global public health professionals as agents of change, capable of understanding social, geographic, economic, and cultural determinants of health with a vision not of individuals but of populations.

The Universidad Peruana Cayetano Heredia is the first in the Latin American region to acknowledge the gap in training and has created a new 5-year, integrated bachelor degree in Public Health and Global Health (Fig. 1). The development of this program has been guided by the recommendations of the Lancet Commission on Health Professional Training for the 21st Century (*3*) but has been adapted to local realities and public health challenges specific to the region. The integrated curriculum includes (i) a competence-based curriculum; (ii) three integrated levels of learning: informational (acquiring knowledge and skills), formative (promotion of values and professional ethics), and transformative (development of leadership skills); (iii) local realities as well as global knowledge components, including new research developments in medicine and public health; (iv) technologies to enhance learning; and (v) an evaluation component, so that the program can be assessed and learning outcomes can be shared with other regional and global partners. The learning outcomes are a list of metrics to evaluate short-, mid-, and long-term outcomes of the training (such as scholarly activity, academic performance, jobs, entrepreneurship, and impact on policy) via quantitative and qualitative assessment tools.

Research is a core foundation of the program, with experiential rotations in the public and private sectors within and outside the country. These rotations include internships in local municipality, local hospitals, regional health policy think tanks and directorates, international agencies such

as the United Nations Children's Fund (UNICEF) and World Health Organization (WHO), as well as private health insurance companies. Through a thesis and additional 9 months of training, students are also able to achieve a master's degree in Public Health and Global Health.

New models in engineering education

Historically, engineering education—particularly, biomedical engineering education in the United States and the developed world—has focused on integrating the medical challenges of the developed world in the curriculum. Over the past two decades, this has been a highly successful model, with the creation of new technologies, new private ventures, and overall improvement in the functioning of medical facilities. However, with the exception of just a handful of select curricula (for example, the Beyond Traditional Borders program at Rice University, http://rice360.rice.edu/btb) (*5*), the biomedical engineering curriculum nationally and internationally reflects a focus on core engineering principles and their applications in societies that are not resource-constrained. Students graduating from these programs are therefore not only unequipped to fully comprehend challenges in the developing world, they are also not cognizant of ethical and policy challenges of the resource-constrained environments.

The ability of the engineering curriculum to adapt will have major implications for the intellectual capacity of engineering graduates. First, it will allow students, regardless of their future pursuits, to develop an appreciation for new paradigms of design and implementation in environments beyond the developed world. Second, these models of curriculum will have a better chance of scaling and reflection in engineering institutions of the developing world, which currently suffer from curricula that do not address their local challenges. Third, the curricula will also enable more engineers to engage with their public health colleagues, just as in the past two decades engineers have worked closely with clinicians. This would create a new class of entrepreneurs who will be able to develop solutions optimized for large populations in the developing countries with growing economies. Additionally, more engineers and quantitative scientists will enter the domain of global health and allow for a richer understanding of our current global challenges.

Success of any rigorous program, in the short and the long term, will need three key ingredients: (i) early exposure to complex challenges; (ii) incorporation throughout the curriculum of hands-on design for resource-limited situations; and (iii) application of the knowledge and tools from traditional courses to specific, real-world problems. For example, a course in which biomechanics is traditionally studied through understanding stresses on beams and rods can instead use the same principles applied to the design of a crutch for a disabled youth in Zambia, using the resources available in country. Current engineering programs that focus on global health typically incorporate one of the three ingredients but do not focus on all or are unable to integrate them over the entire duration of the program. A more integrated curriculum that introduces and reinforces the key concepts at multiple points and then enables students to apply their core knowledge to address real-world challenges through traditional approaches of problem sets, projects, and papers can have a substantial impact in developing knowledge and making discoveries to solve high-impact global health problems.

These new models of education in engineering, public health, and medicine are not without their critics. Two major criticisms are often brought to the debate. First, it is argued that focusing on interdisciplinary topics leads to better breadth but poor fundamental depth. This criticism is inherent in most interdisciplinary studies, and some of it is fair. However, the rigor of any curriculum should not depend on the examples or the sociocultural context that are used to illustrate the fundamentals. The quality of instruction, the depth of the material, and the appreciation of real-world applications all need to be integrated into the curriculum. The second criticism reflects reluctance based on possible career paths and opportunities. However, our argument is not about exclusivity but instead about inclusion of newer problems and realistic scenarios. In many ways, the career paths for those who are keenly aware of socioeconomic realities of the world are brighter than those who are trained in silos.

In the end, we as practitioners of global health in our respective domains believe that new models of education are needed to recognize the evolution of both the society and its medical challenges. As with any academic discipline that is growing in popularity and intellectual depth and breadth, interdisciplinarity and social realities need to be incorporated in the global health curricula in order to maintain relevance and the ability of the graduates to perform the tasks they are trained for in dynamic and complex environments.

Fig. 1. Practical experiences. Fieldwork conducted by public health students from Universidad Peruana Cayetano Heredia at a slum in Lima, Peru, for their course "Basic Concepts in Global Health." The students are from multiple disciplines, including public health, veterinary medicine, sciences, and psychology. [Credit: Patricia Garcia]

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10.1126/science.1258782

PERSPECTIVE

Prioritizing integrated mHealth strategies for universal health coverage

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As countries strive toward universal health coverage, mobile wireless technologies—mHealth tools—in support of enumeration, registration, unique identification, and maintenance of health records will facilitate improved health system performance. Electronic forms and registry systems will enable routine monitoring of the coverage of essential interventions for individuals within relevant target populations. A cascading model is presented for prioritizing and operationalizing the role of integrated mHealth strategies.

The goals of universal health coverage (UHC) are shaping the global health agenda (*1*, *2*), emphasizing that all people, irrespective of socioeconomic status, should have access to health services they need, without incurring financial hardship. These ideas, echoing the 1948 Universal Declaration of Human Rights, were reinforced as a central strategy for global health in the World Health Organization (WHO) 2010 World Health Report and a 2012 United Nations General Assembly Resolution (*3*, *4*). Achieving UHC, while addressing proximal and distal contributors to poor coverage and quality of care, often requires rectifying decades-old dysfunction in supply-and-demand aspects of health systems. Creative financing and private-sector engagement may offset some basic health service costs but are unlikely to do so at the scale required to cover the millions who need care. Innovations are required that improve unrestricted access to, and full use of, affordable quality services.

Meanwhile, for two decades, plummeting costs have led to nearly ubiquitous access to telecommunications technologies (*5*); this has occurred independently of the global health or development communities, driven by market forces and consumer demand. The ability of even simple phones to connect to complex digital systems is proving transformative for low- and middle-income country (LMIC) populations (Fig. 1). As health program implementers struggle to scale up and integrate public health interventions of known efficacy (such as vaccines, micronutrients, and skilled birth attendance), strategies leveraging mobile wireless technologies—mHealth—are increasingly part of a systems-thinking approach to resolving these challenges (*6*).

Global health agencies are beginning to advocate the prudent use of mHealth solutions, guided by evidence demonstrating their usability, functionality, reliability, and impact under real-world conditions (*7*). Country-level health information systems that enable national aggregate record-keeping and facility-level medical records have been facilitated by low-cost, open-source robust systems such as District Health Information Systems (DHIS2) and Open Medical Record Systems (OpenMRS). Enterprise-grade mHealth systems are also gradually emerging. The past 5 years have seen substantial maturation of the field, with at least three WHO-led initiatives [mHealth Technical and Evidence Review Group (mTERG), eHealth Technical Advisory Group (eTAG), and the International Telecommunications Union–WHO Mobile Health for Non-Communicable Diseases Initiative], accompanied by numerous other mHealth communities of practice. Several hundred randomized trials are under way worldwide, measuring the efficacy of mHealth strategies (*8*). However, most of these innovations remain vertical in their approach; that is, addressing single problems faced by health systems. A framework to help prioritize investments and opportunities for collaboration has been absent, especially given the complexity of health systems, where the receipt of quality care by clients is often contingent on several preceding layers of enabling conditions. Knowing who is in need of services, having the necessary human resources and commodities in adequate supply, and connecting these together at the right time and place are elements critical to success. Government agencies have been slow to consider innovations that help with each of these challenges, such as those being developed in the mHealth space, perhaps because the harmonization of innovations across these health system layers is a daunting task. Drawing from seminal work in health systems analysis, we provide a novel framework for visualizing and planning how mHealth innovations can be applied to help surmount common and persistent constraints along the pathways toward the achievement of UHC goals.

Fig. 1. D-tree International nutrition program to identify and treat early childhood malnutrition, Urban District, Zanzibar. [Credit: Mark Leong/World Health Organization]

People-centered integrated care requires numerous coordinated inputs. In 1978, Tanahashi (*9*) proposed a cascading model to illustrate how health systems lose performance because of bottlenecks at successive levels, each dependent on the previous layer. Gaps in information, training, quality, and equitable distribution contribute to accumulated losses of potential, leading to diminished health system performance. Tanahashi's model helps identify opportunities for improvements in particular strata of the system, with cascading benefits to each layer above it (*10*). As mHealth moves from an era of experimentation—dominated by targeted vertical solutions—toward cross-sectoral health systems that integrate numerous mHealth strategies, we have adapted Tanahashi's model to illustrate where digital investments can have the greatest impact toward the achievement of UHC. Previously, we proposed that mHealth innovations be viewed not as a direct way to achieve a health outcome but as a strategy to overcome obstinate barriers to the delivery of known efficacious interventions (*7*). This reframing allowed researchers to focus on measuring the catalytic role of mHealth tools: how a mobile intervention was able to improve efficiency or the coverage of an intervention, such as childhood vaccinations, that has previously been shown to save lives. Although this helped bridge the dialogue between mHealth innovators and health systems policy-makers,

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frustration remained because of the proliferation of these solutions without a roadmap to guide their integration into health systems. One can imagine the chaos that would be created by 100 architects trying to repair a Victorian mansion without a blueprint. Our modification of Tanahashi's model extends this approach to illustrate how integrated mHealth systems solutions can be leveraged to improve existing implementation plans and reduce health service bottlenecks, to realize improvements in the quality, cost, and coverage drivers necessary for UHC (Fig. 2). In essence, we drew from a time-tested conceptual framework that captures the complexity and interdependences of health systems to build a new guide for policy-makers to conceptualize how distinct mHealth strategies, even if developed independently, could be integrated and applied together, simultaneously strengthening the various layers of a health system.

Increasing accountability—capturing denominators

Population enumeration, which is the responsibility of government, poses a challenge to constructing a true denominator of those to whom the health system is accountable. Paper-based civil registration systems have degraded; few LMICs can claim universal citizen registration. Routine paper registers, used to profile and track client subpopulations and account for services, often contain suspect data. Systems are typically slow to aggregate data and extract disease trends or performance patterns, resulting in inefficient or delayed health system responses. Equitable delivery of health services by frontline health workers (HWs) often relies on highly localized microcensuses of specific target populations (such as pregnant women).

In Fig. 2, the "total population" represents all individuals, known and unknown, in an area; "target population" is a subset characterized by demographic or health profile, defining eligibility for specific interventions (such as infants receiving vitamin A supplementation). "Accountability coverage" is the proportion of those in the target population registered into the health system; e.g., through civil registration and vital statistics (CRVS) mechanisms, population census, issuance of a national or health identifier, or electronic medical record (EMR).

Because total and target populations are extant but unknown quantities, accountability coverage becomes the point of entry for mHealth interventions. mHealth tools can bolster population enumeration and CRVS systems in remote areas (*11*), even at national scale, as in Uganda (*12*), where community-based volunteers report births via mobile device to the appropriate civil agency, which verifies the information and issues a birth certificate. HWs in India and Bangladesh use tablet-based Open Smart Register Platform (OpenSRP) and mCare systems that connect registers with EMR systems and, through the use of shared unique identifiers, facilitate continuity of care across providers and facilities.

Registration and EMR approaches, combined with electronic forms for profiling client health status, establish a common denominator that can be shared between civil and health systems, as well as tools to document individuals' health profiles and service needs and the ability to monitor and respond promptly to epidemiological trends. Digital reporting offers supervisors real-time insight about performance issues and opportunities for responsive actions to time-sensitive information.

Improving supply of services

In most LMICs, poor finances, inadequate facilities, a strained health workforce, and aging supply management operations have resulted in health systems struggling to meet populations' needs. Clients may encounter a lack, or poor quality, of supplies ("availability of commodities and equipment"); insufficient, unmotivated, or

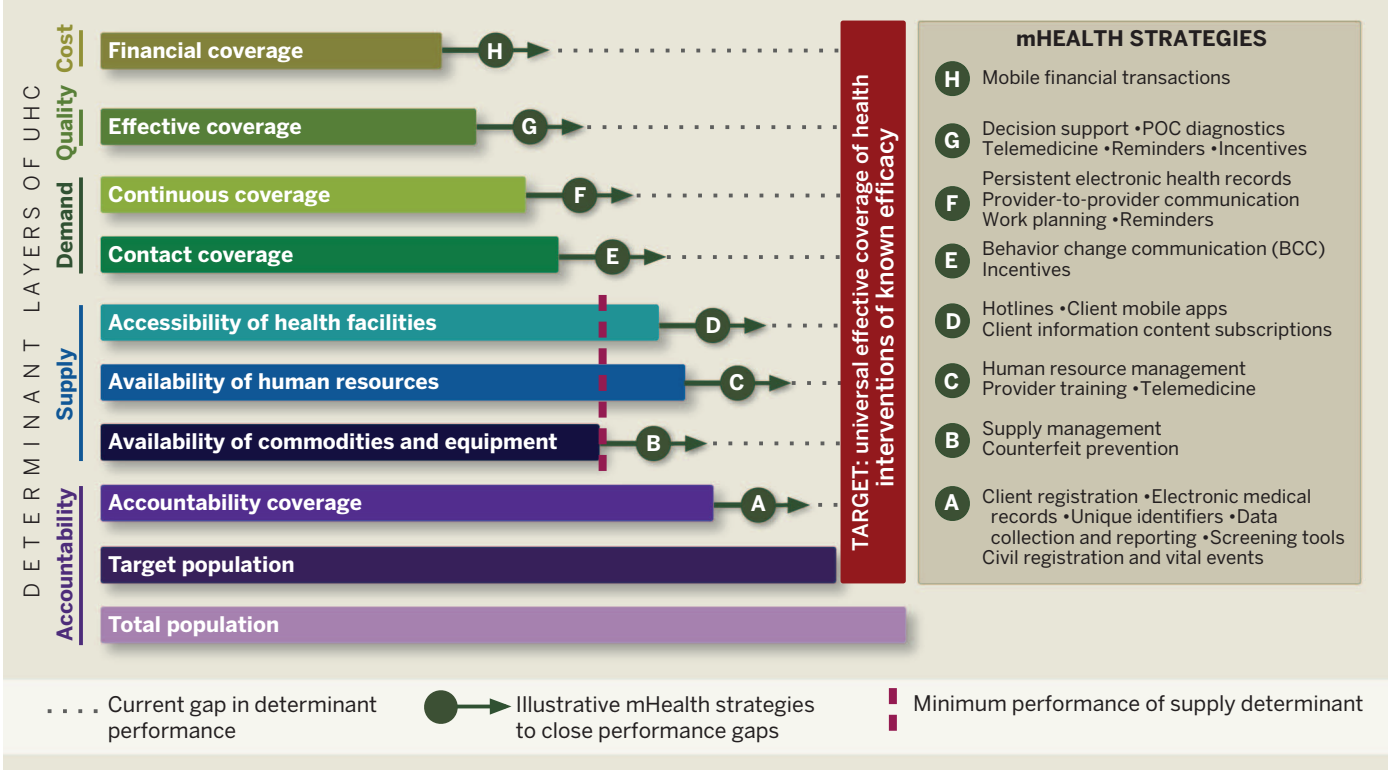

Fig. 2. A cascading model to prioritize and select integrated mHealth strategies for achieving UHC [adapted from (*7*) and (*9*)]. This framework shows how eight key ingredients are required before a target population (a subset of the total population at the bottom of the cascade) can all receive the quality, timely, and adequate care they need. These components of health system performance are grouped into categories of accountability, supply, demand, quality, and cost. As illustrated, each layer not only builds on the performance of the layer below it but also falls short (dotted lines) of the optimal, desired level. A suite of mHealth strategies (labeled A through H) exists that could contribute to efforts in strengthening any given category. This framework helps facilitate dialogue between mHealth innovators and health system policy-makers to strategically plan how independently developed solutions can work in an integrated approach to addressing health system challenges across numerous layers.

dysfunctional health staff ("availability of human resources"); and lack of "accessibility of health facilities"—any of which would hinder the supply of appropriate services.

mHealth innovations, in the form of text messaging with linked Web dashboards, have been used to track essential supplies and reduce the time for restocking at health centers. Sproxil and mPedigree enable HWs and clients to scan barcodes to verify counterfeit and expired commodities; in Tanzania, mHealth supply-chain management tools reduce stockouts of antimalarials (*13*).

mHealth tools targeting the availability of an effective health workforce focus on training approaches, incentive mechanisms, and supervisory support to strengthen skills and enforce compliance with standards. Training and in-service refresher content [for example, eMocha TB Detect's multimedia content for continuous medical education (*14*)] can strengthen HWs' ability to provide quality care. In Rwanda, mHealth tools facilitate documentation of service delivery, providing real-time data streams designed for benchmarking and data-informed feedback by supervisors.

Many barriers prevent or delay clients from accessing needed information, commodities, or other services in a timely manner (*15*). To reduce barriers to accessing services, mHealth strategies use behavior change communication content for client health engagement and mechanisms for remote diagnosis and management of illness. Tools include phone apps for client self-management; hotline and interactive voice response (IVR)–based approaches that facilitate client-initiated, structured information content retrieval; and access to counseling, diagnostics, and referral mechanisms.

Driving demand, increasing access

Even where health interventions are available, there may be barriers to target populations accessing them. "Contact coverage" is the proportion of clients who have contact with relevant facilities, providers, and services among the total population requiring a health intervention. Typically, this reflects a difference in the characteristics defining the available interventions and the target population's expectations, mediated by barriers including affordability, acceptability, and client/provider interaction experiences. Variations in demand or ability to access services within a target population may be characterized by stratifications of age, social or economic status, geography, and gender. To improve access to information, some mHealth strategies provide personalized message content. In South Africa, the MAMA project employs multiple mobile communication channels (voice, text message, IVR, Mxit, and mobile Web) to inform pregnant women from different social strata about appropriate nutrition and to remind them of antenatal care (ANC) practices and available services.

Health interventions often require multiple interactions with the health system for maximum effect. For instance, WHO recommends that pregnant women have at least four ANC visits, a mark seldom achieved in LMICs. "Continuous coverage" refers to the extent to which the full course of intervention required to be effective is achieved. mHealth strategies that support client-side reminders and provider-side alerts for health interventions, such as vaccinations linked to specific schedules, help reinforce timely health services.

mHealth strategies have focused on streamlining health providers' client management tools. HWs with the Ananya Project in India, for example, use mobile phones to register clients, record service delivery information, and consult a client's medical history. Using unique digital identifiers, client health data are synchronized between phones of multiple HWs, and providers receive notifications when scheduled cases are missed, further ensuring continuity of care.

Woman receiving financial voucher confirmation message, Changamka Microhealth maternal health voucher payment program, Kenya. [Credit: Mark Leong/WHO.HRP]

Enhancing quality, improving efficiency

For health interventions to offer maximum benefit, they need to be administered following evidence-based guidelines, representing minimum standards of inputs and processes. "Effective coverage" refers to the proportion of individuals receiving satisfactory health services, among those in need (the target population) (*16*). Health interventions that are delivered suboptimally, such as when health providers are inefficient or fail to abide by treatment protocols, result in reduced effective coverage.

In LMICs, ensuring effective coverage is inherently challenging. mHealth tools that reinforce best practices, or facilitate beneficiary feedback on service quality, can mitigate bottlenecks that compromise the potential of interventions (*17*). mHealth decision support algorithms, such as electronic Integrated Management of Childhood Illness, guide frontline HWs in step-by-step diagnosis and management of diseases (*18*). In Zambia, mHealth technology improves access to cervical cancer point-of-care screening; digital cervicography informs consultative telemedicine expert review determining the need for immediate referral or cryotherapy treatment (*19*).

Telemedicine and closed-network provider-to-provider communication mHealth solutions such as Switchboard work to eliminate obstacles preventing providers from seeking supportive guidance or arranging patient referrals. mHealth provider-to-patient approaches facilitate free communication (voice and messages), enabling direct follow-up about specific care regimens or services.

Ensuring protection from health costs

Direct and indirect financial costs can have catastrophic financial effects (for instance, for the uninsured). "Financial coverage" is the proportion of patients protected from impoverishment due to health interventions.

Across sub-Saharan Africa and Asia, mobile phone–based banking has spawned microinsurance and incentive schemes, leveraging technology to connect health and financing. Mobile money reduces financial barriers: It can incentivize clients to seek health care, subsidize transportation to health facilities, ensure treatment completion, and encourage savings to offset health costs. In Madagascar, Marie Stopes used mobile phone–based short message service money transfer systems to more efficiently deliver vouchers to reimburse service providers, offset client costs, and increase poor people's access to voluntary family planning services (*20*).

Conclusion

For mHealth strategies to be recognized as integral to the achievement of UHC, the field needs to invest intellectual and financial resources to move beyond vertical solutions addressing single problems. Alongside targeted, single-focus innovations, integrated evidence-based packages of interoperable solutions must emerge, optimizing health systems across multiple determinants of UHC. Such "best of breed" agglomerates may be championed by single public- or private-sector entities, such as a corporate solution provider or health agency, or be fostered through the adoption of global standards. Granted, horizontal solutions are seldom easy to develop or adopt, especially in the public sector, but guiding frameworks such as this one can help governments set realistic expectations and prioritize investments across critical health system layers. Likewise, in the absence of targets for integrated mHealth strategies, donors will continue to invest primarily in siloed innovation, neglecting comprehensive approaches with real potential for scale. A coordinated donor investment mechanism, such as the CRVS Scaling Up Investment Plan and Trust Fund, is needed for mHealth to ensure that governments have the

means to guide mHealth into this next era of maturity, with integrated solutions becoming the norm. WHO and other stakeholders will need to issue guidance to help prioritize and accelerate government mHealth adoption. Already, multiple efforts are under way to synthesize evidence, from WHO's mTERG to USAID's periodic Evidence Summits. In the future, these efforts could be guided by this framework to direct strategic investment toward key foundational layers of struggling health systems in an integrated manner. Our modified Tanahashi model facilitates a systematic approach toward constructing integrated mHealth strategies that together address multiple gaps in the pathway to UHC, improving performance in the quality, cost, and coverage necessary to provide care to all in need.

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ACKNOWLEDGMENTS

The authors alone are responsible for the views expressed in this article, and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated. The authors thank T. Seimon, L. Gonsalves, T. Tamrat, L. Say, S. Vanderpoel, N. Tran, V. Chandra-Mouli, C. Bailey, and the anonymous reviewers. G.M. acknowledges support from the Bill and Melinda Gates Foundation, the United Nations Foundation, the Wellcome Trust, and the Norwegian Agency for Development Cooperation.

10.1126/science.1258926

PERSPECTIVE

How to transform the practice of engineering to meet global health needs

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More of the world's population has access to cell phones than to basic sanitation facilities, a gap that can only be closed if the engineering and international aid communities adopt new approaches to design for scarcity and scalability.

Engineers have known how to produce safe drinking water and how to build toilets and roads in developing countries for more than 100 years. Yet, global access to such technologies is far from uniform. Approximately 768 million people do not have access to safe drinking water; 2.5 billion lack basic sanitation, and 1 billion practice open defecation (*1*). More than 50% of people who have no access to water and sanitation live in middle-income countries (*1*). Use of these technologies can mean the difference between life and death; diarrheal illness, 90% of which is related to inadequate access to clean water and sanitation, kills more children under 5 than AIDS, malaria, and measles combined (*2*).

Why is it so difficult to translate technologies that have improved public health in wealthy countries into solutions that equitably improve lives around the world? It is primarily because these solutions were developed to satisfy constraints of high-resource settings. In many cases, they cannot be easily adapted to work in low-resource settings; they are too expensive or rely on infrastructure or expertise that does not exist. For example, a recent survey of anesthetists in Uganda reported that only 20% had a constant supply of electricity for the equipment necessary for basic surgery (*3*). Between 2005 and 2011, the President's Emergency Plan for AIDS Relief (PEPFAR) invested over $1 billion to strengthen clinical laboratories to improve HIV/AIDS care, primarily in sub-Saharan Africa (*4*); yet maintenance and repair of the necessary laboratory equipment, designed for high-resource settings, is a continued challenge across PEPFAR countries (*4*), where intermittent power can render equipment unusable, and there is limited in-house technical support to repair medical equipment (*5*). If we are to resolve global inequities in access to innovations that improve health, we must adopt new approaches to engineering design that reflect the unique needs and constraints of low-resource settings.

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Design for scarcity

Engineers design new technologies to meet societal needs in the face of economic constraints; in contrast, frugal design—designing through the lens of scarcity—begins first with the assumption that material and human infrastructure are limited and not systematically integrated. These resource and infrastructure limitations dictate the constraints that frugal designs must satisfy but may also lead to reuse or repurposing of available commodities in ways that are not anticipated. For example, early efforts to scale up provision of injectable vaccines in low-resource settings led to a wave of unsafe injections, where disposable syringes were reused. It has been estimated that as many as 30% of injections in low-resource settings are unsafe because of reuse of syringes (*6*); this practice continued despite efforts to educate practitioners about the dangers of reusing disposable syringes. A "cultural resistance to waste" drove continued reuse of syringes, "regardless of training, advocacy, and regulatory factors" (*7*).

Next, it's important to engage users early. Projects pursued from the perspective of adapting high-resource design principles to low-resource settings without firm evidence of user need beg the question of adoption and can lead to one-off projects that are scaled on the basis of donor priority without evidence that they improve outcomes. For example, the nongovernmental organization (NGO) PlayPumps (Fig. 1) was initially heralded for its ability to use children's play on a merry-go-round to provide a much-needed community service: pumping of water to a community storage tank. With relatively little target community feedback, the U.S. government and other donors committed $16 million to scale up the implementation, and PEPFAR announced a plan to raise an additional $44 million. However, it quickly became apparent to users that, not only were the spare parts and technical expertise required to fix the PlayPump difficult to find, but also that the 27 hours of playtime needed to meet the required minimum daily water requirement was simply infeasible (*8*). In the end, the community users preferred the efficiency and reliability of traditional hand pumps.

Finally, there are numerous design trade-offs to be made in developing and scaling frugal technologies; rigorous experimentation is required to understand which features are most important to support positive impact at scale. For example, the rapid growth in global access to cell phones provides an opportunity to use mobile phone technology to improve health in low-resource settings. Yet, despite more than 500 pilot studies of mobile technologies for health (mHealth), there is still not sufficient programmatic evidence to inform scale-up (*9*). In most pilot studies, investigators treat the mHealth application as a "black box" and examine the effects of using or not using the intervention. As a result, there are no data to indicate which individual mHealth features might be most effective. There is an important need for multifactorial pilot study designs that identify and empirically test a range of features that might contribute to variation for a particular application (*9*).

Design for scalability

Although successful frugal design begins with constraints that are dictated by scarcity, it must also integrate this perspective with a systems-level approach centered on how new designs can be successfully implemented, scaled, and sustained. The frugal design cycle begins with a user-centric focus that accounts for the available infrastructure and economic resources. Scale-up requires evidence of efficacy at the community and national levels. Whereas aid from the international community can help jump-start efforts to scale up a project or program, business development and private sector partnerships are required to sustain implementation. Ensuring long-term access to new health technologies requires a coordinated architecture that integrates efforts to make new technologies affordable, makes certain the technologies are available where they are needed, and facilitates adoption of the technologies within health systems (*10*).

Developing a compelling value proposition requires both data to support the health benefit of a new technology and identification of a paying customer—a significant challenge within the context of the world's poorest health systems. Yet, there are examples of success. Unsafe injection practices have been dramatically reduced by developing new injection technologies that cannot be reused. One example is Uniject, a blister pack prefilled with the proper dose of vaccine and connected to a needle via a one-way valve that prevents the device from being refilled and reused (*11*). By eliminating the need to properly fill a syringe, Uniject simplifies injection practices for users like midwives and community health workers in rural communities. Tests of the device to deliver the hepatitis B vaccine to newborns in Indonesia showed that use of a prefilled device simplified logistics of vaccine delivery immediately after birth by midwives, reduced vaccine wastage, and was preferred by midwives and mothers (*6*).

The role of the international and business communities was central to the development and scale-up of Uniject. Indeed, the technology was developed in response to a 1987 meeting convened by the World Health Organization (WHO) to highlight the challenges of unsafe injection practices. With support from the U.S. Agency for International Development (USAID), the NGO PATH worked to improve technology originally developed by Merck. Merck transferred its intellectual property rights to PATH, which licensed the technology to Horizon Medical and went into pilot production of the Uniject device. On the basis of the positive results of early implementation trials, the Uniject technology was licensed to Becton Dickinson & Company (BD) in 1996 (*11*). BD invested $25 million to establish a dedicated manufacturing line for empty Uniject packages in Singapore and $10 million to launch the product on the global market; today, vaccine manufacturers buy empty Uniject containers and prefill them for global distribution (*11*). Since 2000, millions of doses of hepatitis B and tetanus vaccines have been delivered with Uniject, and efforts are under way to use Uniject to expand access to injectable contraceptives in low-resource settings (*12*). Through a partnership with Biodel, Uniject is now being developed for emergency delivery of liquid glucagon to treat severe hypoglycemia, where a simple, portable solution is of particular importance for first responders or parents of children with diabetes.

"If we are to resolve global inequities in access to innovations that improve health, we must adopt new approaches to engineering design that reflect the unique needs and constraints of low-resource settings."

Yet, the technology still faces the challenge of articulating an effective value proposition for purchasers in health systems, who are often evaluated on the basis of their ability to maximize the number of lots of vaccine they can purchase with a fixed sum. The incremental cost to deliver prefilled vaccine in Uniject packaging is approximately $0.06 higher per immunized child than with disposable syringes (*11*). Although Uniject packaging is more costly than disposable syringes, its use for newborn hepatitis B vaccination saves money when one takes into account reductions in vaccine wastage and costs of home visits. Nonetheless, critical gatekeepers often resist suggestions to purchase Uniject because of higher initial costs (*11*).

Template for success

Design simple solutions

Sometimes inexpensive, nontechnical solutions are best. Roughly 1.3 million people die annually in road traffic accidents, 90% of whom live in low- and middle-income countries. The number of deaths due to road traffic accidents is anticipated to double by 2030, rising to the third leading cause of global mortality; most of this increase will occur in low- and middle-income countries, where the number of motor vehicles is projected to increase sixfold without improvements in road infrastructure or traffic safety (*2*). Modifying driver behavior is an inexpensive alternative to building better road systems. For example, in an experiment aimed at examining the influence of social pressure on driver safety in Kenyan minibuses, signs were posted in half of a fleet of vans encouraging passengers to collectively speak out about unsafe driving practices (*13*). When compared with the control group, passengers riding in vans with signs filed about one-third as many insurance claims, and injury and fatality claims dropped nearly 50%. Behavior as a frugal design solution is low cost and easily adapted to different contexts, which makes it highly scalable.

Don't overlook traditional solutions

Investments to eradicate malaria have resulted in dramatic reductions in mortality, as much as 42% globally since 2000, with child mortality rates in Africa dropping by nearly 54% during the same time period (*14*). But with this has come increasing resistance to antimalarial medicines and heavily used insecticides such as pyrethoids. With the likelihood that new drugs are still many years out, environmental management could emerge as a key means of vector control. In the early 20th century, engineers worked with malaria control personnel to manage the mosquito population through environmental design features, many of which still show efficacy. For example, mosquito-proofing houses and better water management and irrigation methods have been highly successful at helping to reduce the incidence of malaria (*15*). This low-cost approach to governance, combining simple water resource management together with public education, can be successfully applied globally. In places like California, outreach is now emphasizing environmental controls: the elimination of standing water and using biological control measures (e.g., mosquito larvae–consuming fish) (*16*).

Think long-term, while solving short-term

Point-of-use water treatment with chlorine is widely considered one of the most effective strategies for providing safe drinking water in water-scarce settings (*17*). As much as a 29% reduction

in diarrheal illness in children was seen with point-of-use chlorine treatment compared with traditional disinfection methods, a protective effect that was nearly universal across populations and conditions in short-term trials. But with rapidly increasing urbanization, it might be more efficient to begin to extend design innovation to technologies that increase the production of potable water through reuse, which would also help to address water scarcity. In Windhoek, Namibia, highly treated reclaimed water has been combined with potable water directly in the water distribution system since the late 1960s (*18*). The reclaimed water meets all drinking water standards, which makes it a viable option under both financial and water provision terms.

Engage students in frugal design

Students must be educated to become successful practitioners of frugal design from a systems perspective (*19*). Curricular reforms are even more crucial in low-resource settings where a lack of engineering capacity and infrastructure severely limits economic development (*20*) and where knowledge of contextual constraints is paramount to the success of frugal designs. Sub-Saharan Africa suffers a chronic lack of indigenous engineering capacity: In the early 2000s, the number of engineers emigrating annually from South Africa matched the number of engineers graduating (*21*). Where available, tertiary education in engineering has not received anywhere near the investment required to keep pace with the developed world. Learning foci are too theoretical, based on outdated curricula, and not relevant to local needs. The teaching and learning approaches that emphasize rote memorization stunt students' potential to be innovative. Faculty lack resources for providing lab experiences and salaries are often so low that many take on additional jobs. Students who graduate from such programs face notable levels of unemployment, most likely because they graduate without needed skills and experience to be employable. Over $130 million has been invested to strengthen medical school education through the Medical Education Partnership Initiative by the U.S. National Institutes of Health, with a focus on developing human capacity, retaining faculty and graduates, and developing regionally relevant research programs (*4*); similar investments are critical if tertiary engineering education is to develop sufficient and relevant engineering capacity in the region. To fully leverage such investments, preuniversity science and math education must also be strengthened.

Design for context

Sustained implementation of a new frugal technology that performs well compared with technologies designed for higher-resource settings requires successful navigation of a number of contextual and political challenges. The explosive global growth in the availability of mobile phone technology illustrates the kind of success that is possible when the value proposition of a new technology is clear at all levels—users were willing to pay for inexpensive handsets and airtime; communities where electricity was not widely available established charging banks; and countries and the private sector invested in both the necessary private and public infrastructure to establish a network of base stations, powered by diesel generators where reliable electricity was not available (*22*). Today, 6 billion of the world's 7 billion people have access to a mobile phone, whereas only 4.5 billion have access to a latrine or toilet (*23*).

Why do more people have access to mobile phones than toilets? In part, it is more difficult to overcome the implementation challenges for technologies that require substantial investment in public infrastructure. The infrastructure to provide clean water and sanitation in developed countries requires robust vertical governance, from national to local levels. In most low-resource settings, local governments have insufficient capital to build community-level infrastructure and even less human capital for long-term maintenance. Resource constraints exist in every setting, but the nature and type of constraints in developing countries requires rethinking traditional processes. For example, the traditional design cycle for public infrastructure projects may require adjustment. Civil engineers are currently trained to optimize a design, then bid the project and accept the low bid. An alternative approach where the design engineer and end-user participate directly in a design process with feedback that is aimed at lowering the end-user costs could help designers maintain perspective about context and yield innovation that is more frugal in nature.

Fig. 1. Meeting the need. Approximately 768 million people do not have access to safe drinking water; new engineering approaches are needed to develop point-of-use technologies that meet the needs of community users and can be sustained over time. Efforts by aid organizations to scale the PlayPump (shown) failed because community users preferred the efficiency and reliability of traditional hand pumps. [Photo credit: German Chauluka]

Adoption is facilitated when end-users see a direct personal benefit associated with purchase of a new technology. Access to mobile phones increased profits for fishermen in India and market participation for farmers in Uganda (*22*). In contrast, the benefits of health or sanitation technologies may not be as apparent to end-users. The public sector, which is usually charged with promoting such technologies, is not good at market research.

Finally, adoption is facilitated in competitive markets that can drive down the price of technology services; market liberalization was associated with a 90% drop in average mobile phone call prices and an increase in traffic volumes (*22*). In the global health care industry, two recent trends may help to accelerate the implementation of promising technologies. First, rapidly expanding health care markets in emerging economies are drawing the interest of multinational corporations (*3*). Inflation-adjusted biomedical research and development expenditures increased in India and China by 6.7% and 32.8% per year, respectively, from 2007 to 2012; in contrast, expenditures in the United States, Canada, and Europe decreased over the same period (*24*). Likewise, an increase in accountable care organizations may drive investment in resource-saving technologies in the United States.

Conclusion

We are not the first to suggest a transition to frugal design—a number of recent "grand challenges" design efforts have engaged the technology

development community in frugal design efforts. Efforts like The Gates Foundation's Reinvent the Toilet Challenge reflect the kind of integrative thinking that must occur at the beginning of a design initiative; support is being directed toward strategies to create a next-generation toilet that can not only manage waste but also harvest water and energy resources. The toilet will also need to operate without the usual infrastructure, be financially sustainable, and be valued by users. Although such competitions highlight important challenges, funders often solicit solutions with a high degree of technical innovation. An unintended consequence of this premium on innovation can be to complicate downstream implementation efforts. It is time for the engineering and international aid communities to adopt approaches that can improve global health in ways that can be sustained.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge helpful discussions with P. Ondeng, E. Molyneux, and S. Hanson (National Academy of Sciences).

10.1126/science.1257085

PERSPECTIVE

Strengthening the evidence base for health programming in humanitarian crises

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Given the growing scale and complexity of responses to humanitarian crises, it is important to develop a stronger evidence base for health interventions in such contexts. Humanitarian crises present unique challenges to rigorous and effective research, but there are substantial opportunities for scientific advance. Studies need to focus where the translation of evidence from noncrisis scenarios is not viable and on ethical ways of determining what happens in the absence of an intervention. Robust methodologies suited to crisis settings have to be developed and used to assess interventions with potential for delivery at scale. Strengthening research capacity in the low- to middle-income countries that are vulnerable to crises is also crucial.

Health interventions in humanitarian crises—situations where disasters or conflicts constitute a critical threat to the health, safety, security, or well-being of a population—are an important focus within the broader field of global health. Such crises affect increasingly large numbers of people worldwide (*1*). There have been notable advances in programming, specifically in immunization and treatment of acute malnutrition, over the past 20 years. However, despite the increasing professionalization and standardization of humanitarian work (*2*), there is a consensus that the evidence base for much current practice remains weak (*3*, *4*).

It is not coincidental that the evidence base for health programming is frail in crisis conditions that cause high mortality and morbidity. Such health care contexts also present many challenges to scientifically rigorous research. Prime among these challenges is the acute vulnerability of populations (*5*), which requires prompt intervention rather than exploration of the comparative benefits and limitations of alternative approaches. In the face of acute needs and against a typical backdrop of limited funding, poor security, and shortages in human resources and logistics, simply providing immediate minimal standards of health services becomes an overriding concern. The space for research—particularly that involving experimental interventions or randomization or, more generally, offering different standards of care within the same population—dramatically shrinks (*6*, *7*). Acutely vulnerable populations have a compromised capacity to give meaningful informed consent. Refusing study participation may be seen as rejecting vital medical assistance (*8*, *9*).

The rapid response required in humanitarian crises contributes to an unpredictable programming environment. Although many health risks in the aftermath of disasters or conflict are predictable and minimum standards for response and best-practice interventions have already been established, health needs can evolve rapidly, and adaptable program strategies are required. Political sensitivities and security concerns may also have a substantial influence on the timing, coverage, and delivery of health interventions (*10*). Different sectorial interventions that affect health (including provision of shelter, water and sanitation, food security, livelihoods, nutrition, and vaccination) may be introduced with limited coordination and varying population coverage (*11*). This makes identification of comparison or control groups and attribution of outcomes to any single intervention methodologically challenging.

Difficulties in coordination are not only cross-sectoral but also reflect the more general complexity of multiple intervening actors and initiatives that characterize humanitarian responses. A population will typically receive services through a complex web of national and local governmental institutions, local civil society partners, United Nations agencies, nongovernmental organizations, and, in some emergencies, foreign

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medical teams (*5*). In coordination meetings, scores of agencies, each with discrete capacities and mandates, are often represented (*10*, *11*). Many will have little expertise in research and a limited understanding of, or incentive to address, gaps in the evidence base in a coordinated fashion.

Research is also heavily constrained by fundamental data challenges (Fig. 1). There is frequently a lack of reliable preexisting data on disease burden and population demographics in humanitarian settings. Even where relevant data are present, the breakdown of health information systems and displacement of populations often mean that there is little access to these data for analysis or the data are no longer valid. When new data are collected, agencies may have little capacity or incentive to publicly share data on programming outcomes and even less to publish findings in peer-reviewed publications (*5*, *12*). As a result, information and lessons learned on health interventions in crises often remain in reports that are not easily accessible to the broader humanitarian community.

Themes critical to scientific progress

Notwithstanding these challenges and given the growing scale and profile of humanitarian response, there is increasing interest in strengthening the evidence base for health interventions in such contexts. (*3*, *13*). The authors serve as the funding committee of the Research for Health in Humanitarian Crises (R2HC) program (*14*). We were recently afforded the opportunity of reviewing preliminary expressions of interest and full proposals for funding for this initiative. By bringing us together as researchers and program managers from academic and humanitarian organizations across four continents, this process led us to formulate the following five themes as critical to scientific progress in this field.

Use of evidence from nonhumanitarian settings

Evidence from noncrisis settings serves as a fundamental basis for programming interventions in emergency contexts. For example, in most circumstances, evidence on drug efficacy or the effectiveness of insecticide-treated nets for malaria control can be appropriately generalized across populations. However, issues occurring rarely in noncrisis contexts, such as severe acute malnutrition, may require research to be conducted in a crisis setting. In addition, extrapolation from nonhumanitarian to humanitarian settings can overlook the many contextual factors that shape the particular vulnerability and fragility of humanitarian crisis conditions, requiring either different or modified interventions for such contexts. Acknowledging these alternative scenarios, researchers have an obligation to articulate the specific conditions where empirical work in the complex context of a humanitarian crisis is both adequately safe and evidentially necessary.

Identification of ethical bases of counterfactual analysis

To inform humanitarian programming, it is important not only to evaluate the outcomes of an intervention but also to understand what outcomes would be without the proposed intervention or with alternative interventions. Identifying ethical and rigorous means of such counterfactual analysis is crucial. Randomization to a control condition may be possible and appropriate. Where it is not, however, other means of attributing the effect of an intervention need to be adopted. Where operational constraints limit the initial coverage of an intervention, step-wedge designs and "waitlist controls" (*15*) may be considered. Changes in government policy, such as ages of vaccination, may allow a comparison of different interventions (*16*).

The development of robust methodologies appropriate to crisis settings

Although humanitarian contexts present many barriers to rigorous research, the same standards of methodological and statistical rigor used in other fields need to be adopted if a sound evidence base for response in crises is to be established. Meeting such standards in crisis settings requires methodological adaptation and evolution. Methodologies have to be matched not only with the specific practical and ethical constraints of a humanitarian setting but also with the current status of knowledge in their field of focus. A combination of studies is needed that address proof-of-concept research, acceptability and feasibility evaluations, and the creation of new tools, in addition to evidence of comparative effectiveness. For the latter, comparative clinical trials are required, but a range of methodological approaches (e.g., time series analyses and rigorous observational studies) will also be relevant (*17*). Particular methodological innovation is required to tackle cross-cutting issues of access, capacity, accountability, and coordination (*3*).

Capacity and potential for scaling up of evidence-based intervention

Any research on humanitarian interventions must inform future practice, but this often requires consideration of a complex set of factors. Strong local engagement is important to ensure the specific demands of a humanitarian context are addressed and requires cultural adaptation and contextual sensitivity. The challenge with interventions suited to a unique context and the particularities of a single implementing agency is that they lack external validity and/or offer little prospect for wider scale-up. Interagency initiatives are strong candidates to shape wider practice. Strong local engagement, wide global ownership, and a detailed dissemination plan is a challenging prescription, but crucial to the translation of valid research evidence into accepted practice.

Research capacity development in countries vulnerable to emergencies

It is vital for donors and implementing agencies to establish effective engagement with research institutions from low- and middle-income countries (LMICs) to ensure ownership of the developing research agenda within settings vulnerable to humanitarian crisis. Although many research proposals include partnerships with LMIC institutions, established institutions from high-income countries still typically hold leadership. This requires sensitivity and commitment

Fig. 1. The REFAH AID director begins data collection procedures before aid distribution at Jalozai refugee camp, Peshawar, Pakistan. [Credit: Mukhtar Ahmad/REFAH AID (2009). Courtesy of Photoshare.]

to issues of both long-term research capacity development and means of ensuring that research initiatives respond to field-based challenges and concerns.

Conclusions

The circumstances of humanitarian crises present many barriers to the conduct of scientifically rigorous research, and yet these same circumstances shape the profound risks to the health of affected populations that make a solid evidence base for health programming in such settings crucial. There are clear opportunities for scientific advance. We consider that progress regarding the five themes elaborated above will be an important driver for assembling and using a more functional evidence base for future health programming and prioritization in humanitarian crises.

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ACKNOWLEDGMENTS

The authors are members of the funding committee responsible for review of submissions made in response to request for proposals from the R2HC program. The findings and conclusion of this report are those of the authors and do not reflect the official position of their organizations or of R2HC.

10.1126/science.1254164

PERSPECTIVE

Emerging, evolving, and established infectious diseases and interventions

M. Elizabeth Halloran[1,2]* and Ira M. Longini Jr.[3]

Planning, implementing, and evaluating interventions against infectious diseases depend on the nature of the infectious disease; the availability of intervention measures; and logistical, economic, and political constraints. Infectious diseases and vaccine- or drug-based interventions can be loosely categorized by the degree to which the infectious disease and the intervention are well established. Pertussis, polio, and measles are three examples of long-known infectious diseases for which global vaccination has dramatically reduced the public health burden. Pertussis vaccination was introduced in the 1940s, polio vaccination in the 1950s, and measles vaccination in the 1960s, nearly eliminating these diseases in many places.

Many known infectious diseases tend to be epidemic, but exactly when and where these epidemics will occur is uncertain. For such sporadic infectious diseases, it may be most efficient to implement a reactive vaccination campaign once an outbreak has begun. Planning for reactive vaccination requires keeping a mobile vaccine stockpile available that can be quickly moved for emergency vaccination. This strategy could be used for epidemic cholera, such as occurs in parts of Africa (*1*). When cholera was introduced into Haiti in 2010, such a stockpile did not exist. Limited supplies of cholera vaccine were scattered in different locations, and a decision was made not to vaccinate the local population, even though mathematical models showed that with limited quantities of vaccine, concentrating vaccination in high-risk areas would be most efficient (*2*). Now that such a stockpile of 2 million doses of oral cholera vaccine exists (*3*), it can be used for future cholera epidemics. Mobile stockpiles, such as the oral cholera vaccine, often have a finite shelf life, and their use can be valuably reassigned in endemic locations, such as Bangladesh, that experience annual cycles of high incidence.

For long-term intervention strategies, sustainability is an issue, and strategies can change as the economic constraints change or as new products become available. For example, a new product against meningococcal A meningitis was developed that overturned the reactive vaccination strategy in the meningitis belt of sub-Saharan Africa. An international commitment working through the Meningitis Vaccine Project developed an inexpensive vaccine against meningitis A (*4*) that can be used in proactive vaccination. Initial introduction has been carried out or is planned in 26 African countries, with mass vaccination of people up to the age of 29 years to be followed by routine vaccination of young children.

Emerging infectious diseases sometimes lend themselves to effective vaccination. In light of the ongoing human cases of avian influenza A (H7N9) in China, the United States is preparing to stockpile vaccines for human cases of H7N9. When the pandemic influenza A (H1N1) emerged suddenly in 2009, influenza vaccine manufacturers turned their production lines to developing and producing appropriate vaccines for immediate administration. Yet for other emerging diseases like HIV/AIDS, which was discovered more than 30 years ago, no effective vaccine has successfully been developed for human use. For several newly emerging infectious diseases, it is questionable when or if vaccines or drugs can be developed at all. One example is Middle East respiratory syndrome (MERS), a viral respiratory illness caused by a coronavirus and first reported in Saudi Arabia in 2012. Another example is the arbovirus chikungunya, which is spreading explosively in the Americas, with 650,468 confirmed and suspected cases reported by the Pan American Health Organization as of 29 August 2014. Although antivirals, vaccines, and monoclonal antibodies are under early development, Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases, wrote that "in the meantime, we can only keep our fingers crossed" (*5*) that the epidemic in the Americas will decline on its own before becoming more widespread. According to (*5*), even if a vaccine were available, chikungunya outbreaks spread too rapidly for reactive vaccination to be effective. In the current Ebola outbreak in West Africa, there is no pharmaceutical intervention. For these newly emerging infectious diseases, surveillance and containment, education, and avoidance are the main responses available until vaccines or drugs may be developed.

Interventions in infectious diseases can have more than just direct protective effects in the

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individuals receiving the intervention (*6*). An intervention strategy can also have indirect protective effects in associated unvaccinated individuals. Accounting for both the direct and indirect effects will give a more accurate measure of the cost-effectiveness of a program; hence, the combined direct and indirect effects need to be assessed. Dynamic mathematical transmission models offer valuable tools for planning infectious disease strategies, particularly for taking potential indirect effects into account. Especially when vaccines confer modest direct protection, the indirect and overall effects of widespread vaccination may affect the cost-effectiveness of vaccination. An example is the cholera vaccine, for which indirect effects play an especially large role in the cost-effectiveness of large-scale vaccination programs. A dynamic mathematical model of cholera vaccination showed that the percentage of cases expected to be averted by vaccinating different proportions of the population is much higher when both indirect and direct effects, as opposed to only direct effects, are taken into account (Fig. 1). When taking only direct protection into account, the same model showed that the cost per disability-adjusted life years (DALYs) averted is barely cost-effective at all levels of coverage. But when taking both indirect and direct protection into account, a vaccination program is much more cost-effective (Fig. 2).

However, reliable mathematical models depend on good epidemiological data. Often the data required to inform the models are not collected in field trials. For example, in most vaccine field trials, cases are ascertained based on clinical symptoms, then the specific infectious agent is confirmed by a laboratory test, so efficacy against clinical disease is determined. But much more information about how vaccination affects transmission is needed for the mathematical models. For example, data are needed on the effect of vaccination on asymptomatic infection and the degree to which infectiousness is reduced in breakthrough infections. Whether the vaccine protects some people completely and others not at all, or whether it reduces the probability of becoming infected in everyone equally, has important population-level consequences. The time of infection, the onset and end of infectiousness and its temporal variability, and contact patterns are seldom measured but greatly influence the outcomes of simulated interventions. Uncertainty regarding these important inputs results in uncertainty of the potential epidemiological effects and cost-effectiveness of a vaccination program. Policy-makers are increasingly asking for mathematical models to aid in decision-making. Moving forward, vaccine studies need to include plans to measure as many of these inputs as possible for well-informed mathematical modeling, as pointed out 20 years ago (*7*).

"Focused field epidemiology and surveillance are needed to provide the information required to make informed policy decisions about interventions to further global public health."

As vaccines against dengue (*8*) and malaria (*9*) become available, models can help us understand how these emerging vaccines might be used most effectively. In a phase III trial, a dengue vaccine has been shown to be effective against clinical dengue and has proven twice as effective in individuals with preexposure to natural dengue (*8*), providing an important input for modeling vaccination in populations with different levels of dengue exposure. However, the trial does not provide information on whether the vaccine protects against infection or just disease, or whether it reduces the infectiousness of infected individuals for mosquitoes. The exact nature of the interaction of vaccine-induced immunity with preexisting immunity and exposure to infection is also not well understood and can affect the interpretation of vaccine efficacy and effectiveness (*10*). All of these inputs are necessary to model the potential population-level effects and cost-effectiveness of dengue vaccination programs in different populations.

Evaluation

In addition to modeling indirect and overall effects of vaccination, such effects of widespread vaccination can be assessed by appropriate field studies and surveillance. Either randomized or observational studies can provide assessment of indirect and overall effects of intervention programs. For example, studies of cholera (*11*) and typhoid (*12*) vaccination, both of which confer only modest direct protection, have been conducted to evaluate the indirect effects of widespread vaccination. Plans for evaluation should be an integral part of implementing a vaccination strategy. For example, before introducing the meningitis A vaccine in Africa, the capacity to evaluate the indirect and overall effects of the strategy on nasopharyngeal carriage was established in several countries by the MenAfriCar (the African Meningococcal Carriage Consortium) project. But studies can also be undertaken after introducing vaccination. A study analyzing insurance data to evaluate direct, indirect, total, and overall effectiveness of rotavirus vaccines in the United States compared the prevaccination baseline years with the years after vaccination (*13*). This study revealed important indirect effects that need to be taken into account when estimating the sustained impact of the vaccination program. Delivery of a new dengue vaccine would offer the opportunity for a well-planned phased introduction to

Modeling the effectiveness of mass vaccination

Percent of effectiveness

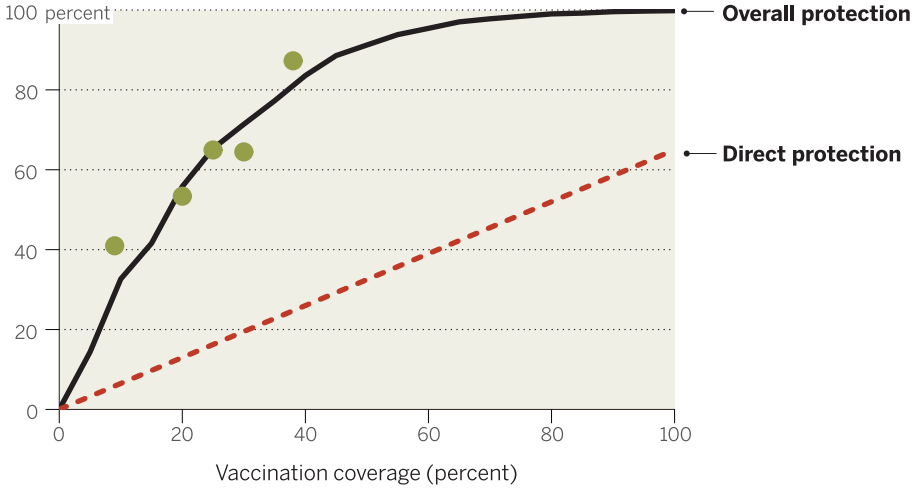

Fig. 1. A mathematical model of cholera transmission was used to estimate the effectiveness (number of cases averted) and cost-effectiveness (in disability-adjusted life years, or DALYs) when a given fraction of the population is vaccinated. The model predicts that the fraction of cases averted by mass vaccination (black solid line) exceeds the estimates when only direct protection is assumed (red dashed line). The green dots indicate levels of protection observed in a cholera vaccine trial (*20*). In addition, the relation between vaccine coverage and effectiveness is not linear when assuming overall protection, unlike direct protection. [Courtesy of C. Troeger and D. Chao]

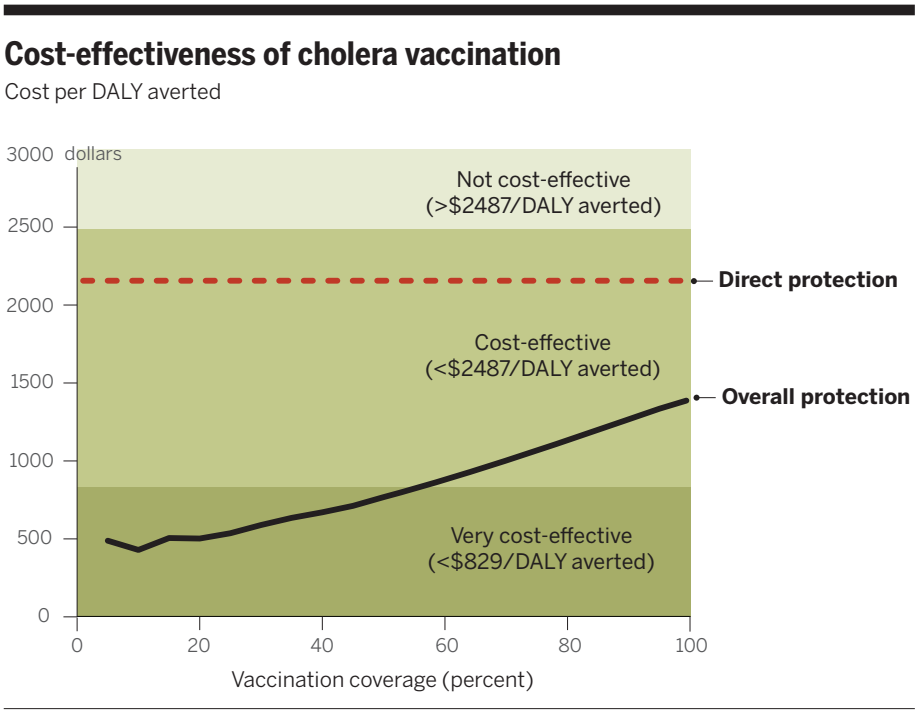

Fig. 2. Mass cholera vaccination is more cost-effective when overall protection is considered compared with calculations that only account for direct protection. According to WHO convention, the ratio of program cost to DALYs averted in a cost-effectiveness analysis is classified by the per capita national gross domestic product (GDP) of the country of interest: Less than the GDP per capita classifies an intervention as very cost-effective; between one and three times the GDP per capita is cost-effective; and more than three times the GDP per capita is cost-ineffective (*21*). [Courtesy of C. Troeger and D. Chao]

evaluate not only the direct effect of vaccination, but also the indirect and overall effects. In all instances, good field epidemiology and surveillance are required.

Changing fronts

Even when a good intervention is available, problems can arise, requiring ongoing careful epidemiological assessment of apparently excellent interventions. Interventions can put selective evolutionary pressure on pathogens, resulting in drug resistance and immune escape. The emergence of drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant tuberculosis, as well as drug-resistant malaria parasites, is the result of evolutionary pressure. The United States is experiencing a resurgence of pertussis cases, despite high coverage and booster vaccination of teenagers. The reasons are poorly understood, but it is possible that the current acellular pertussis vaccines do not produce long-lasting immunity (*14*). In addition, pertussis strains that lack one of the important vaccine antigens have evolved because of immune escape and have spread in recent years (*15*). Careful epidemiological assessment of the changing pertussis situation is required to guide development of new vaccines and intervention strategies (*16*).

Influenza viruses evolve antigenically over time, escaping both natural and vaccine-induced immunity. Challenges arise from producing an influenza vaccine each year, and constant monitoring of the influenza viruses is needed. Global influenza virological surveillance has been conducted through the World Health Organization's (WHO) Global Influenza Surveillance and Response System for more than half a century. Twice a year—once for the Northern Hemisphere and once for the Southern Hemisphere—the WHO advisory panel decides what viruses will be incorporated in the next seasonal influenza vaccine (*17*).

Although global vaccination has eliminated polio in many parts of the world, choosing between live-attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV) is a crucial policy decision once elimination has been achieved. OPV is less expensive and easy to administer; can be transmitted to close contacts and in the environment, helping to spread vaccination; and induces more natural immunity in the gut, preventing onward transmission. However, OPV can revert, causing very occasional cases of polio. IPV is more expensive, but it induces the production of antibodies that can prevent paralytic disease. IPV does not induce gut immunity and thus does not prevent onward transmission, but it also does not raise the same safety concerns as OPV. When polio has been essentially eliminated and the vaccine is affordable, then the switch to IPV from OPV is a sensible policy decision. However, this choice can also be fraught with difficulties. Israel switched to IPV for 9 years, but through intense environmental surveillance discovered circulation of type 1 wild poliovirus and thus reintroduced OPV (*18*, *19*). This excellent environmental surveillance facilitated the rapid response to the changing situation.

The shifting landscape of infectious diseases and interventions poses challenges. Focused field epidemiology and surveillance are needed to provide the information required to make informed policy decisions about interventions to further global public health.

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ACKNOWLEDGMENTS

We are funded by grants from NIH National Institute of Allergy and Infections Diseases (R37-AI032042) and NIH National Institute of General Medical Sciences (U01-GM070749). C. Troeger and D. Chao kindly provided Figs. 1 and 2.

10.1126/science.1254166

PERSPECTIVE

Virus sharing, genetic sequencing, and global health security

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This Perspective focuses on the future of the Pandemic Influenza Preparedness (PIP) Framework, which was initially established to promote the fair sharing of public health–related pandemic influenza samples between countries. We examine the changes that need to be made to address the growing likelihood that genetic sequence data might be shared instead of physical virus samples, as well as the need to expand the PIP framework's scope and to improve its fairness.

The Pandemic Influenza Preparedness (PIP) Framework (*1*) was a milestone agreement in promoting international sharing of biological samples to develop vaccines, while ensuring that poorer countries would have access to those vaccines. Now genetic sequencing data (GSD) can allow scientists to develop synthetic viruses rapidly for product development. Electronic transfer of GSD could enable wider access to researchers to innovate and share findings, speeding development of life-saving technologies in a time-sensitive global emergency. But access to GSD may also have major implications for biosecurity, biosafety, and intellectual property (IP). By rendering physical transfer of viruses antiquated, GSD may also undermine the effectiveness of the PIP Framework itself, with disproportionate impacts on poorer countries.

In December 2006, Indonesia challenged the fairness of the global preparedness system by refusing to share samples of avian influenza A (H5N1). Concerned that wealthier countries would gain disproportionate access to vaccines while creating cost barriers for others, Indonesia invoked sovereign ownership of a virus isolated in its territory. The government claimed that the Convention on Biological Diversity prevents exploitation of its "biological and genetic resources," including novel pathogens (*2*). Indonesia's decision—supported by many low- and middle-income countries—risked significantly impeding the capacity to monitor and respond to novel influenzas.

It took more than 4 years, but the World Health Organization (WHO) was able to broker the PIP Framework, finalized in May 2011 (Fig. 1). It used a novel strategy to advance global justice by affording more equitable access to the benefits of research. Rather than having governments commit to providing a share of their vaccine stockpiles with lower-income countries in exchange for access to biological samples, the PIP Framework created legally enforceable contractual obligations on participating private and academic partners that are involved in the manufacture of vaccines to make vaccines available to poorer countries.

Fig. 1. By sharing influenza viruses with human pandemic potential and building financial and research partnerships, countries can unite to widen access to vaccines.

Although it has the potential to be transformative, the PIP Framework leaves fundamental gaps in health security and in equity.

Gaps in equity, research, and security

The dynamics behind the PIP Framework were unusual because Indonesia had something to leverage—virus samples critical to the functioning of the global public health system (Fig. 2). The agreement changed an unfair status quo, whereby lower-income countries shared viruses, while relying on ad hoc "charitable" donations of vaccines. For example, during the 2009 influenza (A) H1N1 pandemic, vaccine donation pledges often were not fulfilled until high-income countries had protected their domestic populations (*3*). In exchange for access to biological materials, the PIP Framework requires researchers and manufacturers to fulfill contractual obligations, such as providing an agreed percentage of vaccines, diagnostics, or pharmaceuticals to the WHO stockpile or granting developing countries "fair and reasonable" or royalty-free licenses to manufacture such products.

In addition to potential limitations in light of GSD discussed below, the PIP Framework is limited in scope as it applies only to influenza viruses with pandemic potential. Seasonal influenza viruses are excluded, as are noninfluenza pathogens irrespective of their pandemic potential. In a globalized world, with myriad emerging infections, the framework's narrow scope excludes the vast majority of future threats. The ownership and sharing of the Middle East respiratory syndrome coronavirus (MERS-CoV), for example, has been controversial, with Saudi Arabian officials circumspect about global cooperation (*4*). Countries lack the leverage to withhold pathogens already in wide circulation, such as HIV, because scientists can acquire samples from multiple sources.

Gaps in global governance

The private sector's incentive to participate is its commercial need for viral samples to develop pandemic influenza vaccines. But in May 2013, scientists demonstrated that GSD could be used to rapidly develop synthetic viruses for product development (*5*). If pharmaceutical researchers no longer require access to biological materials to develop products, private sector cooperation in the framework could decline.

The PIP Framework defines "biological materials" as influenza viruses with human pandemic potential, including extracted RNA and cDNA, which appears to exclude GSD. Although the framework also defines genetic sequences, the framework's sharing and benefits scheme does not explicitly apply to GSD. Noting that some countries treat the publication of pathogen sequencing data as highly sensitive, the PIP Framework left for the future the handling of GSD—which suggests that WHO member states and stakeholders failed to agree during the negotiations.

The omission of GSD in the PIP Framework's sharing and benefits scheme is out of step with global preferences for the inclusion of genetic information of global health importance in public domain or open access databases, to increase transparency and to advance social justice. The National Institutes of Health's genetic sequencing database GenBank, for example, offers an annotated collection of publicly available DNA sequences, which enables data exchange. Similarly, the Global Initiative on Sharing All Influenza Data (GISAID) platform promotes sharing influenza-type virus sequences. Although these sequences are publicly accessible, researchers must acknowledge the originating laboratories that contributed data to the GISAID platform and ensure fair use of research findings.

Biosafety and biosecurity

Biosafety refers to the maintenance of safe conditions in biological research to prevent inadvertent escape of hazardous materials that could harm workers, persons outside the laboratory, or the environment. Unlike physical virus sharing, sequencing data are not inherently hazardous, and sharing information instead of physical samples mitigates the risk of inadvertent release of the infectious agent by limiting access to biological agents.

Biosecurity refers to the precautions taken to protect against the spread of harmful biological substances. This includes pathogens of dual-use

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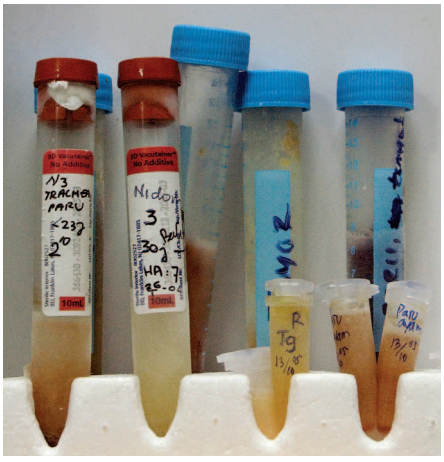

Fig. 2. Bird flu in Indonesia: freewheeling toward a pandemic. Biological samples (poultry and pigs) for the detection of H5N1 virus at the Virology Laboratory Control Center for Tropical Diseases, University of Surabaya, Java. [CORBIS]

research of concern: life sciences research intended for benefit, but which could be misapplied to do harm, such as through bioterrorism. GSD pose unique biosecurity threats because of their potential for broad global dissemination, which results in insecure laboratories or potential terrorists gaining access. At present, the technical sophistication to use genetic sequencing to create a hazardous agent is beyond the expertise of most scientists, and presumably more so of potential bioterrorists. However, the future risk of recreating a lethal virus cannot be discounted, especially as sequencing data become more widely available on the Internet and through scientific publications (*6*).

Governments could also use export controls to limit sharing of biological materials or GSD. Many countries require a license to export particularly dangerous pathogens, although export licenses are routinely granted for legitimate scientific research. In rare instances, governments have used export controls to limit dissemination of pathogen GSD—as when the Netherlands restricted publication of enhanced H5N1 influenza research—but this is uncommon for unclassified basic science research (*7*).

Intellectual property

Claiming IP rights is highly polarizing because it can make essential medicines and vaccines less affordable. To ensure greater fairness, the PIP Framework imposes contractual prohibitions on IP asserted over biological materials transferred within the WHO Global Influenza Surveillance and Response System. The framework's intent is to prevent recipients of biological materials from exploiting a global resource for their own financial benefit. Although a contract between the WHO and a recipient may not invalidate IP rights afforded in the researcher's home country, it could permit a lawsuit for breach of contract.

Countries have varying laws as to whether naturally occurring biological samples are patentable. A biological sample, such as a virus sample, would likely fall under the scope of "naturally occurring substances" and thus not be patentable as defined by the World Intellectual Property Organization (*8*). GSD, however, entail additional intellectual steps from simply isolating the virus. Still, it is conceivable that GSD might be deemed a nonpatentable "naturally occurring substance" in major jurisdictions such as the United States (*9*).

Although the PIP Framework does not displace the IP regimes of domestic jurisdictions, extending the framework to cover GSD would contractually prohibit unauthorized assertions of property rights over GSD, similar to biological materials. Such an expansion of coverage would further the framework's objectives of social justice, while encouraging open access to GSD.

Global health governance

The private sector (e.g., pharmaceuticals, for-profit health care providers, food, alcohol, and tobacco) has powerful effects on the public's health, security, and safety. Yet, the WHO finds it hard to govern these and other nonstate actors. When it does mobilize multiple stakeholders, the norms set are usually voluntary, such as through action plans or codes of practice. A perennial weakness of international agreements is their inability to assure compliance. The PIP Framework not only includes the private sector in global health negotiations but also creatively applies civil contract law to enforce the agreement—a model for future global health diplomacy.

Thus, despite its weaknesses and omissions, the PIP Framework offers an innovative template for future global health agreements. The framework brought together the WHO secretariat, member states, and the pharmaceutical industry in an unprecedented way. Traditional international law creates state obligations, but rarely, if ever, binds the private sector. The PIP Framework—which is not in the form of a treaty—uniquely creates legally enforceable contractual obligations on participating private and academic partners that manufacture vaccines, antivirals, or diagnostics. Consequently, as a method of global health governance, the framework has the potential to be transformative.

Filling the gaps in the PIP framework

A recent WHO review of the PIP Framework offered an opportunity to close major gaps but also revealed the text's rigidity in adapting to rapid scientific progress (*10*). GSD fundamentally change the paradigm within which the PIP Framework operates, potentially rendering the framework less effective.

In the short run, it would be best simply to interpret the framework to include GSD as biological materials. Keeping GSD under the umbrella of a global agreement would facilitate tracking sequencing data, while holding users accountable—facilitating socially productive research, while limiting harmful use. A tracking system need not be burdensome or restrict the free flow of scientific information. A simple registration system that monitors who has downloaded GSD would help ensure that recipients abide by global research norms and could be incorporated into existing databases such as the GISAID platform. Moreover, allowing free exchange of GSD without enforcing the obligations owed to the donating country essentially unravels the PIP Framework "bargain" after the fact, undermining both the framework's legitimacy and the WHO's credibility to negotiate future agreements.

In the long run, the gaps in the framework that we describe open an opportunity to resolve other existing weaknesses by revising the text. A revised framework should be driven by norms of equity and shared global vulnerability, with the vital goals of open access to research materials, accessible license agreements, and more rapid development of therapeutic countermeasures. Note that fairness requires that lifesaving vaccines and pharmaceuticals, as well as the results of research, are equitably distributed in low- and middle-income countries that lack the ability to purchase products in the market or produce them domestically.

Further, the sharing of biological materials, as well as GSD, should be extended beyond influenza to all other hazardous pathogens with pandemic potential, such as novel coronaviruses. If the intent of the framework is to facilitate research and speed the development of vaccines and antiviral medications, a narrow scope of application diminishes the effectiveness of global health governance (*11*). The use of GSD in product development could prove to be a remarkable achievement. However, this and other scientific advancements need to be harnessed to safeguard populations worldwide not simply countries that can afford the products derived from innovative research. Global health security is vital but so, too, is equitable access. Not only will this ensure a more secure and healthy world, but also a more just world, for humanity.

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ACKNOWLEDGMENTS

Disclosures: L.O.G. was a member of the WHO Technical Expert Working Group (TEWG) on Genetic Sequence Data to the PIP Advisory Group and is the Director of the WHO Collaborating Center on Public Health Law and Human Rights.

10.1126/science.1257622

PERSPECTIVE

Monitoring parasite diversity for malaria elimination in sub-Saharan Africa

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The African continent continues to bear the greatest burden of malaria and the greatest diversity of parasites, mosquito vectors, and human victims. The evolutionary plasticity of malaria parasites and their vectors is a major obstacle to eliminating the disease. Of current concern is the recently reported emergence of resistance to the front-line drug, artemisinin, in South-East Asia in *Plasmodium falciparum*, which calls for preemptive surveillance of the African parasite population for genetic markers of emerging drug resistance. Here we describe the Plasmodium Diversity Network Africa (PDNA), which has been established across 11 countries in sub-Saharan Africa to ensure that African scientists are enabled to work together and to play a key role in the global effort for tracking and responding to this public health threat.

Africa is home to the most successful malaria vector (*Anopheles gambiae* complex) and the deadliest malaria parasite (*Plasmodium falciparum*). Despite efforts to eradicate malaria in the 1950s and early 1960s, 207 million cases and 627,000 malaria deaths were estimated to have occurred in 2012, mostly in sub-Saharan Africa and in children under 5 years of age (*1*). The inability to eradicate malaria reflects the complex coevolution of the three eukaryotic genomes involved and represents the major biological challenge to vaccine and drug development. Since publication of the full genome of a reference clone, many other model and population isolates have been sequenced (*2*), which has provided some insight into the genetic variation responsible for phenotypes like drug resistance and virulence. However, the global genome-wide pattern of genetic diversity across most endemic regions is poorly understood, as few natural *Plasmodium falciparum* populations have been analyzed.

Malaria elimination efforts in Africa are heavily reliant on artemisinin combination therapies (ACT) yet no coordinated effort for monitoring emergence or prevention of the spread of artemisinin resistance is in place in Africa. In addition, introduction of new malaria vaccines or mass therapeutic approaches to control and elimination will increase selection for resistance in parasite populations that could damage the effectiveness of these and future tools (Fig. 1).

Rationale and mission for an African Plasmodium Diversity Network

The emergence of antimalarial resistance to artemisinin in Southeast Asia (*3*, *4*) calls for global monitoring of the parasite population (*5*). The MalariaGEN community has already built a successful framework for data-sharing (*6*); hence, we have adopted this model to form the Plasmodium Diversity Network Africa (PDNA). The concept of PDNA was seeded by several African scientists during the Genomic Epidemiology of Malaria conference at Hinxton, United Kingdom, in 2012. In May 2013, the network was formally launched in Accra, Ghana, when scientists and clinical researchers met from 13 research groups from across Africa (Fig. 2).

The mission of PDNA is to explore the malaria parasite's genetic and phenotypic diversity to advance the malaria elimination and eradication agenda in sub-Saharan Africa. We have initiated or will initiate projects to investigate *Plasmodium* population structure and evolution, to survey the frequencies of current and novel molecular markers of antimalarial drug resistance, and specifically to monitor the emergence and spread of artemisinin resistance in sub-Saharan Africa.

How will PDNA contribute to malaria elimination?

To succeed, malaria elimination requires knowledge of parasite genome variation in different geographical locations and of recent evolutionary selection and a better understanding of the factors that determine gene flow between locations, such as rates of inbreeding and population structure. The PDNA membership represents the range of malaria diversity in Africa. The Network is in its infancy and is bound to evolve with time. Each PDNA member brings specific scientific expertise (table S1) and the capacity to collect local biological material, as well as clinical data. *Plasmodium* DNA will be either analyzed on site or dispatched

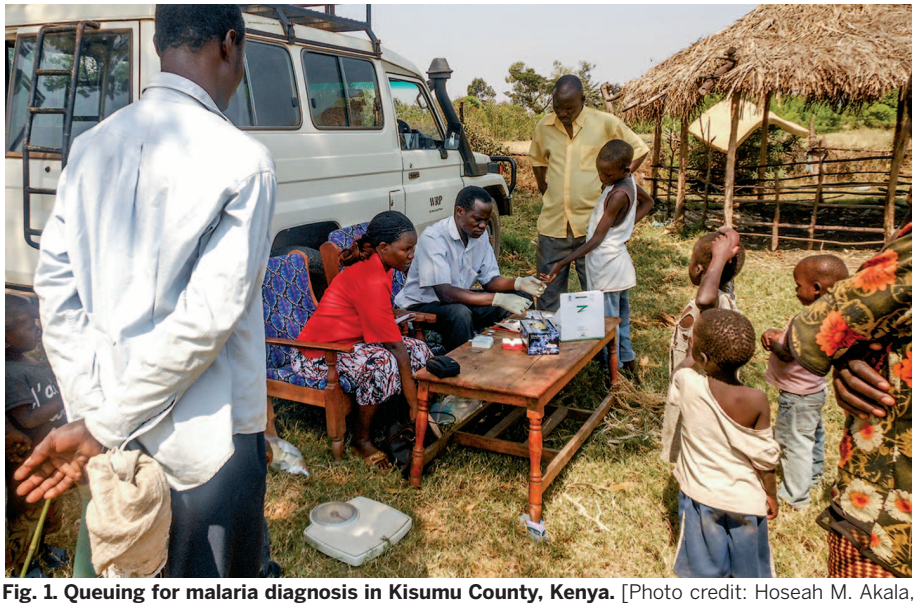

Fig. 1. Queuing for malaria diagnosis in Kisumu County, Kenya. [Photo credit: Hoseah M. Akala, Kenya Medical Research Institute/U.S. Army Medical Research Unit—Kenya]

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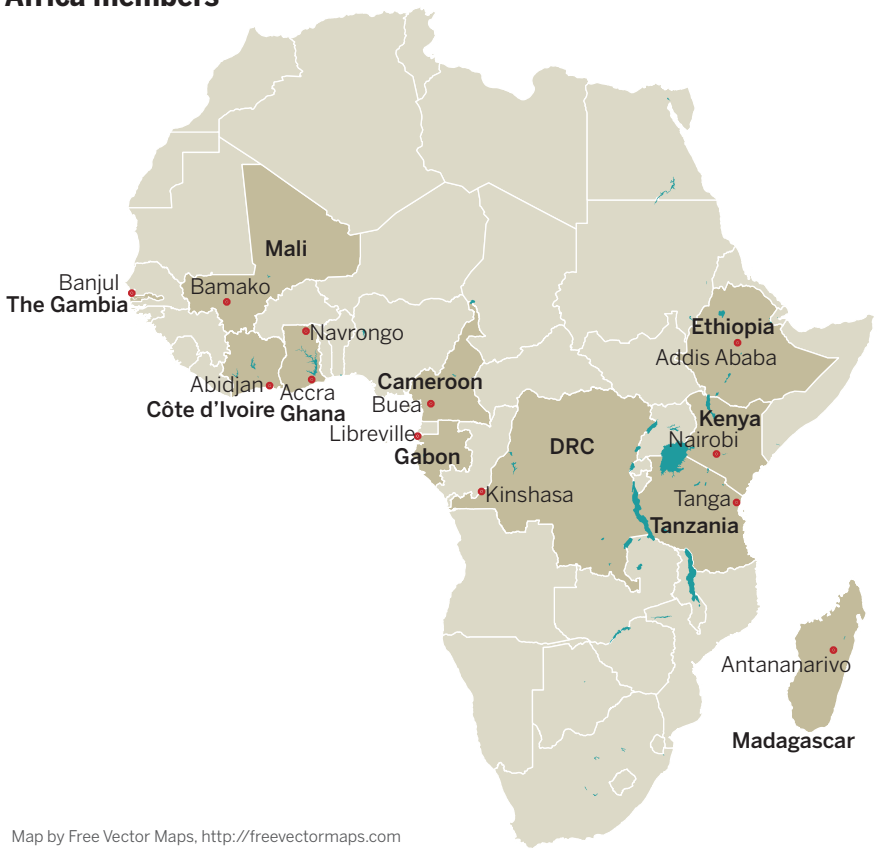

Fig. 2. Countries represented by the Plasmodium Diversity Network Africa members.

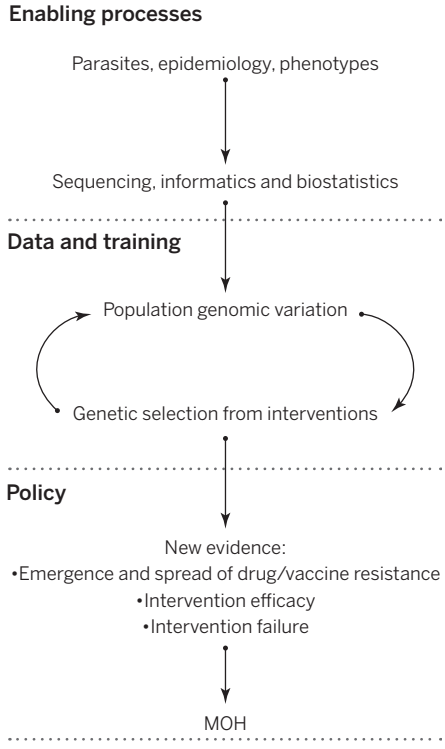

Fig. 3. Flow diagram of how PDNA will contribute to malaria elimination policy decisions.

to various laboratories, such as the Wellcome Trust Sanger Institute, UK, for sequencing, genotyping, and various wet laboratory investigations to survey for evidence of evolution in response to recent or ongoing elimination measures. PDNA studies will translate genomics data on *Plasmodium* diversity into evidence to be shared with the sub-Saharan Ministries of Health; national malaria control programs; regional and international health authorities, such as the Africa Bureau of the World Health Organization (WHO) and the WHO global malaria program; and other stakeholders (Fig. 3).

Pooled analysis of data will provide larger sample size and comparable information across the African continent, as well as the detection of rarer genetic events, site-specific issues, and opportunities for modelling. Hence, PDNA will have an Africa-wide dynamic view of malaria diversity and recent evolution. Through real-time knowledge-sharing and dissemination, lessons learned will be openly available to the various stakeholders.

Current projects

Each study site will adopt common protocols; common standard operating procedures; and, whenever possible, the same reagents and supplies. For example, using seed funding from the UK Medical Research Council, we have initiated a baseline investigation of *P. falciparum* diversity in sub-Saharan Africa. Each site collected 100 leukocyte-depleted *P. falciparum*–infected blood samples. DNA was extracted and shipped to the Wellcome Trust Sanger Institute for whole-genome sequencing, and data analysis is under way with support from the MalariaGEN resource center. As the new K13 propeller polymorphisms have been described as a molecular marker for artemisinin resistance (*7*), we have also been investigating their presence and prevalence in each of our African sites. Within weeks, each PDNA site collected, processed, and shipped genomic DNA or dried blood spots for sequencing. Together, these projects show how a coordinated network of African scientists can mount a rapid surveillance response.

The institutions involved in PDNA are at different levels of scientific expertise, and hence, a priority will be to engage in training and capacity-development at each participating institution for know-how as well as ethics. Although the primary focus of the PDNA project is *Plasmodium*, the process of obtaining parasite DNA involves the collection of blood samples from human volunteers. Thus, in accordance with international ethical guidelines, voluntary informed consent of research participants is sought in each of the projects, and all PDNA research proposals are submitted to relevant ethics committees for review and approval.

Conclusion

The PDNA represents an African initiative for the assessment of endemic-country malaria parasite diversity at the genotype and phenotype levels to address scientific questions most relevant to the successful elimination of malaria in sub-Saharan Africa. PDNA will use its range of scientific, geographic, epidemiologic, political and ethno-linguistic diversity to engage local and international stakeholders, including health policy decision-makers, to ensure that we contribute directly to the global agenda for malaria elimination.

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ACKNOWLEDGMENTS

Seed funding and support was obtained from Medical Research Council UK (G0600718). We thank R. Giacomantonio for help with Fig. 1.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1297/suppl/DC1
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References

31 July 2014; accepted 8 August 2014
10.1126/science.1259423

PERSPECTIVE

Antibiotic effectiveness: Balancing conservation against innovation

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Antibiotic effectiveness is a natural societal resource that is diminished by antibiotic use. As with other such assets, keeping it available requires both conservation and innovation. Conservation encompasses making the best use of current antibiotic effectiveness by reducing demand through vaccination, infection control, diagnostics, public education, incentives for clinicians to prescribe fewer antibiotics, and restrictions on access to newer, last-resort antibiotics. Innovation includes improving the efficacy of current drugs and replenishing effectiveness by developing new drugs. In this paper, I assess the relative benefits and costs of these two approaches to maintaining our ability to treat infections.

Since their introduction into modern medicine in 1941, antibiotics have saved millions of lives. Although access to antibiotics remains a problem—more than a million children with untreated pneumonia and sepsis die each year—the effectiveness of these drugs is declining globally, driven by ever-higher rates of antibiotic use and selection pressure for resistance (*1*). For example, gonorrhea, which was entirely susceptible to penicillin in the 1970s, is now becoming increasingly resistant to third-generation oral cephalosporins and is reemerging as a threat (*2*). Resistance elements, such as extended-spectrum β-lactamase (ESBL), NDM-1, and *Klebsiella pneumoniae* carbapenemase (KPC)–producing Enterobacteriaceae, have made many Gram-negative infections untreatable globally (*3*).

Antibiotic resistance is a problem of managing an open-access resource, such as fisheries or oil (*4*). Individual patients, doctors, pharmaceutical companies, hospitals, and even countries have little incentive to use antibiotics judiciously. Maintaining antibiotic effectiveness in the long term requires conservation, defined broadly as technological, medical, and incentive-based solutions to keep existing antibiotics working (Fig. 1); and innovation, defined as efforts to develop new antibiotics.

The two approaches are essentially linked: Just as incentives for finding new sources of oil reduce incentives to conserve oil, large public subsidies for new drug development discourage efforts to improve how existing antibiotics are used. And just as the cost of discovering new sources of oil becomes more expensive as the resource is depleted, new antibiotics are likely to cost more than existing ones—not only because the easiest-to-find resources may have been discovered but also because of higher regulatory costs (of clinical trials in the case of antibiotics and environmental protection in the case of oil) (*5*).

Conservation

Antibiotic use by humans is a significant driver of resistance. Global sales of antibiotics for human consumption increased 36% between 2000 and 2011, with Brazil, Russia, India, China, and South Africa accounting for 76% of the increase (*6*). Higher consumption was also noted for two "last-resort" classes of antibiotics, carbapenems (45%) and polymyxins (13%). Newer antibiotics tend to be more expensive but also used less frequently (Fig. 2). Reducing the need for antibiotics and reducing unnecessary antibiotic use will help keep existing antibiotics working. Reducing need is best achieved by reducing the burden of infections by: (i) improving public health and sanitation, especially in low-income countries where antibiotics are used to fill the gap created by unsafe water, poor sanitation, and deficient public health (approaches to deal with these problems are dealt with elsewhere in the special section); (ii) expanding the use of existing vaccines and investing in new vaccines; and (iii) improving hospital infection control.

The introduction of the pneumococcal conjugate vaccine has reduced the burden of pneumococcal disease, avoided many prescriptions for antibiotics, and lowered rates of invasive disease caused by strains not susceptible to penicillin. In the United States, resistant pneumococcal strains decreased by 59% between 1999 and 2004 to 1.7 cases per 100,000 (*7*). However, fewer than a quarter of the world's children are protected by pneumococcal conjugate vaccination, which is not yet part of the routine immunization programs in India and China. Furthermore, only 14 to 48% of health care workers in Europe reported being vaccinated against seasonal influenza (*8*), a common trigger for an inappropriate antibiotic prescription or for use against secondary infections. Unfortunately, a vaccine against *Staphylococcus aureus*, the most common cause of postoperative infection, has remained elusive, and recent trials have not been promising (*9*).

Reducing antibiotic overuse is the other part of conservation. In many low- and middle-income countries, nonprescription antibiotic use contributes to resistance (*10*). Even when prescriptions are needed to obtain antibiotics, physicians may not adequately screen for appropriate use. A potential solution is to allow over-the-counter access to first-generation antibiotics while more strictly regulating newer-generation antibiotics. Addressing fevers in low- and lower-middle–income countries through integrated management of childhood illnesses could reduce the burden of disease while also lowering antibiotic use (*11*, *12*). Improving hospital infection control through surgical infection prophylaxis, skin preparation, and preoperative

Fig. 1. Activists of the alliance My Agriculture protest against the use of antibiotics in intensive livestock farming in front of the Federal Chancellery in Berlin, Germany, on 18 January 2012. Several protesters dressed as chickens advertised another demonstration to take place on 21 January 2012. [Credit: EPA/Maurizio Gambarini]

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screening and decolonization can reduce the need for antibiotics, particularly in resource-poor settings that have made less progress in limiting health care–associated infections (*1*).

In many high-income countries, patient norms drive expectations for antibiotic prescribing (*13*). Public education, such as the French effort that led to a 5% reduction in the number of antibiotic prescriptions over the first 5 years (*14*), or the annual mass media campaigns in Belgium that reduced antibiotic prescriptions by 36% between 1999–2000 and 2006–2007 (*15*), should be deployed in high-prescribing countries (*16*).

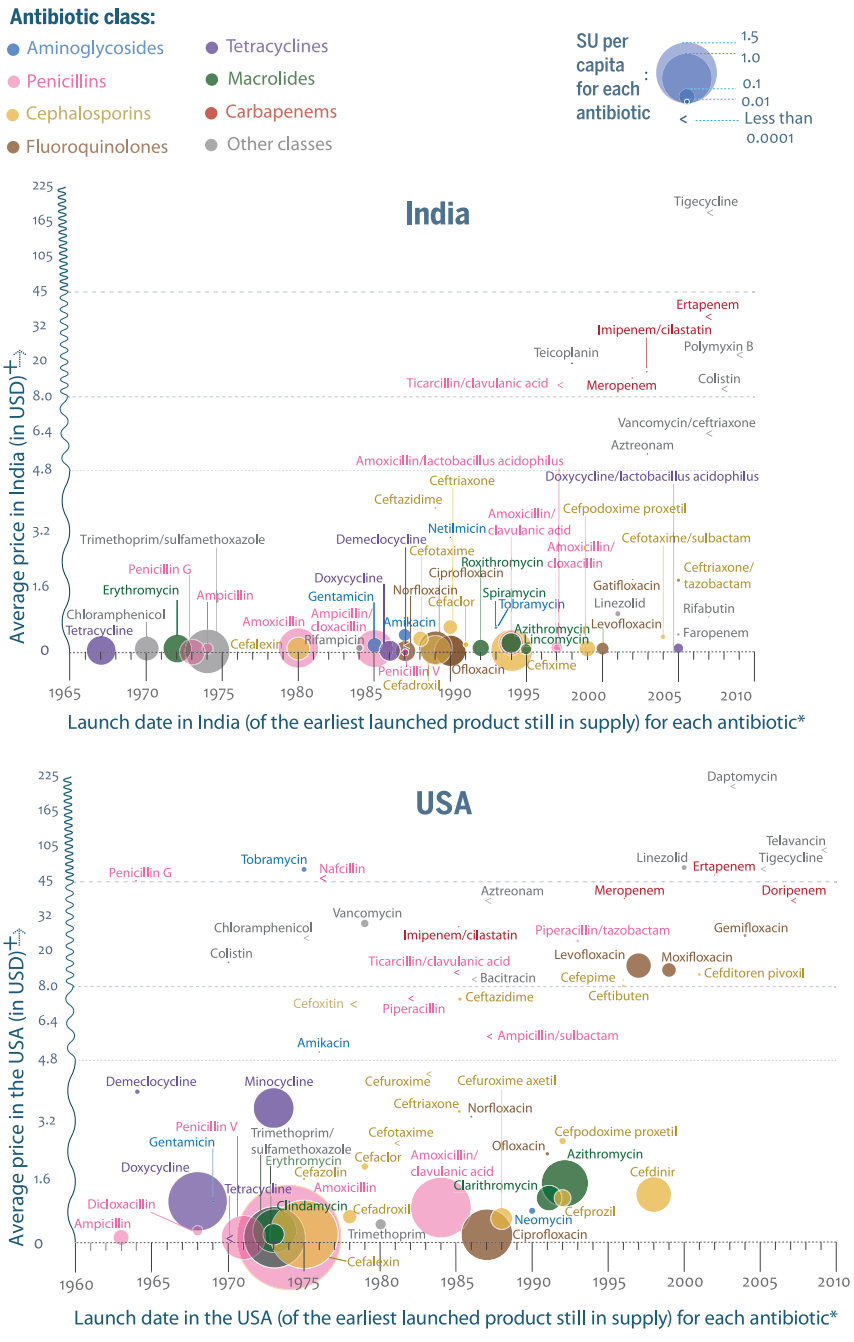

Fig. 2. Consumption of select key retail antibiotics in the United States and India in 2010, by year of market introduction and price. Bubble sizes correspond to the estimated quantity of standard units (SUs) per capita dispensed in the retail sector in 2010. Variable scaling is used on the y axis. Antibiotics that do not have sales data, revenue data, or the launch date reported are not shown. In the U.S. section, aztreonam/lysine, launched in 2010 with estimated sales of 318 SUs at an average price of $3750 USD during 2010, is not shown. *The date of market launch may differ from this measure. +The average price for each antibiotic is estimated by dividing total retail sales revenue by the number of SUs sold in the retail sector. [Data source: IMS MIDAS]

In China, hospitals that rely on pharmaceutical sales for income have an incentive to overprescribe; one study estimated that a quarter of the revenue in two hospitals came from antibiotic sales (*17*). In India, doctors routinely receive compensation from drug sellers in exchange for directing patients to their pharmacies (*18*). In Taiwan (*19*) and other parts of the world, antibiotic prescribing may also be influenced by competition between health care providers and from unsanctioned providers. Mass drug administration of antibiotics for the management of severe acute malnutrition (*20*) and trachoma control (*21*) could contribute to resistance at a mass scale if not monitored carefully (*22*).

Antibiotics are also used extensively in agriculture, in subtherapeutic concentrations, to promote growth and prevent disease in livestock. Associations have been reported between time spent on farms and colonization with multidrug-resistant *Staphylococcus aureus* (MRSA) (*23*, *24*). Medically important antibiotics have been withdrawn from subtherapeutic use for growth promotion in the European Union (by regulation) and in the United States (by nonbinding advisories).

Finally, genes for resistance to biocides, which include alcohols, phenols, and quaternary ammonium compounds, are often carried on the same multidrug resistance plasmid as antibiotic resistance genes (*25*). The widespread use of biocides coselects for antibiotic resistance genes and could promote the spread of multidrug resistance plasmids (*26*).

Innovation

Even if antibiotics are used appropriately, resistance is an inevitable consequence of drug selection pressure, necessitating the discovery, testing, and development of new antibiotics. The thin pipeline of new antibiotics reflects a declining trend in new drug development across all therapeutic areas, caused by the difficulty of innovation and the challenges of regulatory hurdles (*27*). Some new agents treat soft-tissue skin infections caused by MRSA and other conditions (*28*), but the newest antibiotics are at least two orders of magnitude more expensive than first-line antibiotics, putting them out of reach of many.

New antibiotics are given 5 years of additional market exclusivity under the Generating Antibiotic Incentives Now (GAIN) section of the 2012 Food and Drug Administration Safety and Innovation Act. Efforts to lower antibiotic development costs (such as by basing approval on more limited clinical evidence) are fruitful when they do not compromise safety and efficacy. Of the 61 new antibiotics approved between 1980 and 2009, 26 (43%) were withdrawn, compared with a 13% withdrawal rate for other therapeutic categories (*29*).

Pharmaceutical firms have little incentive to sell fewer antibiotics to reduce the likelihood of resistance. If anything, resistance makes existing antibiotics obsolete and creates demand for newer, more expensive antibiotics; market incentives may thus work against conservation's effectiveness.

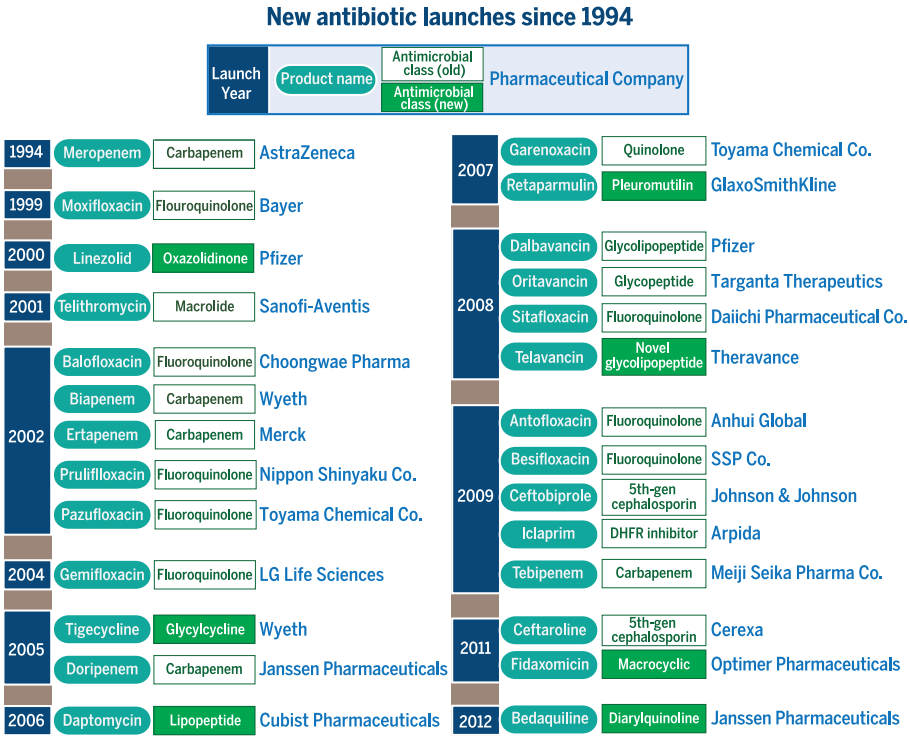

Fig. 3. Antibiotic pipeline for the past 20 years.

Insurers' and third-party payers' low reimbursement rates for antibiotics partly reflect value perceptions shaped by the low prices of older antibiotics; they discourage both conservation and new drug development.

Some have proposed delinkage as a solution to this problem: A different entity (such as the government) would purchase an antibiotic at a fixed price unrelated to the quantity sold and then ration it (*30*). However, it is unclear how a drug could be allocated to its highest-value use and whether government could perform this function any better than private markets.

What other kinds of innovation are most useful? Innovation could involve combination therapies, such as amoxicillin-clavulanate, that target both essential functions and resistance factors. Development efforts could repurpose old drugs to optimize dosing levels and the duration, and route of administration, and leverage pharmacokinetics and pharmacodynamics to identify promising combination drug therapies. For example, optimizing dosing of colistin, a drug first introduced in the 1950s, can reduce toxicity and improve efficacy (*31*).

Innovation could also aim at preventing the development of resistance and protecting nontarget gut bacterial flora during antibiotic treatments. For example, absorbents can prevent active antibiotic residues from reaching the gastrointestinal flora (*32*).

Innovation in point-of-care diagnostics to identify both the cause of an infection and its sensitivity to common antibiotics would be particularly appropriate for countries such as India, Brazil, and China, which need to preserve their ability to treat bacterial infections but are unlikely to be immediate markets for any new broad-spectrum antibiotics. More affordable diagnostics could significantly reduce unnecessary antibiotic prescriptions and ensure that the right antibiotic is prescribed.

Balancing conservation and innovation

The issue of antibiotic resistance has often been framed as a crisis in new drug development, but with little supporting evidence. The pharmaceutical industry has responded to the threat of soft-tissue MRSA infections by developing new antibiotics, including most recently a single-dose Oritavancin or a two-dose Dalbavancin for treatment in outpatient settings (*33*, *34*). Contrary to popular belief, many new antibiotics have been introduced during the past two decades (Fig. 3), but their cost is much greater than that of older antibiotics, and many have been pulled from the market. Direct public financing such as the recent $200 million grant to GlaxoSmithKline by the Biomedical Advanced Research and Development Authority to produce a new antibiotic (*35*) may increase the number of drugs in the pipeline but does not address issue of cost to consumers or of missing incentives for conservation.

The benefits of conserving existing drugs are significant. A 1-year delay in the need for a $1 billion investment in a new antibiotic is worth roughly $60 million, even at a modest 6% discount rate (according to my calculations). We should therefore be willing to collectively spend at least that amount to shift the resistance curve back 1 year for a single antibiotic. It would cost hundreds of billions of dollars to replace our entire portfolio of antibiotics, so conservation would appear to be a viable strategy. Until recently, however, the United States has spent less than $5 million per year on conserving antibiotic effectiveness through public education, research, and surveillance, and even with recent increases in allocations to antibiotic resistance programs at the U.S. Centers for Disease Control and Prevention, the imbalance remains. As long as we inhabit a world of microbes, we must focus more resources and effort on the conservation of existing antibiotics by ensuring their appropriate—and less frequent—use, while finding ways to incentivize the development of new antibiotics, using mechanisms that promote appropriate use.

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10.1126/science.1254163

PERSPECTIVE

Creating a global observatory for health R&D

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A global map of health R&D activity would improve the coordination of research and help to match limited resources with public health priorities, such as combating antimicrobial resistance. The challenges of R&D mapping are large because there are few standards for research classification and governance and limited capacity to report on R&D data, especially in low-income countries. Nevertheless, based on developments in semantic classification, and with better reporting of funded research though the Internet, it is now becoming feasible to create a global observatory for health R&D.

The issue of knowing what research is currently being undertaken—where, by whom, and which organizations are supporting it—is a black hole in the public health landscape. In a funding environment still reeling from the economic crisis, being able to do more with what we have—thinking inside the box—is a priority, especially for those diseases disproportionately affecting developing countries and for which normal market incentives fail. Market failure describes those situations where the members of a population that suffer most from a disease—for example, leishmaniasis carried by sand flies—are too poor to provide a market that incentivizes industry to undertake research and development (*1*, *2*).

How to finance research and development where normal market forces are absent has been the focus of a number of studies organized by the World Health Organization (WHO), culminating in 2012 with a report that assessed the strengths and weaknesses of more than 100 new financing mechanisms (*3*). One of the issues that became clear in compiling this report was the absence of good data. There is no global health R&D map that provides a comprehensive picture of research funding, ongoing research, and results that could be used to guide the allocation of the limited available funding. Consequently, the member states of WHO have called for the establishment of a global observatory on health R&D to address this lack of information (*4*). The vision is of an observatory that would compile data from bibliometrics and available databases on patents, registered clinical trials, the product pipeline, and the data related to current and ongoing R&D held in the databases of research funders (Fig. 1) (*5*).

Although some of the information necessary to create a global health R&D map can be found in existing databases, there is nothing that covers all diseases for all countries. There are a number of regular R&D surveys undertaken by, for example, the Organisation for Economic Cooperation and Development (OECD) for its members; Red de Indicadores de Ciencia y Tecnología (RICYT), covering Spain, Portugal and 29 countries in the Americas; and the UNESCO Institute. However, these data are not interoperable because of the varied methods of classifying the research and the different degrees of detail reported—for example, information on medical research as opposed to different diseases. This means that if you wanted to understand a global picture of, for example, research into new antibiotics, you would be left with undertaking a lengthy and expensive survey with all the difficulties such surveys entail.

One of the biggest challenges we face is developing capacity for collecting and reporting data on R&D funding when only 37% of countries are currently able to publicly report data of this type (*5*). Work in the WHO Regional Office for the Western Pacific has begun to address this challenge. The office has developed software that creates a management system to register a research project and prepare it for ethics approval. Technical assistance has been provided to five developing countries within the region: Cambodia, Laos, Fiji, Mongolia, and Papua New Guinea; subsequently some of them have launched national registries (*6*). Although this will not capture all health research, the approach of combining ethics approval with reporting on R&D funding provides a good example of getting multiple uses out of a single collection of data.

As with many WHO projects of this type, it is a new activity and will require new and additional funding outside of its existing budget. A conservative estimate is that $11.5 million will be needed in the first 5 years to cover project

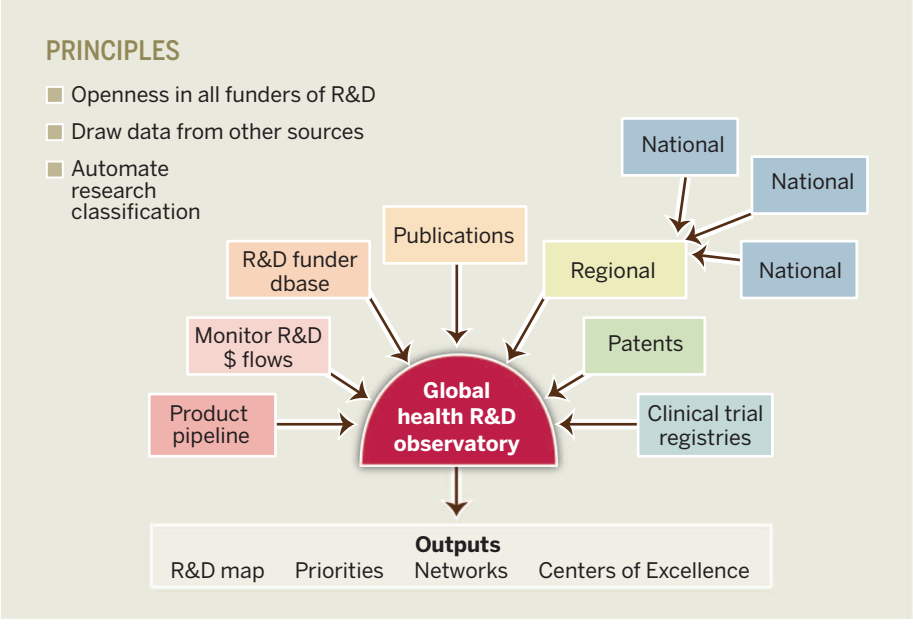

Fig. 1. Components of a global health observatory. Much of the initial data for the observatory will be garnered from available sources such as the following (examples rather than complete lists are shown). Publications: Medline and PubMed, the Biblioteca Virtual em Saúde (BIREME), Thomson Reuters, and others. Regional R&D hubs: Health Research Web, Regional Platform on Access and Innovation for Health Technologies (PRAIS), and others. Patents: World Intellectual Property Organization (WIPO) Patent Scope, Re:Search, WIPO Gold, and national and regional patent databases. Clinical trial registries: WHO International Clinical Trials Registry Platform. Product pipeline: Global Health Primer. Monitoring of R&D funding flows: UNESCO Institute for Statistics, OECD, Eurostat, RICYT, G-FINDER (31 neglected tropical diseases), Association of Southeast Asian Nations (ASEAN) science and technology indicators, Organization of Islamic States R&D, African Union disease-specific tracking (e.g., HIV). R&D funder databases: ÜberResearch, World RePORT, and others.

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staff and software development and to build capacity in those countries (the majority) that do not report health R&D data.

A proposal: Automated mapping of the research landscape

The aim is to create a global health R&D observatory by adopting the latest approaches in data mining to maximize the collection, synthesis, and interpretation of data, and to minimize the cost of bringing the data together.

As more of the administration of R&D becomes digital, more of it comes online on the Web sites of individual researchers, their institutions, and the research funders. This provides raw data but adds little knowledge. For a global R&D observatory to be feasible, automating the coding and subsequent association of available data will be essential. When operational, this would enable data on research funding to be recorded locally in whatever form suits that context, but, through automated translation, it could be reported against a global standard (*7*).

This requires a culture shift to create greater transparency in the R&D system by publishing more information on current research that is open access, allowing automated reading by machines such as Web crawlers. To create a map of current activity, all public research funders and academic institutions (and eventually industry) should publish metadata, abstracts, or even whole texts of current research protocols on the Web in a machine-readable code, such as XML. This will require standards, because there are numerous variations in the current research information systems (CRIS) used by many universities, particularly in Europe and North America. However, if the funders of research could publish a common set of metadata points—for example, principal investigator, host institute, project title, project abstract, time scales, and amount and source of funding, then it would be technically feasible to create a current research map by means of search engines rather than surveys. This is not as insurmountable an obstacle as it might seem; access to Medline via PubMed only began in 1997, and the system has quickly moved from manual deposition of citation data to an automated system that is integrated into the workflows of medical publishers. We need to build a similar system into our research-funding processes.

The major challenge is classifying the research itself. There are high-level research classification systems that exist, and the OECD's Frascati manual is a good example used to estimate gross domestic expenditure on R&D (GERD). However, the Frascati methodology aggregates R&D funding under medical and health sciences, with subcategories, but does not report the detail below this. It is used mainly by OECD countries.

So the opportunity cost of first agreeing on a standard and then getting every researcher and funding body to use it accurately is large. The alternative is to develop semantic-based classification using search engines that can automate the classification step. Health has an advantage over other sciences because it already has a number of comprehensive vocabularies with agreed-upon definitions or ontologies—for example, the Unified Medical Language System of the NIH and the International Classification of Diseases (ICD). Although there is no single global standard, these ontologies provide the seed from which automated classification can grow.

In South America at the end of the 1990s, the Brazilian Council of Science, Technology, and Innovation (CNPq) launched the Lattes Platform, with a goal of providing information services for researchers, students, institutions, research groups, and agency staff. Although originally conceived as a centralized database, during the first years of operation it developed a roadmap for improving access to data and providing an ontological classification to help share information (ConscienTias LMPL (Comunidade para Ontologias em Ciência, Tecnologia e Informações de Aperfeiçoamento de Nível Superior/Linguagem de Marcação da Plataforma Lattes)) (*8*). Most of this standardization work was coordinated by the Stela Institute/Stela Group, a nonprofit Brazilian research organization that has been responsible for the development of other Brazilian government systems, including Aquarius, a newly launched "all federal funds" investment tracking system, and many other federal- and state-based infrastructure systems. Today, it is an exemplar of how individual researcher data (for example, standardized biographical data) can be integrated with other information (such as publications) to create a picture of research at institutional and national levels.

> ***"There is no global health R&D map that provides a comprehensive picture of research funding, ongoing research, and results that could be used to guide the allocation of the limited available funding. --The vision is of an observatory that would compile data from bibliometrics and available databases on patents, registered clinical trials, the product pipeline, and the data related to current and ongoing R&D held in the databases of research funders"***

Using the experience of the Lattes Platform Stela Institute, the National Science Foundation, the National Institutes of Health, and the Office of Science and Technology Policy have discussed a similar architecture for the American Research Profile System. The intention is to use semantic technology and a data adapter to transform proprietary data (researcher IDs, CVs, funded protocols, patents, and publications) from different data sources to a common format to create Linked Open Data (LOD).

By starting with the individual researcher profile, an R&D map could be built from the bottom up—as opposed to surveys, which tend to be top down. Such a system enables social network analysis, identifying research collaborations, coauthorship distance networks, and the degree of crossdisciplinary research (*9*).

This vision of semantic, automated classification sounds a long way off, but current efforts demonstrate more than just proof of principle. For example, ÜberResearch has recently produced an analytical tool and an R&D database from a selection of public health research funders, including NIH and the Wellcome Trust. It analyzed the abstracts of current research projects available on their Web sites by means of semantics and coded them to the Medical Subject Headings (MeSH) of the National Library of Medicine (*10*). The resulting database allows searches to be conducted from a user's desktop and demonstrates the value of analyzing research trends, visualizing research networks, and identifying centers of excellence, even using the limited data set that ÜberResearch can currently access from public sites.

Next steps

At a technical workshop hosted by the Wellcome Trust in February 2013, a number of agencies that collect R&D data were brought together to discuss the feasibility of creating a global resource for health research. The key recommendations were to begin by building on data that already exists, to understand what users want and what they will use in planning their own R&D activities. Understanding user needs will be vital for creating a resource that can be sustainably supported.

Using these criteria, two existing resources on R&D were investigated as a first step. Emory University manages a database called the Global Health Primer that seeks to map the pipeline for health technologies (diagnostics, vaccines, and drugs) in development for the neglected tropical diseases (NTDs). Separately, the G-FINDER database operated by Policy Cures tracks funding for R&D in the NTDs area by more than 3600 research funders (*11*, *12*). We therefore commissioned a stakeholder analysis to design a platform linking these data together.

A consultation was conducted that included interviews with 27 of the major stakeholders of R&D in NTDs responsible for 73% of global

funding for neglected diseases R&D in 2012. In addition, an online survey attracted 91 responses from 30 different countries. The findings support the need for a new platform, with the majority of respondents wanting a resource that they can rely on in terms of quality, that is regularly updated for use in planning, and that is relatively simple to use. Their key request was for a common tool or portal to provide access to both R&D funding and pipeline information, including detail on diseases, products, funders, and developers. The stakeholders preferred to have the ability to access the underlying data for their own analyses rather than being given a suite of ready-provided analyses Presenting the data as simple tables and graphs was preferred over interactive animations and maps, even if these were seen as "nice-to-haves." With regard to content, in addition to the data providing a current picture of R&D funding by disease, product, and organization, there were requests for predictive analysis trying to estimate future funding needs. An example was the need for advance warning to fund expensive late-stage clinical trials if early development looks promising (*13*). On the basis of these findings, a business case for this platform—which would form one part of a global health observatory—is being designed, with a beta version expected to be launched in 2015.

The political driver for establishing such an observatory grew from discussions about noncommercial areas of health research in the NTDs, and this is the initial focus of the observatory. However, market failure affecting R&D is not only an issue in the prevention and treatment of diseases of poor countries. As antimicrobial resistance continues to rise, the pipeline for new antibiotics is not supplying enough new drugs (*14*, *15*). So, among other factors, new financial incentives and more public money will be needed to cover the risk of R&D for antibiotic development, increasing the need to ensure efficiency in the public R&D system. Consequently, there have already been calls from the WHO member states for the observatory to expand its remit to include a mapping of antimicrobial resistance R&D as one way to facilitate global collaboration and improve efficiency (*16*).

Open access to research publications and, increasingly, to data is now well established as a principle of good research practice. To a large degree, this has been facilitated by the policies of the health research funders. In moving forward, what would be really beneficial is if those open-access principles could also be applied to the data sets that the research funders hold themselves.

With the call from health ministers through WHO and the recent findings of our consultation, there is a desire for better understanding of global health R&D and a need to improve the coordination of research, particularly around global efforts. This desire in itself is not new, but with the potential of the Internet and innovations in automated research mapping techniques, it is now becoming feasible to create a global observatory for health R&D.

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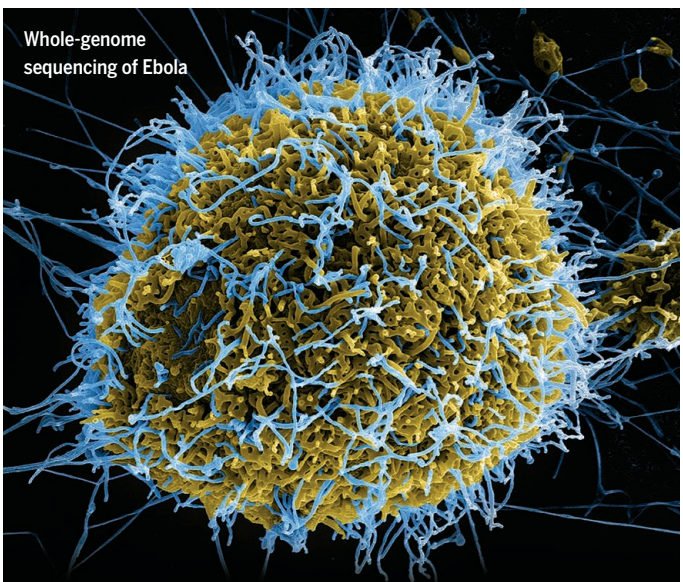
10.1126/science.1258737

RESEARCH

The black, silver, blue, yellow-orange, and gold of zebrafish stripes
Mahalwar et al. *and McMenamin* et al., *pp. 1362 & 1358*

IN *SCIENCE* JOURNALS

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



VIRAL EVOLUTION

Evolution of Ebola virus over time

The high rate of mortality in the current Ebola epidemic has made it difficult for researchers to collect samples of the virus and study its evolution. Gire *et al.* describe Ebola epidemiology on the basis of 99 whole-genome sequences, including samples from 78 affected individuals. The authors analyzed changes in the viral sequence and conclude that the current outbreak probably resulted from the spread of the virus from central Africa in the past decade. The outbreak started from a single transmission event from an unknown animal reservoir into the human population. Two viral lineages from Guinea then spread from person to person into Sierra Leone. — LMZ

Science, this issue p. 1369

PLANETARY DYNAMICS

Orbits don't forget their chaotic pasts

Many exoplanets orbit in planes that don't correspond to their star's spin axis. This phenomenon may be the fossil record of a dynamical history. Storch *et al.* demonstrate in simulations that the evolution of a star's spin is key in determining how its planets' orbits are eventually oriented. A binary companion body can drive a hot Jupiter to migrate inward, which induces chaotic behavior in the stellar spin axis and feedback down the line. If we can accurately replicate the statistics observed in planetary surveys, we may understand better how planets form. — MMM

Science, this issue p. 1317

WATER SPLITTING

Scheduling hydrogen release from water

Photosynthesis splits water to provide protons and electrons for plant growth; oxygen is a by-product. When chemists split water, they're also more interested in making fuel, and the simplest product is hydrogen (a combination of protons and electrons). One challenge is keeping the reactive hydrogen and oxygen product streams separate. Rausch *et al.* present a scheme that captures the protons and electrons in a molecular cluster of silico-tungstic acid. Later, they expose the cluster to platinum, coaxing the acid into releasing hydrogen. Eliminating the mixing risk increases the potential for household use. — JSY

Science, this issue p. 1326

PHYSIOLOGY

Regulating nitric oxide production

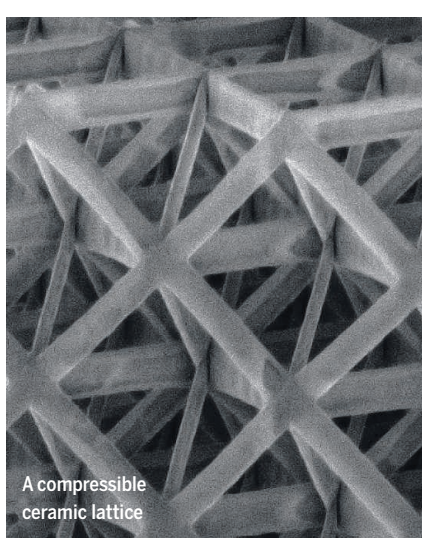
Blood vessels relax and enable increased blood flow when the enzyme eNOS produces nitric oxide (NO) in the endothelial cells lining the vessels. The activity of eNOS varies with modifications to specific sites in the enzyme, such as S-sulfhydration, a process that is triggered by the gas hydrogen sulfide (H_2S). Altaany *et al.* found that S-sulfhydration of eNOS increased its activity by affecting other modifications to the enzyme. Endothelial cells from mice that could not produce H_2S also produced less NO than those from normal mice. Enhancing the S-sulfhydration of eNOS may be an effective strategy to increase blood flow in patients. — WW

Sci. Signal. **7**, ra87 (2014).

LOW-DENSITY MATERIALS

Compressive, ductile ceramic nanolattices

Ceramics are strong and stiff, but their limited ability to stretch like putty or steels makes them unsuitable for many engineering applications. Meza *et al.* constructed ceramic nanolattices from aluminum oxide, in which the beams are designed to stretch rather than bend. A key parameter in lattice design is the ratio of the wall thickness to the beam radius. When that ratio is small enough, compressing the beams does not break them. That way, the nanolattices



PHOTOS: (TOP TO BOTTOM) MAHALWAR *ET AL.*; NIAID; MEZA *ET AL.*

Downloaded from www.sciencemag.org on September 12, 2014

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can be highly compressed and recover to something close to their original shape when the stress is removed. – MSL

Science, this issue p. 1322

SOCIAL PSYCHOLOGY

Moral homeostasis in real life vs. the lab

Individuals who witnessed a moral deed are more likely than nonwitnesses to perform a moral deed themselves and are also more likely to allow themselves to act immorally. Hofmann *et al.* asked smartphone users to report their encounters with morality (see the Perspective by Graham). Most moral judgment experiments are lab-based and don't allow for conclusions based on what people experience in their daily lives. This field experiment revealed that people experience moral events frequently in daily life. A respondent's ideology influenced the kind of event reported and the frequency, which is consistent with moral foundations theory. — GJC

Science, this issue p. 1340; see also p. 1242

MICROBIAL ECOLOGY

Patterns of life in the ocean wave

The open ocean is not uniform. The seas teem with unseen life that has evolved distinctive patterns of species distribution, much as the land-bound world contains a spectrum of biological provinces. Taking data from genome studies, Hellweger *et al.* devised a model showing how marine bacteria diversified throughout the world's oceans into stable geographic provinces independently of natural selection (see the Perspective by Giovannoni and Nemergut). All that is needed to create these distributions are mutations and ocean currents. — CA

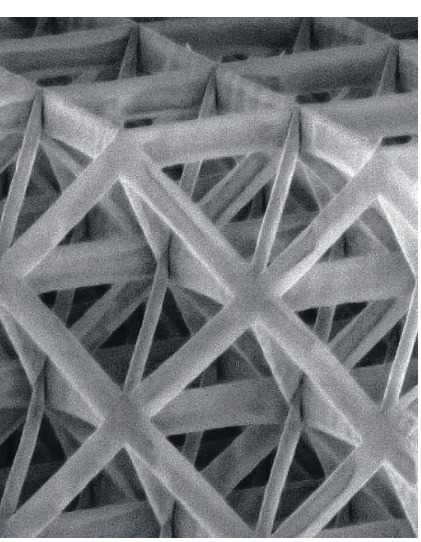
Science, this issue p. 1346; see also p. 1246

ERROR MEMORY

Practice makes perfect – or does it?

How do we learn from past errors? Herzfeld *et al.* found that when we practice a movement, the human brain has a memory for errors that is then used to learn faster in new conditions. This memory for error exists in parallel with motor memory's two traditional forms: memory of actions and memory of external perturbations. They also proposed a mathematical model for learning from errors. This model explained previous experimental results and predicted other major findings that they later verified experimentally. — PRS

Science, this issue p. 1349



MAGNETISM

All-optical magnetic state switching

Magneto-optical memory storage media, such as hard drives, use magnetic fields to change the magnetization of memory bits, but the process is slow. Light can often reveal information about the magnetization state of a sample, such as its field direction. Lambert *et al.* show that under the right circumstances, light can also switch the magnetization state of a thin ferromagnetic film. Using light pulses instead of magnetic fields led to ultrafast data memory and data storage. — ISO

Science, this issue p. 1337

Damage in Hyde Park, London, after the October 1987 storm

PHOTO: GEORGE BERNARD/NHPA/PHOTOSHOT/NEWSCOM

FOREST ECOLOGY

Legacy of a storm

A severe windstorm has increased plant species diversity in British broadleaved woodlands. In September 1987, hurricane-force winds uprooted an estimated 15 million trees in southeastern England. Smart *et al.* monitored the understory plant community in forest plots before and after the storm, and compared affected and unaffected sites. In the 15 years after the storm, the number of species in wind-damaged plots increased by 32%. Most were native woodland species rather than invasives. This single massive event reversed a 40-year background trend toward decreased plant diversity in undisturbed woodlands. — AMS

J. Ecol. 10.1111/1365-2745.12291 (2014).

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ANTIBIOTIC RESISTANCE

Giving bacteria the old one-two...three-four

Is it possible to streamline the complex task of finding new drugs to fight resistant bacteria and other disease targets? Most biological processes are controlled by complicated regulatory networks, so combinations of two or more drugs are likely to be more effective than any single agent. Finding combinations that work means first screening enormous numbers of possibilities. Cheng *et al.* examined mixtures of genetic elements in millions of different combinations. Those combinations with the desired effect in a biological test could be identified afterward by high-throughput sequencing capable of detecting associated DNA "barcode" identifier sequences. Results are promising and revealed combinations of transcription factors that enhanced lethal effects of an antibiotic by a millionfold. — LBR

Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1400093111 (2014).

INFLUENZA

Flu survivors are an inflammatory club

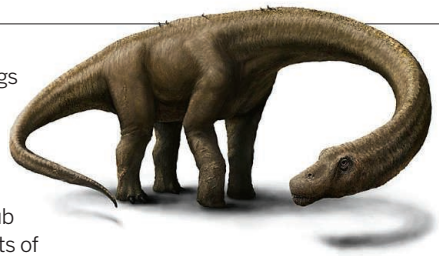
Kill it: That is the immune system's response to most viral infections, including influenza. Eliminating infected cells rids the body of the infection. Heaton *et al*. now report that a special type of epithelial cell in the lungs of mice—called club cells—survive influenza infection. How do they do it? Gene expression analysis suggests that club cells express high amounts of antiviral genes in response to infection. Although this process probably helps the animal contain the virus during early infection, club cells also produced pro-inflammatory molecules that cause lung pathology. Whether club cells play a role in inflammation-induced mortality, as seen in the H5N1 and H1N1 influenza pandemics, remains to be seen. — KLM

J. Exp. Med. 10.1084/jem.20140488 (2014).

PALEONTOLOGY

New titanosaur unearthed in Argentina

Researchers working in Argentina have discovered the most complete skeleton of a titanosaur, giant plant-eating dinosaurs that dominated the Southern Hemisphere beginning 90 million years ago. *Dreadnoughtus schrani* was 26 m long and weighed 59 metric tons—twice as long as *Tyrannosaurus rex* and as heavy as a herd of elephants. Lacovara *et al*. recovered 70% of the dino's skeleton, including most of its vertebrae but not the head. The researchers say the beast was so big it would have had no fear of predators (*Dreadnoughtus*, from Old English, means "fearing nothing"). And an examination of its back and shoulder bones indicates that the animal was still growing when it died. — MB



Sci. Rep. 10.1038/srep06196 (2014).

OPTICAL METROLOGY

Shining light on precision time-keeping

Keeping time typically works by counting the ticks on a clock. A new generation of clocks based on the optical transitions of atoms can sub-divide each second into billions of finer ticks, making it possible to measure even the fleetest of moments. The physical mechanism (or clockwork) of such accurate timepieces is complex, limiting them largely to national laboratories. Papp *et al.* show that a chip-based approach, in which optics are integrated with semiconductor-based microresonators, can provide an easy way to engineer such complex clockwork. Their approach promises a new era of metrology that makes precision timekeeping widely available. — ISO

Optica 10.1364/OPTICA.1.000010 (2015).

DRUG DELIVERY

A complementary refill? Yes, please!

Drugs delivered throughout the body often cause collateral damage to healthy tissues. When disease or injury is localized, patients can avoid this problem by using a drug-delivery device implanted in the target tissue. However, such devices eventually run out of drugs and must be removed surgically and refilled. Brudno *et al*. designed a drug-delivery device that can be refilled noninvasively and tested it in a mouse tumor model. They made the device from a gel tethered to short DNA sequences. To refill it, they coupled gel strands to drugs and tethered them to complementary DNA sequences, then injected the strands intravenously into the mice. Because of the complementary DNA sequences, the strands homed directly to the device. — PAK

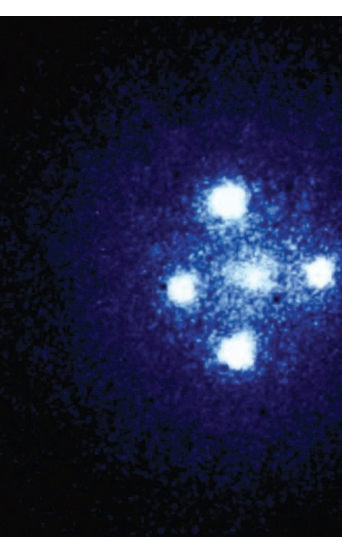
Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1413027111 (2014).

EDUCATION

Collaborating on assessments

When teachers collaborate with educational researchers, everyone wins: Teachers focus on their practice and researchers learn about the challenges teachers face. Education reforms require these often separate communities to overlap. Szteinberg *et al.* merged both groups and tasked them with developing a sequence of knowledge that students are expected to learn in their chemistry class, as well as a way to assess their learning. Participants evaluated their own work, and responses suggest that the approach to merge theory and practice greatly influenced teachers' views of assessment. Specifically, teachers reported a greater value on measuring the substance of their students' thinking and less emphasis on the selection of assessments themselves. — MM

J. Chem. Educ. 10.1021/ed5003042 (2014).



BLACK HOLES

The spinning heart of the Einstein Cross

Supermassive black holes power entire galaxies from their cores, but how they grew so large is uncertain. When astronomers describe a black hole, they rely on two features: its mass and how fast it spins. The spin rate provides information about how the black hole grew, but it is tricky to measure. Reynolds et al. have determined the spin for the most distant quasar yet, a source called the Einstein Cross. This source got its name because the quasar's light is bent around a massive galaxy and reimaged four times. The high spin rate suggests that several dramatic accretion events produced the black hole rather than many small ones. — MMM

Astrophys. J. Lett. 10.1088/2041-8205/792/1/L19 (2014).

PHOTOS: (TOP TO BOTTOM) JENNIFER HALL; NASA/ESA/STSCI

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ICE SHEETS

Top-down rather than bottom-up change

The Larsen-B Ice Shelf in Antarctica collapsed in 2002 because of a regional increase in surface temperature. This finding, reported by Rebesco *et al.*, will surprise many who supposed that the shelf's disintegration probably occurred because of thinning of the ice shelf and the resulting loss of support by the sea floor beneath it. The authors mapped the sea floor beneath the ice shelf before it fell apart, which revealed that the modern ice sheet grounding line was established around 12,000 years ago and has since remained unchanged. If the ice shelf did not collapse because of thinning from below, then it must have been caused by warming from above. — HJS

Science, this issue p. 1354

QUANTUM INFORMATION

Manipulating a defect in diamond

Like magnetic storage in today's classical computers, tiny "magnets" associated with electronic and nuclear states—spins—are promising qubits (quantum bits) for the future. Electronic spins in special defects in diamond called nitrogen-vacancy (NV) centers are one example. Whereas most applications focus on the least energetic (ground) state of an NV center, Bassett *et al.* explored the properties of the higher-energy (excited) state (see the Perspective by Childress). They used light pulses to bring the system into the excited state and to vary the time it stayed there. In this way, they could both deduce the electronic structure of the excited state and manipulate the ground state spin. Similar methods may be applicable to other quantum information systems. — JS

Science, this issue p. 1333; see also p. 1247

MAKING STRIPES

Origin of fish pigment cell for pattern

Zebrafish stripes arise from the interactions of pigment cells: black melanophores, iridescent iridophores, and yellow-orange xanthophores. Melanophores and iridophores develop from nerve-associated stem cells, but the origin of xanthophores is unclear. Two studies now reveal that adult xanthophores originate from xanthophores in embryonic and larval fish, when they proliferate to cover the skin before the arrival of black and silver cells in a striped arrangement. Mahalwar *et al.* show that xanthophores change their final shape and color depending on their location. In black cells, xanthophores appear faint and stellate, but in silver cells, they are bright and compact. Precise superposition creates the blue and golden colors. McMenamin *et al.* observe the loss of pigment in embryonic xanthophores and the later reappearance in the adult. They show that redifferentiation depends on the thyroid hormone that also limits melanophore population expansion. – BAP

Science, this issue p. 1362 and p. 1358

PHOTO: LARISSA B. PATTERSON AND DAVID M. PARICHY

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BLACK HOLE PHYSICS

Sheltered black hole seeds grow faster

Astrophysicists have struggled to find a way to grow supermassive black holes within the first billion years of the universe, in the first 6% of its current age. These objects power the extremely bright and distant quasars whose light from that era has finally reached us. To grow a black hole so massive so quickly seemed to violate the expected balance of both radiation pressure and angular momentum. Alexander and Natarajan report that the predicted accretion rate can be exceeded with the help of a protective gas cloud at the heart of a star cluster. There, the radiation from accretion is reverted back onto the black hole, enabling a phase of supra-exponential growth for a brief 10 million years. — MMM

Science, this issue p. 1330

MUCOSAL IMMUNOLOGY

Immune cells and bugs make a sugary coat

Epithelial cells line the intestinal tract and help to keep the peace between our immune system and our trillions of gut microbes. Such peacekeeping requires glycosylated proteins (proteins with attached carbohydrate chains) present on the epithelial cell surface, but how glycosylation occurs is unclear. Goto *et al.* find that fucosylation (a type of glycosylation) of gut epithelial cells in mice requires gut microbes (see the Perspective by Hooper). This process also requires innate lymphoid cells there, which produce the cytokines interleukin-22 and lymphotoxin, presumably in response to microbial signals. These cytokines signal epithelial cells to add fucose to membrane proteins, which allows the détente between microbes and immune cells to continue. – KLM

Science, this issue p. 1310; see also p. 1248

ORIGIN OF NOTOCHORD

Origin of the spine lies in a worm

The notochord, the developmental backbone precursor, defines chordates—the group of animals to which humans belong. The origin of the notochord remains mysterious. Lauri *et al.* report the identification of a longitudinal muscle in an annelid worm that displays striking similarities to the notochord regarding position, developmental origin, and expression profile. Similar muscles, termed axochords, are found in various invertebrate phyla. These data suggest that the last common ancestor of bilaterians already possessed contractile midline tissue that, via stiffening, developed into a cartilaginous rod in the chordate line. — BAP

Science, this issue p. 1365

IMMUNOGENETICS

Response to pathogens is in the genes

T cells are important in mounting immune responses to a host of pathogens, and they have also been implicated in autoimmune disease. By examining the variability in gene expression in stimulated T cells over time from a multi-ethnic cohort of healthy humans, Ye *et al.* identified specific genetic polymorphisms that explain differences among individuals in response to pathogens. Furthermore, a candidate gene approach led to the identification of a single-nucleotide polymorphism that controls the response of the autoimmune-associated IL2RA gene. This study helps us understand the degree to which immune responses are driven by the environment or by an individual's physiological or genetic factors. — LMZ

Science, this issue p. 1311

BIODIVERSITY LOSS

Costa Rican birds of a feather lost together

Evolutionary history is lost when land is converted for farming, and recently evolved species may cope better with changing land use. Frishkoff *et al.* compared bird diversity over 12 years in three different kinds of landscape in tropical Central America. They mapped their data onto the bird evolutionary tree and found that more evolutionary branches were lost in intensive agricultural landscapes than in mixed landscapes. In turn, mixed landscapes lost more evolutionary branches than forest reserves. This is not just because of species loss; in fact, mixed agricultural landscapes contained similar numbers of species to those in forest reserves. Evolutionary history is lost because the more evolutionarily distinct species—those with fewer extant relatives and a longer evolutionary history—are more likely to become extinct in agricultural land. – AMS

Science, this issue p. 1343

PHOTOACCLIMATION

Making sure leftover light gets in

Bacteria that harvest sunlight through photosynthesis are everywhere and are vital for absorbing carbon dioxide. Like plants in a jungle, these cells have to compete for access to light. Gan *et al.* found that one species of such cyanobacteria, *Leptolyngbya*, survives on the scraps of leftover light that other photosynthetic cells can't use. When the light is crowded out by competing organisms, *Leptolyngbya* changes the pathways in its cells that make the light-harvesting molecules and remodels them into structures that can use redder light at longer wavelengths that is unused by other cells. — CA

Science, this issue p. 1312

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RESEARCH ARTICLE SUMMARY

MUCOSAL IMMUNOLOGY

Innate lymphoid cells regulate intestinal epithelial cell glycosylation

Yoshiyuki Goto, Takashi Obata, Jun Kunisawa, Shintaro Sato, Ivaylo I. Ivanov, Aayam Lamichhane, Natsumi Takeyama, Mariko Kamioka, Mitsuo Sakamoto, Takahiro Matsuki, Hiromi Setoyama, Akemi Imaoka, Satoshi Uematsu, Shizuo Akira, Steven E. Domino, Paulina Kulig, Burkhard Becher, Jean-Christophe Renauld, Chihiro Sasakawa, Yoshinori Umesaki, Yoshimi Benno, Hiroshi Kiyono*

INTRODUCTION: The combination of food intake and the resident gut microbiota exposes the gastrointestinal (GI) tract to numerous antigens. Intestinal epithelial cells (ECs) compose a physical barrier separating the internal organs from the gut microbiota and other pathogenic microorganisms entering the GI tract. Although anatomically contained, the gut microbiota is essential for developing appropriate host immunity. Thus, the mucosal immune system must simultaneously maintain homeostasis with the gut microbiota and protect against infection by pathogens. Maintenance of the gut microbiota requires epithelial cell-surface glycosylation, with fucose residues in particular. Epithelial fucosylation is mediated by the enzyme fucosyltransferase 2 (Fut2). Polymorphisms in the *FUT2* gene are associated with the onset of multiple infectious and inflammatory diseases and metabolic syndrome in humans.

RATIONALE: Despite its importance, the mechanisms underlying epithelial fucosylation in the GI tract is not well understood. In particular, although commensals such as *Bacteroides thetaiotaomicron* induce epithelial fucosylation, how mucosal immune cells participate in this process is unknown. We used a combination of bacteriological, gnotobiological, and immunological techniques to elucidate the cellular and molecular basis of epithelial fucosylation by mucosal immune cells in mice, especially innate lymphoid cells (ILCs). To explore the role of ILCs in the induction and maintenance of epithelial fucosylation, we used genetically engineered mice lacking genes associated with the development and function of ILCs. To investigate the physiological functions of ILC-induced epithelial fucosylation, we used a Fut2-deficient mouse model of *S. typhimurium* infection.

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Read the full article at http://dx.doi.org/10.1126/science.1254009

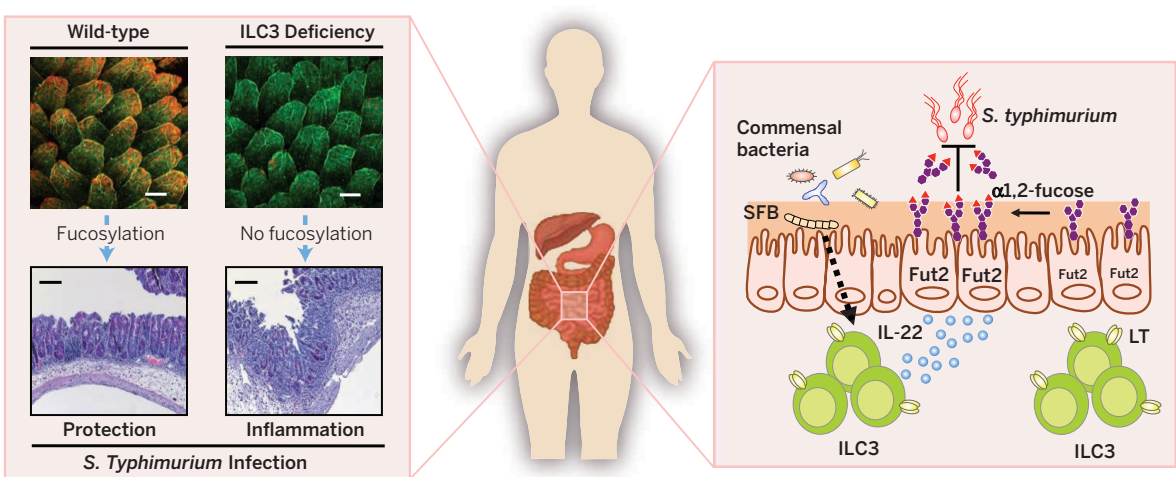
RESULTS: The induction and maintenance of Fut2 expression and subsequent epithelial fucosylation in the GI tract required type 3 ILCs (ILC3s) that express the transcription factor RORγt and the cytokines interleukin-22 (IL-22) and lymphotoxin (LT). Commensal bacteria, including segmented filamentous bacteria (SFB), induced fucosylation of intestinal columnar ECs and goblet cells. Expression of IL-22 by ILC3 required commensal bacteria, whereas LT was expressed in a commensal-independent manner. Ablation of IL-22 or LT in ILC3 resulted in a marked reduction in epithelial fucosylation, demonstrating that both cytokines are critical for the induction and regulation of epithelial fucosylation. Fucosylation of ECs in response to the intestinal pathogen *S. typhimurium* was also mediated by ILC3. Compared with control mice, Fut2-deficient mice were more susceptible to pathogenic inflammation as a result of *S. typhimurium* infection, suggesting that epithelial fucosylation contributes to host defense against *S. typhimurium* infection.

CONCLUSION: We demonstrate the critical role of the cytokines IL-22– and/or LT-producing ILC3 in the induction and regulation of intestinal epithelial fucosylation. We also show that ILC3-mediated epithelial fucosylation protects the host from invasion of *S. typhimurium* into the intestine. Our results provide important details of the glycosylation system and homeostatic responses created by the trilateral ILC3–EC–commensal axis in the intestine. Modulation of mucosal immune cell–mediated epithelial glycosylation may provide novel targets for the treatment or prevention of infectious diseases in humans. ■

RELATED ITEMS IN *SCIENCE*

L. V. Hooper, Innate lymphoid cells sweeten the pot. *Science* **345**, 1248–1249 (2014).

The list of author affiliations is available in the full article online.
*Corresponding author. E-mail: kiyono@ims.u-tokyo.ac.jp
Cite this article as: Y. Goto *et al.*, *Science* **345**, 1254009 (2014). DOI: 10.1126/science.1254009



ILC3s regulate epithelial glycosylation. Commensal bacteria, including segmented filamentous bacteria (SFB), induce IL-22 production by ILC3. LT is produced by ILC3 in a commensal bacteria–independent manner. ILC3-derived IL-22 and LT cooperatively induce the production of Fut2 and subsequent epithelial fucosylation, which protects the host against *Salmonella typhimurium* infection.

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RESEARCH ARTICLE

MUCOSAL IMMUNOLOGY

Innate lymphoid cells regulate intestinal epithelial cell glycosylation

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Fucosylation of intestinal epithelial cells, catalyzed by fucosyltransferase 2 (Fut2), is a major glycosylation mechanism of host–microbiota symbiosis. Commensal bacteria induce epithelial fucosylation, and epithelial fucose is used as a dietary carbohydrate by many of these bacteria. However, the molecular and cellular mechanisms that regulate the induction of epithelial fucosylation are unknown. Here, we show that type 3 innate lymphoid cells (ILC3) induced intestinal epithelial *Fut2* expression and fucosylation in mice. This induction required the cytokines interleukin-22 and lymphotoxin in a commensal bacteria–dependent and –independent manner, respectively. Disruption of intestinal fucosylation led to increased susceptibility to infection by *Salmonella typhimurium*. Our data reveal a role for ILC3 in shaping the gut microenvironment through the regulation of epithelial glycosylation.

In the gastrointestinal tract, bilateral regulation between the gut microbiota and the host creates a mutually beneficial environment. The intestinal epithelium is a physical barrier that separates the environments inside and outside the mucosal surface. Intestinal epithelial cells (ECs) are the first line of defense against foreign antigens, including those from commensal and pathogenic bacteria. ECs play key roles in initiating and maintaining an immunologically appropriate and balanced environment in reaction to constant foreign stimulation (*1*). Resident commensal bacteria support the development of this functional mucosal immune system, and in turn, mucosal immune cells control the homeostasis of the gut microbiota and protect against pathogenic bacterial infection through intestinal ECs. In particular, type 3 innate lymphoid cells (ILC3) produce interleukin-22 (IL-22), which not only regulates the homeostasis of the commensal microbiota but also protects against *Citrobacter rodentium* infection, presumably by inducing EC-derived antimicrobial molecules such as RegIIIγ (*2*–*5*).

Fucosylated carbohydrate moieties expressed on intestinal ECs are involved in the creation of an environmental niche for commensal bacteria in mice and humans (*6*–*10*). Fucosylated glycans are generated by the addition of an L-fucose residue via an α1-2 linkage to the terminal β-D-galactose residues of glycan in a process catalyzed by fucosyltransferase. Two fucosyltransferases, Fut1 and Fut2, mediate intestinal epithelial fucosylation, and each enzyme acts on a distinct subset of epithelial cells. Fut1 regulates fucosylation of Peyer's patch (PP) M cells, whereas Fut2 is a key enzyme regulating intestinal columnar epithelial fucosylation and the production of secretory fucosylated ABO(H) histo-blood group antigens (*11*). Defective Fut2 has been shown to result in susceptibility to *Candida albicans* infection in mice (*12*). In addition, inactivating polymorphisms of *FUT2* are associated with metabolic abnormalities and infectious and inflammatory diseases in humans (*13*–*19*).

The importance of epithelial fucose has been explored through studies of host–microbe interactions. Signals from commensal bacteria are required for epithelial fucosylation (*6*). Specific commensals, in particular *Bacteroides*, have been shown to induce epithelial fucosylation and are able to catabolize fucose for energy or incorporate it into bacterial cellular components—capsular polysaccharides—that give microbes a survival advantage in competitive environments (*8*, *9*). Indeed, a lack of Fut2 alters the diversity and composition of the fecal microbiota in humans and mice (*20*, *21*). Therefore, epithelial fucose functions as a mediator between the host and commensal microbiota. Although a previous report proposed a model in which *Bacteroides*–EC interaction mediates epithelial fucosylation (*7*), the precise mechanisms by which Fut2 regulates fucosylation remain largely unknown.

Microbiota induces epithelial fucosylation

Epithelial fucosylation, a major glycosylation process, occurs in the small intestine (*10*, *11*). To assess the inductive mechanism of intestinal epithelial fucosylation, we first investigated the localization of fucosylated ECs (F-ECs) along the length of the small intestine, divided equally into four parts from the duodenum (part 1) to the terminal ileum (part 4), in naïve mice (Fig. 1A). The frequency of F-ECs, detected with the α(1,2)-fucose–recognizing lectin *Ulex europaeus* agglutinin-1 (UEA-1), was low in the duodenum and jejunum (part 1 and a portion of part 2; <15% F-ECs) and gradually increased toward the ileum (part 4; 40 to 90% F-ECs) (Fig. 1, A to C). Consistent with epithelial fucosylation, epithelial Fut2 expression was also higher in the ileum (Fig. 1D). Because greater numbers of microorganisms are present in the distal ileum than in the duodenum (*22*), it may be possible that high numbers of ileal F-ECs are induced and maintained through microbial stimulation. To test this hypothesis, we examined the fucosylation status of ileal ECs (part 4) in mice treated with a mixture of antibiotics (AB), as well as in germ-free (GF) mice. The number of F-ECs was dramatically reduced in AB-treated and GF mice (Fig. 2A and fig. S1A). Furthermore, expression of epithelial *Fut2* was also reduced in AB-treated mice (Fig. 2B). Epithelial fucosylation was restored after cessation of AB treatment and in conventionalized GF mice (Fig. 2A and fig. S1A). In addition, fucosylation of goblet cells, but not Paneth cells, was lost in AB-treated and GF mice (Fig. 2C), indicating that commensal bacteria induce fucosylation of columnar epithelial cells and goblet cells, but not Paneth cells.

It has been shown that epithelial fucosylation can be induced by the mouse and human commensal *Bacteroides thetaiotaomicron* (*6*). However, on the basis of bacterial 16*S* ribosomal RNA (rRNA) gene clone library data obtained from

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isolated ileal mucus samples from naïve mice (Fig. 2D), we did not detect *B. thetaiotaomicron* in our colony, suggesting that other commensals can induce epithelial fucosylation. To identify which indigenous bacteria are responsible for the induction of F-ECs, we analyzed mucus-associated bacterial populations residing in the mouse duodenum (part 1) and ileum (part 4). In contrast to the predominance of *Lactobacillus* in the duodenum, segmented filamentous bacteria (SFB) predominated in the ileum (Fig. 2D); this is consistent with previous studies (*23*, *24*). SFB are Gram-positive bacteria that preferentially colonize the epithelial surface of the terminal ileum, where they induce T helper 17 (T_H17) cells (*25*, *26*). Similar to their effect on T_H17 cell–inducing microbiota (*27*), vancomycin, ampicillin, and to some extent metronidazole—but not neomycin—extinguished epithelial fucosylation (fig. S1, B and C). Furthermore, consistent with the emergence of SFB, epithelial fucosylation is initiated after weaning (*6*, *28*). To investigate whether SFB have the potential to induce F-ECs, we examined mono-associated gnotobiotic mice and found that F-ECs were induced in SFB but not in *Lactobacillus murinus* mono-associated mice (Fig. 2E). Together, these results suggest that epithelial fucosylation in the terminal ileum is induced by commensal bacteria, including SFB, under physiological conditions.

ILC3 are required for epithelial fucosylation

We next investigated the cellular and molecular mechanisms of F-EC induction. Commensal bacteria, including SFB, induce the proliferation of intraepithelial lymphocytes and immunoglobulin A (IgA)–producing cells and the development of T_H17 cells; they also modulate the function of ILCs (*3*, *4*, *25*–*27*, *29*). To assess whether epithelial fucosylation is induced directly by commensal bacteria or is mediated by mucosal immune cells, we first analyzed the epithelial fucose status of T cell–, B cell–, and Rag-deficient mice. The number of F-ECs was not decreased in T cell– or B cell–deficient mice (fig. S2), indicating that T cells and B cells are dispensable for the induction of epithelial fucosylation. Although SFB induce T_H17 cells (*25*, *26*), T_H17 cells are not required for epithelial fucosylation because IL-6, a critical cytokine for T_H17 cell differentiation in the intestine (*30*), was also not necessary for the induction of F-ECs (fig. S3, A to C). We next analyzed RAR-related orphan receptor–γt (RORγt)–deficient mice, which lack the ILC3 subset, in addition to T_H17 cells (*30*, *31*). RORγt-deficient mice exhibited a marked decrease in the number of F-ECs, accompanied by a decrease in *Fut2* expression in ileal ECs (Fig. 3, A to D). These findings suggest that ILC3 are critical inducers of F-ECs. This was further supported by our observation of few F-ECs in the ileum of Id2-deficient mice, which do not develop any of the ILC subsets (Fig. 3, E to G) (*31*, *32*). Although both RORγt- and Id2-deficient mice lack PPs (*33*, *34*), PPs are not necessary for epithelial fucosylation because PP-null mice, generated by treatment with monoclonal antibody (mAb) to IL-7R during fetal growth, had normal levels of F-ECs (fig. S4). ILC3 in the small intestine are aberrantly expanded in Rag-deficient mice (*35*), and elevated numbers of F-ECs were observed in these mice (Fig. 3, H and I), supporting the notion that F-ECs are induced by ILC3. Because ILC3 express higher levels of CD90, they

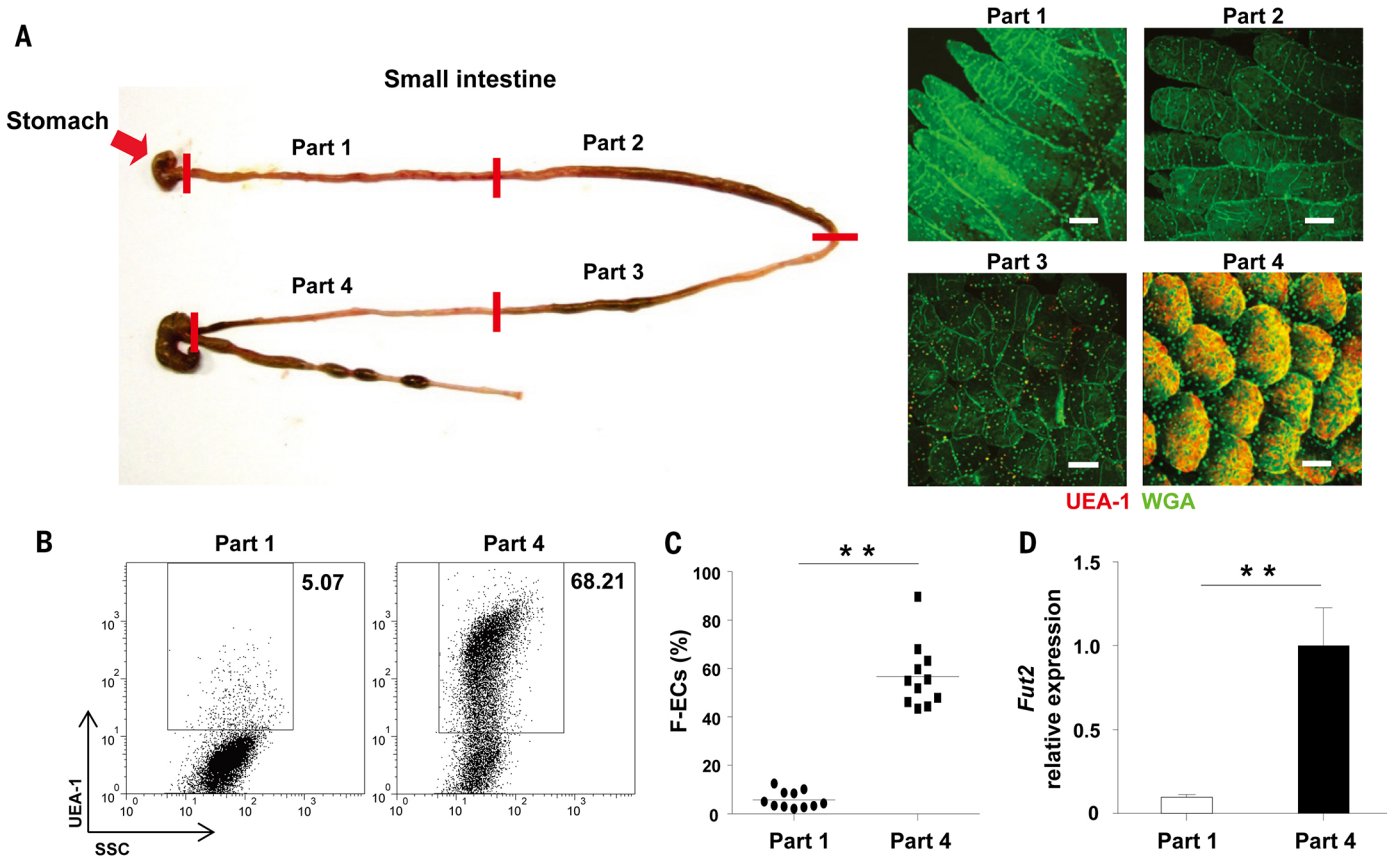

Fig. 1. F-ECs are dominant in the ileum. (**A**) Mouse small intestines were divided equally into 4 parts (parts 1, 2, 3, and 4), from the proximal (duodenum) to the distal (ileum) ends (left), and whole-mount tissues were stained with UEA-1 (red) and WGA (green) to detect F-ECs ($UEA\text{-}1^+$ WGA^+ cells) (right). Scale bars, 100 μm. Data are representative of three independent experiments. (**B** and **C**) Flow cytometric analysis of intestinal ECs isolated from part 1 and part 4 of the small intestines of C57BL/6 (B6) mice. Representative dot-plots are shown in (B). Percentages and mean numbers (horizontal bars) of fucosylated epithelial cells (n = 11 mice per group) are shown (C). SSC, side scatter. Data of two independent experiments are combined. (**D**) Expression of *Fut2* in ECs isolated from part 1 and part 4 of the small intestine isolated from five to six mice per group. Error bars indicate SD. $^{**}P < 0.01$ by using Student's t test. Data are representative of two independent experiments.

can be depleted with a mAb to CD90 (*36*, *37*). To identify whether ILC3 induce F-ECs, we treated wild-type and Rag-deficient mice with a mAb to CD90. *Fut2* expression and the number of F-ECs were markedly decreased after depletion of ILCs in both wild-type and Rag-deficient mice (Fig. 3, J to M, and fig. S5, A and B). Substantial numbers of SFB were still observed in RORγt-, Id2-, and CD90$^+$ ILC–depleted mice (fig. S6, A and B). Therefore, the defective epithelial fucosylation in these models was not attributable to the absence of F-EC–inducing commensals. Collectively, these results indicate that CD90$^+$ ILC3 are required for the induction and maintenance of F-ECs.

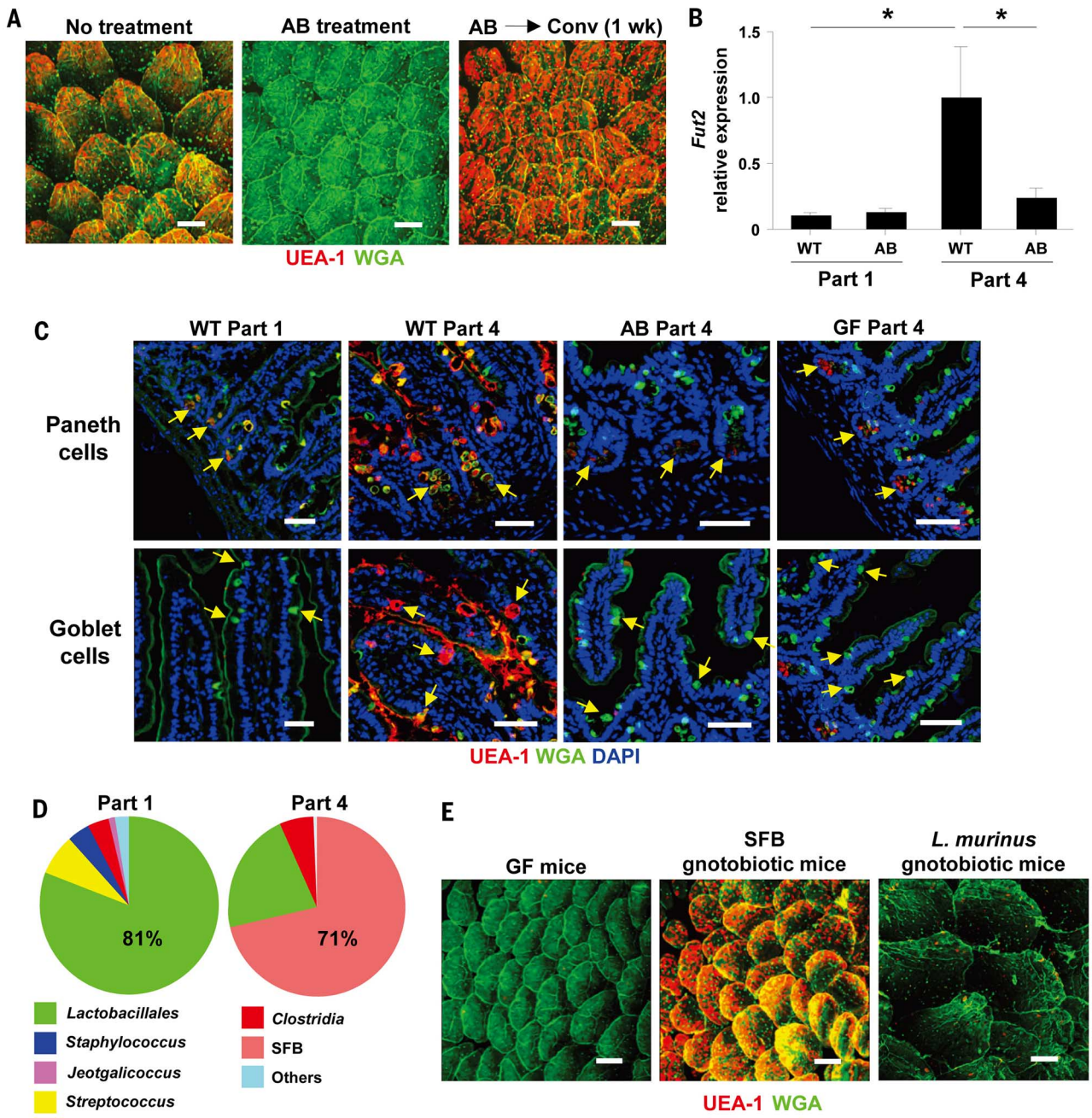

Fig. 2. Commensal bacteria induce epithelial fucosylation under homeostatic conditions. (**A**) Whole-mount ileal tissues of AB-treated mice and conventionalized AB-treated mice were stained with UEA-1 (red) and WGA (green) (n = 3 mice per group). Scale bars, 100 μm. Data are representative of two independent experiments. (**B**) *Fut2* expression in ECs isolated from part 1 (duodenum) and part 4 (ileum) of the small intestines of wild-type (WT) and AB-treated mice (n = 3 mice per group). Error bars indicate SD. *P < 0.05 by using Student's t test. Data are representative of two independent experiments. (**C**) Tissues from part 1 and part 4 of the small intestines of WT, AB-treated, and GF mice were stained with UEA-1 (red), WGA (green), and 4′,6-diamidino-2-phenylindole (DAPI) (blue). Arrows show Paneth cells (top) and goblet cells (bottom). Scale bars, 50 μm. Data are representative of two independent experiments. (**D**) Bacterial populations isolated from the mucus fraction of part 1 and part 4 of mouse small intestine were analyzed by means of 16S rRNA gene clone library. Representative graphs were constructed from samples (part 1, n = 480 clones; Part 4, n = 477 clones) isolated from five different mice (95 or 96 samples were obtained from each mouse). (**E**) Ileal tissues of GF, SFB, or *L. murinus* mono-associated mice (n = 3 mice per group) were stained with UEA-1 (red) and WGA (green). Scale bars, 100 μm. Data are representative of two independent experiments.

IL-22 produced by ILC3 mediates epithelial fucosylation

We next investigated how ILC3 induce epithelial fucosylation. ILC3 cells secrete IL-22, which stimulates the antimicrobial function and maintenance of intestinal ECs (*3*, *4*, *36*, *38*). Indeed, the expression of *Il22* gene was much higher in ILC3 than in any other intestinal immune cell subset (fig. S7A). We therefore assessed whether commensal bacteria regulate ILC3 differentiation and cytokine expression. Although AB-treated or wild-type mice had similar numbers of CD3$^-$ RORγt$^+$

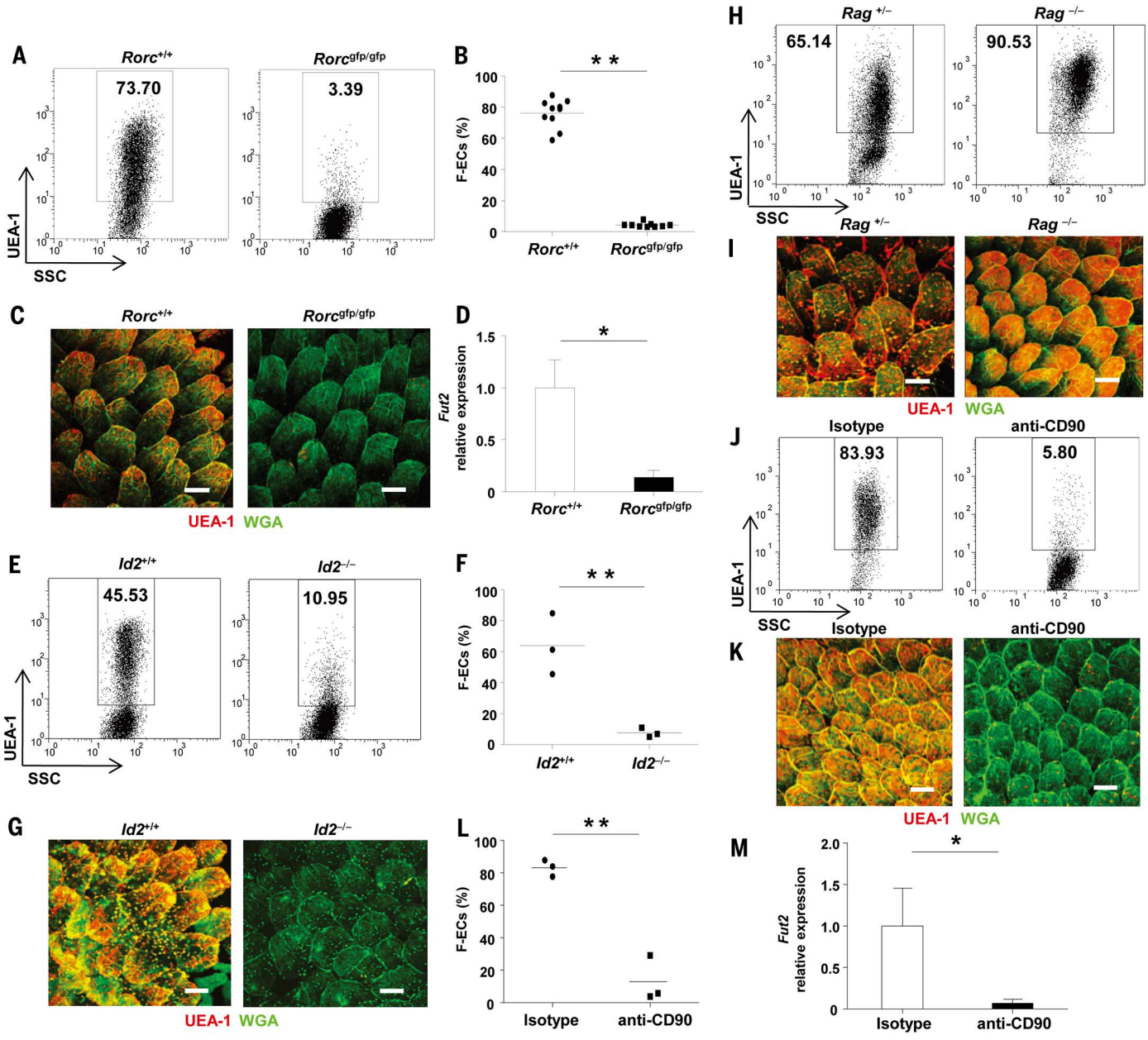

Fig. 3. CD90$^+$ RORγt$^+$ ILC3 induce F-ECs. (**A** and **B**) Representative dot-plots (A) and percentages and means (B) (horizontal bars) of ileal F-ECs isolated from *Rorc*$^{+/+}$ and *Rorc*$^{gfp/gfp}$ mice (n = 10 mice per group). SSC, side scatter. $^{**}P < 0.01$ by using Student's t test. Data of two independent experiments are combined. (**C**) Whole-mount ileal tissues from *Rorc*$^{+/+}$ and *Rorc*$^{gfp/gfp}$ mice were stained with UEA-1 (red) and WGA (green) (n = 10 mice per group). Scale bars, 100 μm. Data are representative of two independent experiments. (**D**) Expression of *Fut2* in ileal ECs isolated from *Rorc*$^{+/+}$ and *Rorc*$^{gfp/gfp}$ mice (n = 5 mice per group). Data are representative of two independent experiments. Error bars indicate SD. $^*P < 0.05$. (**E** and **F**) Representative dot-plots (E) and percentages and means (F) (horizontal bars) of ileal ECs isolated from *Id2*$^{+/+}$ and *Id2*$^{-/-}$ mice (n = 3 mice per group). Data of three independent experiments are combined. (**G**) Whole-mount staining of ileal villi isolated from *Id2*$^{+/+}$ and *Id2*$^{-/-}$ mice. Scale bars, 100 μm. Data are representative of three independent experiments. (**H** and **J**) Representative dot-plots of ileal ECs isolated from *Rag*$^{+/-}$ and *Rag*$^{-/-}$ mice (H) and *Rag*$^{-/-}$ mice treated with mAb to CD90 (anti-CD90 mAb) or isotype control Ab to CD90 (J) (n = 3 mice per group). (**I** and **K**) Whole-mount staining of ileal villi isolated from *Rag*$^{+/-}$ or *Rag*$^{-/-}$ mice (I) and anti-CD90 mAb– or anti-CD90 isotype control Ab–treated *Rag*$^{-/-}$ mice (K) (n = 3 mice per group). Scale bars, 100 μm. Data are representative of two independent experiments. (**L** and **M**) Percentages and means (horizontal bars) of ileal F-ECs (L) and *Fut2* expression (M) isolated from anti-CD90 mAb– or isotype control Ab–treated *Rag*$^{-/-}$ mice (n = 3 mice per group). Data are representative of two independent experiments. Error bars indicate SD. $^*P < 0.05$, $^{**}P < 0.01$ by using Student's t test.

ILC3 (fig. S7, B and C), expression of IL-22 was significantly reduced in AB-treated mice but was restored after cessation of AB treatment (fig. S7D). To identify whether IL-22 is involved in the induction of F-ECs, we analyzed mice lacking IL-22 and found that they had reduced numbers of F-ECs; this was correlated with a decrease in epithelial *Fut2* expression (Fig. 4, A and B). We next examined whether IL-22 alone induced epithelial fucosylation. We used hydrodynamic delivery of an *Il22*-encoding plasmid vector so as to ectopically overexpress IL-22 in AB-treated mice (fig. S8, A and B). In both AB-treated wild-type and *Rorc*$^{gfp/gfp}$ mice, F-ECs were induced in both the duodenum (part 1) and the ileum (part 4) in mice ectopically producing IL-22 but not in mice receiving control vector (Fig. 4, C and D, and fig. S8, C and D). This suggests that IL-22 is sufficient for epithelial fucosylation. Expression of *Fut2* was correlated with the presence of IL-22–induced F-ECs (Fig. 4E). To confirm whether IL-22 produced by ILC3 is necessary for epithelial fucosylation, Rag-deficient mice were treated with an antibody in order to neutralize IL-22. Epithelial *Fut2* expression and fucosylation were interrupted by the neutralization of IL-22 (Fig. 4, F to H). Microbial analyses of IL-22–deficient and

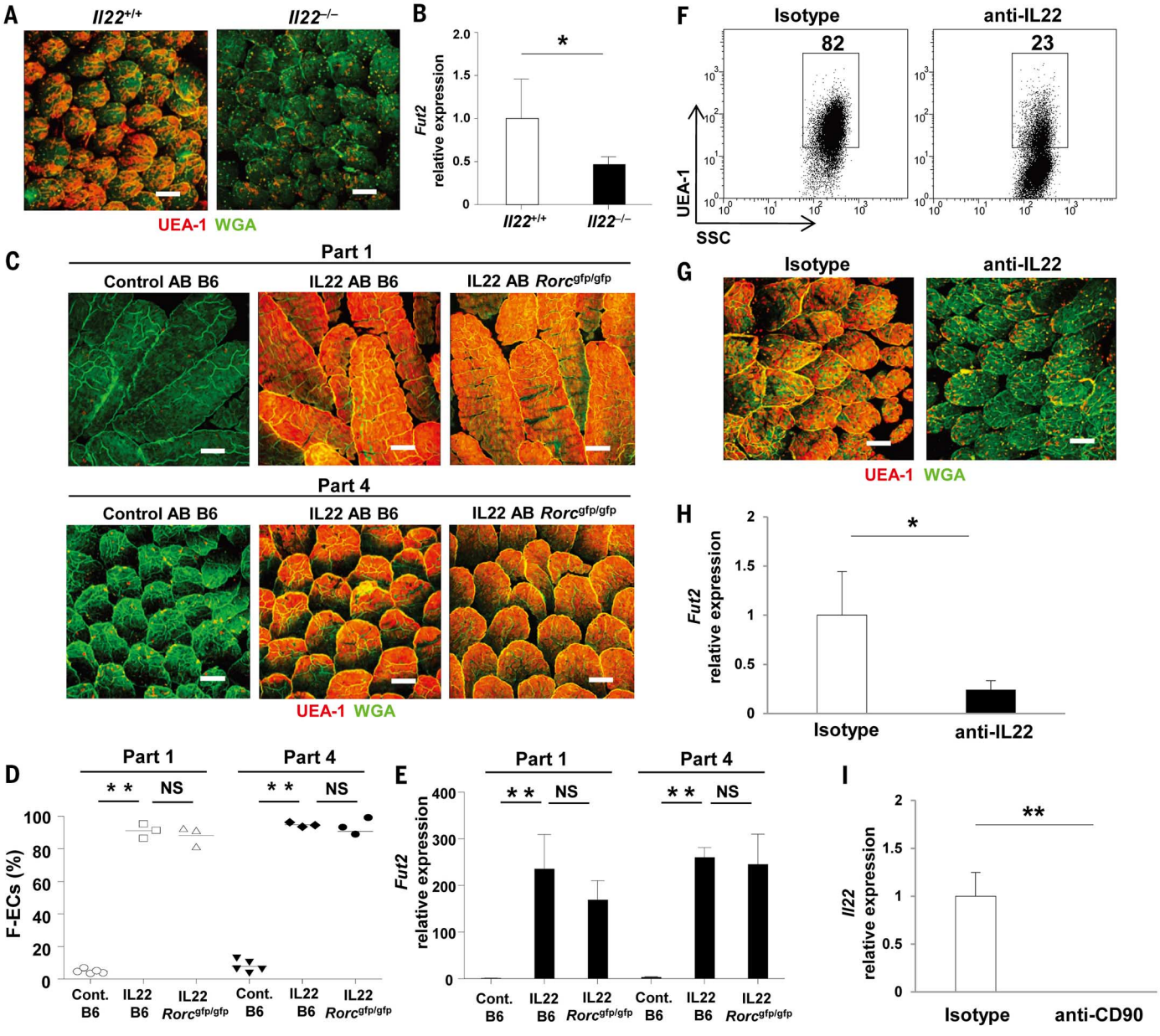

Fig. 4. IL-22 produced by ILCs is involved in the induction of F-ECs. (**A** and **B**) Whole-mount tissues stained with UEA-1 (red) and WGA (green) (A) and gene expression of *Fut2* (B) in ileal villi isolated from *Il22*$^{+/+}$ or *Il22*$^{-/-}$ mice (n = 6 mice per group). Error bars indicate SD. *P < 0.05 by using Student's t test. Scale bars, 100 μm. Data are representative of two independent experiments. (**C** to **E**) AB-treated C57BL/6 (B6) or *Rorc*$^{gfp/gfp}$ mice were intravenously injected with IL-22–encoding plasmid or control vector. Whole-mount staining (C), frequency of F-ECs (D) (mean, horizontal bars), and Fut2 mRNA expression was analyzed by means of rRT-PCR (n ≥ 3 mice per group) (E). Scale bars, 100 μm. Error bars indicate SD. **P < 0.01 by using Student's t test. NS, not significant. Data are representative of two independent experiments. (**F** to **H**) Representative dot-plots (F), whole-mount histological images (G), and expression of *Fut2* (H) of ileal ECs isolated from *Rag*$^{-/-}$ mice treated with antibody to IL-22 or control Ab. Scale bars, 100 μm. Error bars indicate SD. *P < 0.05 by using Student's t test. (**I**) Expression of *Il22* in ileal LP cells from *Rag*$^{-/-}$ mice treated with antibody to CD90 or control Ab. Error bars inidicate SD. **P < 0.01 by using Student's t test. Data are representative of two independent experiments.

antibody-to-IL-22–treated Rag-deficient mice revealed the presence of SFB (fig. S6, A and B). These findings demonstrate that ILC3-derived IL-22 induced by commensal bacteria mediates epithelial fucosylation. Furthermore, depletion of ILC3 by injecting antibody to CD90 into Rag-deficient mice resulted in marked reduction of IL-22 expression (Fig. 4I), supporting the notion that IL-22–mediated signals produced by ILC3 are a key part of the EC fucosylation cascade. IL-22R is composed of two subunits, IL-22R1 and IL-10Rβ (*39*). Whereas IL-10Rβ was ubiquitously expressed, expression of IL-22R1 was specifically detected in intestinal ECs and was not reduced, even after the depletion of commensal bacteria (fig. S9, A and B). Taken together, our findings indicate that commensal bacteria provide signals that prompt ILC3 to produce IL-22, which leads to the induction of Fut2 by IL-22R–positive intestinal ECs.

LTα expressed by ILC3 induces epithelial fucosylation

ILC3 support the development and maintenance of secondary lymphoid tissues through the expression of lymphotoxins (LTs)—especially LTα1β2 (*40*). The expression of *Lta* and *Ltb* genes was higher in ILC3 than in any other intestinal immune cell subset (fig. S10A). In contrast to IL-22, which was induced by commensal bacteria, *Lta* and *Ltb* gene expression in ILC3 was not affected by commensal flora because the AB treatment did not alter the gene expression (fig. S10B). However, intestinal epithelial fucosylation and *Fut2* expression were severely impaired in $Lta^{-/-}$ mice (Fig. 5, A to C). $Lta^{-/-}$ mice possess congenital defects in secondary lymphoid organs (*41*). To elucidate the contribution of LTα to epithelial fucosylation in adult mice that have established secondary lymphoid organs, wild-type mice were treated with LTβR-Ig, which blocks LTα1β2 signaling. Epithelial fucosylation was attenuated by treatment with LTβR-Ig (Fig. 5, D to E), implying that a continuous LT signal is required for epithelial fucosylation. To investigate whether LTα in ILC3 is crucial for the induction of F-ECs, we constructed mixed bone marrow (BM) chimeric mice by transferring BM cells taken from

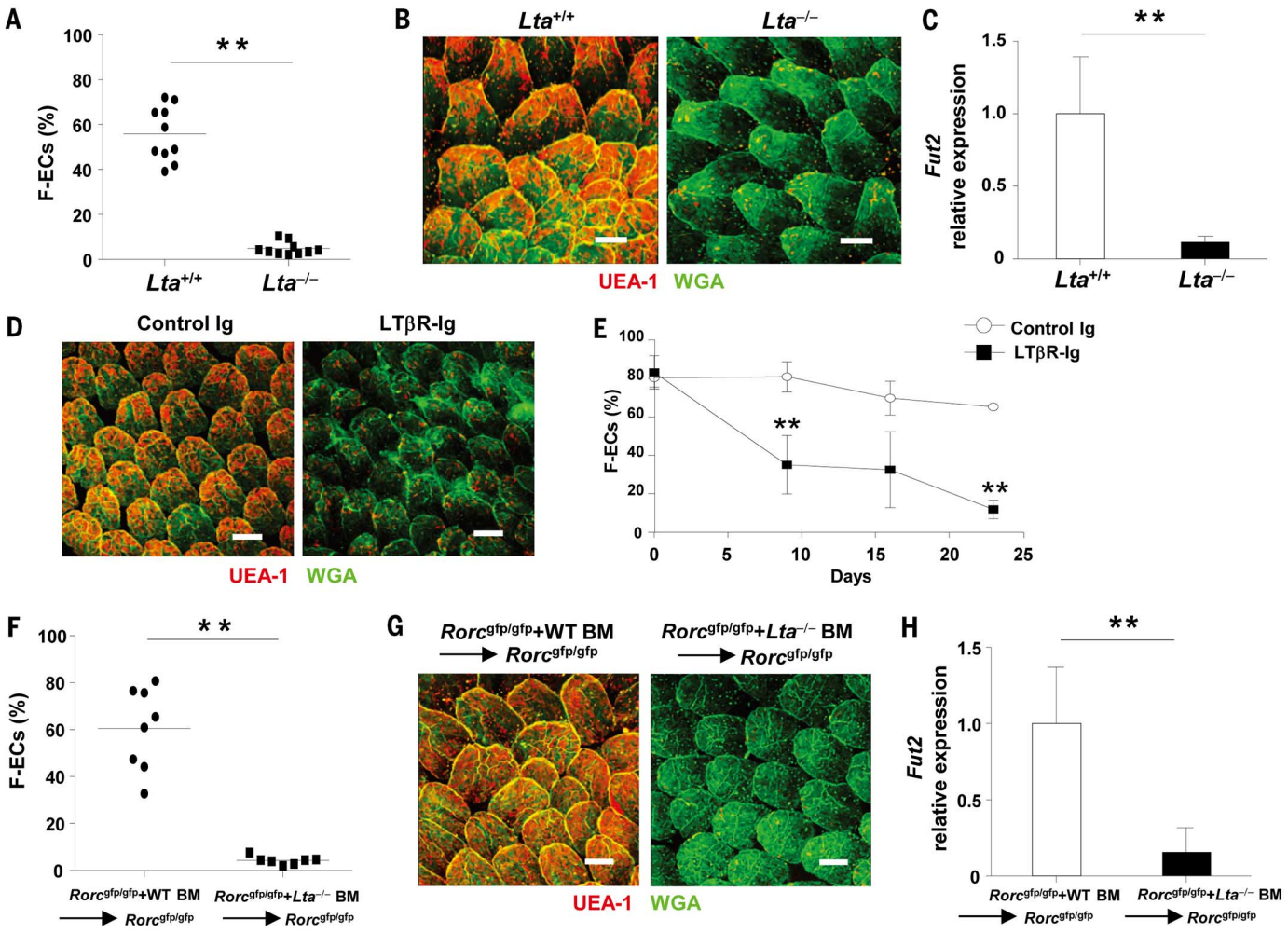

Fig. 5. LTs in innate lymphoid cells induce F-ECs. (**A**) Representative values and means (horizontal bars) of frequency of ileal F-ECs isolated from $Lta^{+/+}$ or $Lta^{-/-}$ mice (n = 10 mice per group). Data of two independent experiments are combined. $^{**}P < 0.01$ by using Student's t test. (**B**) Representative whole-mount staining of ileal villi isolated from $Lta^{+/+}$ or $Lta^{-/-}$ mice (n = 10 mice per group). Scale bars, 100 μm. (**C**) Expression of *Fut2* in ileal ECs isolated from $Lta^{+/+}$ or $Lta^{-/-}$ mice (n = 5 mice per group). Error bars inidicate SD. $^{**}P < 0.01$ by using Student's t test. Data are representative of two independent experiments. (**D**) Representative whole-mount staining of ileal villi from C57BL/6 mice injected with control IgG or LTβR-Ig. Tissues were stained with UEA-1 (red) and WGA (green). (n = 3 mice per group) (**E**) Frequencies of F-ECs in the ileum of C57BL/6 mice injected with control IgG (control Ab) or LTβR-Ig twice (day 9), 3 times (day 16), or 4 times (day 23) (n = 3 mice per group). Error bars indicate SD. $^{**}P < 0.01$ by using Student's t test. (**F** to **H**) Values and means (F), representative whole-mount staining (G), and expression of *Fut2* (H) in ileal ECs isolated from $Rorc^{gfp/gfp}$ mice injected with a mixture of BM cells from $Rorc^{gfp/gfp}$ and WT mice or $Rorc^{gfp/gfp}$ and $Lta^{-/-}$ mice (n = 7 to 8 mice per group). Data of two independent experiments are combined. Error bars indicate SD. $^{**}P < 0.01$ by using Student's t test. Scale bars, 100 μm.

LTα-deficient or -sufficient mice and mixed with BM cells from RORγt-deficient mice into lethally irradiated recipients. F-ECs and *Fut2* expression were diminished in recipient mice reconstituted with BM cells containing LTα-deficient RORγt$^+$ ILC3, whereas substantial numbers of F-ECs, and *Fut2* expression, were induced in recipient mice reconstituted with BM cells containing LTα-sufficient RORγt$^+$ ILC3, indicating the importance of LTα expressed by ILC3 in the induction of F-ECs (Fig. 5, F to H). When the microbiota of LTα-deficient mice or of mixed BM chimeras containing LTα-deficient ILC3 were examined, substantial numbers of SFB were observed (fig. S6, A and B). From these results, we concluded that induction and maintenance of F-ECs were also regulated by ILC3-derived LT in a commensal flora–independent manner.

Epithelial fucosylation protects against infection by *Salmonella typhimurium*

We next investigated the physiological role of epithelial fucosylation. With exception of Paneth cells, the *Fut2* expression and ileal epithelial fucosylation observed in wild-type mice were abolished in *Fut2*$^{-/-}$ mice (fig. S11, A to E). We did not detect any overt changes in mucosal leukocyte populations or in IL-22 or LT expression in ILC3 in these mice (fig. S11F and table S1). Epithelial fucosylation provides an environmental platform for colonization by *Bacteroides* species (*6*–*9*); however, it is unknown whether epithelial fucosylation affects colonization and subsequent infection by pathogenic bacteria. To assess the effects of intestinal fucosylation on pathogenic bacterial infection, we first infected GF mice with the enteropathogenic bacterium *Salmonella typhimurium*, which has the potential to attach to fucose-containing carbohydrate molecules (*42*). After infection with *S. typhimurium*, ECs from both part 1 (duodenum) and part 4 (ileum) of the mouse intestine were fucosylated, and this was correlated with Fut2 expression (Fig. 6, A and B). Previous reports have shown that expression of IL-22 in ILCs is much higher in mice infected with *S. typhimurium* (*43*, *44*). Therefore, *S. typhimurium*–induced epithelial fucosylation may be mediated by ILC3. Indeed, epithelial fucosylation was not induced in RORγt-deficient mice after *S. typhimurium* infection (Fig. 6C). To investigate whether epithelial fucosylation has a role in regulating pathogenic bacterial infection, we infected wild-type or *Fut2*$^{-/-}$ mice with *S. typhimurium* and examined disease progression. Compared with wild-type mice, *Fut2*$^{-/-}$ mice were more susceptible to *Salmonella* infection accompanied with the observation of severe inflamed cecum (Fig. 6D). Consistent with the inflammatory status of diseased mice, the numbers of infiltrating leukocytes in cecum were higher in *Fut2*$^{-/-}$ mice than in wild-type mice (Fig. 6E). Although *S. typhimurium* titers in cecal contents were comparable between wild-type and *Fut2*$^{-/-}$ mice, increased numbers of *S. typhimurium* infiltrated the cecal tissue of *Fut2*$^{-/-}$ mice (Fig. 6F). These results suggest that epithelial fucosylation is dispensable for luminal colonization by *S. typhimurium* but inhibits bacterial invasion of intestinal

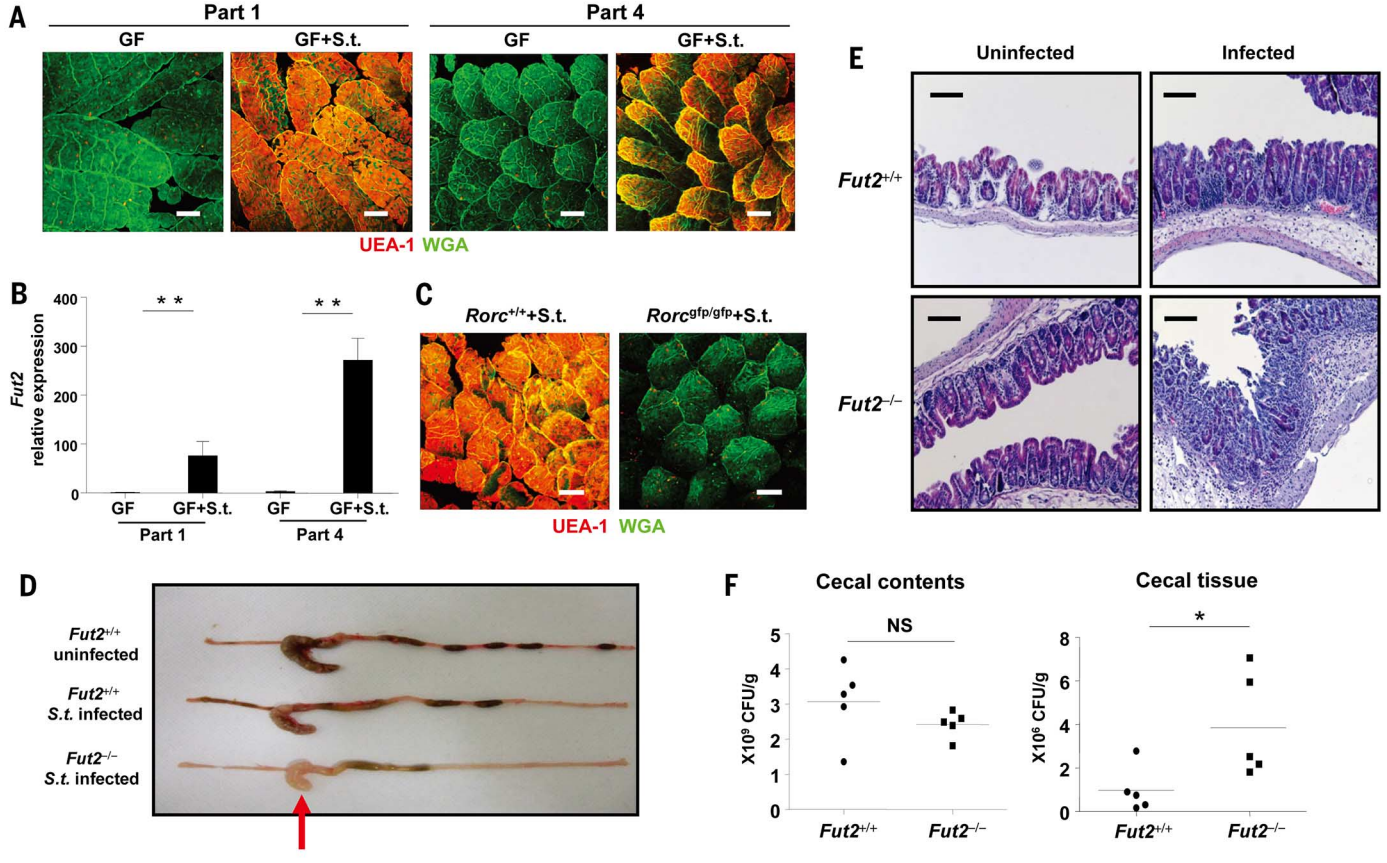

Fig. 6. Epithelial fucosylation protects against infection by *S. typhimurium*. (**A**) Whole-mount tissues from part 1 (duodenum) and part 4 (ileum) of the small intestines of germ-free (GF) or *S. typhimurium*–infected GF mice were stained with UEA-1 (red) and WGA (green) (n = 3 to 4 mice per group). Scale bars, 100 μm. (**B**) Epithelial Fut2 expression in part 1 and part 4 of the small intestines of GF and *S. typhimurium*–infected GF mice was analyzed by using quantitative PCR (n = 3 to 4 mice per group). Error bars indicate SD. $^{**}P < 0.01$ by using Student's t test. (**C**) Whole-mount tissues from ileum of *S. typhimurium*–infected *Rorc*$^{+/+}$ or *Rorc*$^{gfp/gfp}$ mice were isolated and stained with UEA-1 (red) and WGA (green) (n = 3 to 4 mice per group). Scale bars, 100 μm. (**D** and **E**) *Fut2*$^{+/+}$ or *Fut2*$^{-/-}$ mice were infected with *S. typhimurium*. Red arrow shows inflammation of the cecum. Representative macroscopic images (D) and hematoxylin and eosin–stained cecal sections (E) of infected or uninfected mice (n = 5 mice per group). Scale bars, 100 μm. (**F**) Numbers of bacteria in the luminal contents, and within the tissues, of the ceca of *Fut2*$^{+/+}$ or *Fut2*$^{-/-}$ mice were counted 24 hours after infection (n = 5 mice per group). $^{*}P < 0.05$ by using Student's t test. NS, not significant. Three independent experiments were performed with similar results.

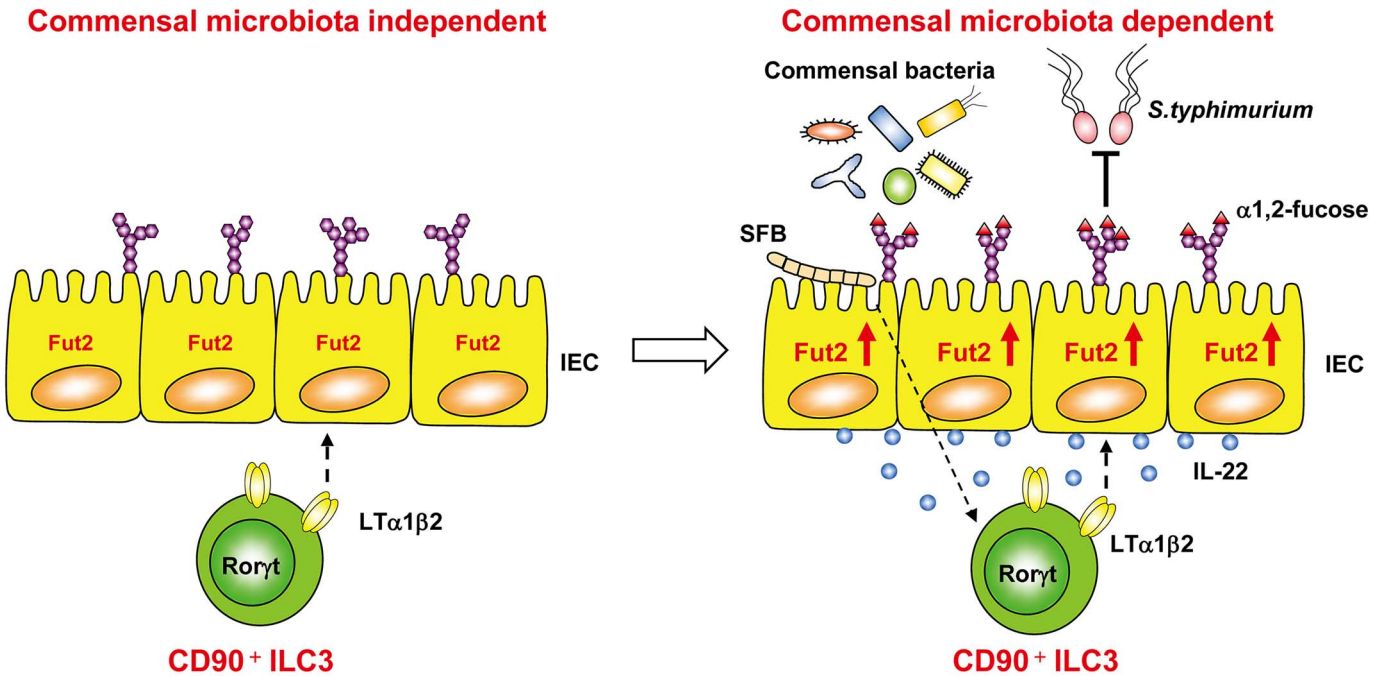

Fig. 7. Scheme for the induction and regulation of epithelial fucosylation by ILC3. IL-22– and LTα-producing ILC3 are critical cells for the induction and regulation of F-ECs. ILC3-mediated fucosylation of ECs is operated by commensal microbiota–dependent and –independent manners. Commensal bacteria, including SFB, stimulate CD90$^+$ ILC3 to produce IL-22 for the induction of Fut2 in ECs. On the other hand, LTα production by ILC3 are operated by a commensal bacteria–independent manner. ILC3-derived IL-22 and LTα induce Fut2 and subsequent epithelial fucosylation, which inhibits infection by *S. typhimurium*. IEC, intestinal epithelial cell.

tissues. Collectively, these results indicate that epithelial fucosylation, regulated by *Fut2*, has a protective role against infection by pathogenic bacteria.

Discussion

The results of recent genome-wide association studies imply that FUT2 nonsense polymorphisms affect the incidence of various metabolic and inflammatory diseases, including chronic intestinal inflammation such as Crohn's disease and infections with pathogenic microorganisms, especially Norwalk virus and rotavirus in humans (*13*–*19*). Understanding the mechanisms of regulation of Fut2 gene expression and fucosylation, one of the major glycosylation events in intestinal ECs, is therefore of great interest. Previously, it was thought that epithelial fucosylation is initiated by direct interaction between commensals and ECs (*7*). Indeed, several reports have shown that epithelial fucosylation is actively induced and used by *Bacteroides* (*8*, *9*). Here, we unexpectedly found that microbiota–epithelia cross-talk is insufficient to induce epithelial fucosylation, and rather, CD90$^+$ RORγt$^+$ ILC3 are necessary for induction of epithelial *Fut2* expression and consequent fucosylation. ILC3 located in the intestinal lamina propria express high levels of IL-22 in a commensal bacteria–dependent manner (Fig. 4I and fig. S7, A and D). This IL-22 then presumably binds to IL-22R expressed by intestinal ECs, leading to the induction of Fut2 and initiation of the EC fucosylation process (Fig. 7). In contrast to the expression of IL-22, ILC3 express LT in a commensal bacteria–independent manner. Spontaneous expression of LT on ILC3 also contributes to the induction of epithelial fucosylation. To explain the mechanism underlying induction of epithelial fucosylation, we propose that epithelial fucosylation is regulated by a two-phase system orchestrated by ILC3 through the microbiota-independent production of LT and the induction of IL-22 by commensal bacteria (Fig. 7). Although other types of stimulation may also affect epithelial fucosylation, our findings reveal a critical role for ILC3.

Our results demonstrated that IL-22 produced by ILC3 is necessary and sufficient for induction of epithelial fucosylation when ILC3 are appropriately stimulated by commensal microbiota (Fig. 4, A to E). In addition to IL-22–mediated epithelial fucosylation, our results also show that the level of epithelial fucosylation is markedly reduced under LTα-deficient conditions (Fig. 5, A to C). Our findings suggest two possibilities for the IL-22/LT–mediated regulation of epithelial fucosylation. The first is that Fut2 expression and subsequent epithelial fucosylation are induced when the intensity of synergistic or additive signals from IL-22 and LT is above the threshold for activation of Fut2. For example, LT produced by ILC3 provides the baseline signal for the minimum expression of Fut2, whereas commensal-mediated IL-22 produced by ILC3 drives the maximum expression of Fut2 for the induction of epithelial fucosylation. The second possibility is that LT directly or indirectly regulates the expression of IL-22R by ECs, and vice versa, and/or the expression of IL-22. Indeed, a previous report has shown that LT produced by ILC3 regulates the expression of IL-23 by intestinal dendritic cells, as well as the subsequent production of IL-22 by ILC3 after infection with *C. rodentium* (*45*). How ILC3-derived IL-22 and LT regulate epithelial Fut2 expression remains to be further elucidated.

Our findings provide further evidence of the critical roles of commensal microbiota, epithelial cells, and innate immune cells (such as ILC3) in the creation of a protective platform against infection by pathogenic bacteria (Fig. 7). Ablation of epithelial fucose allowed severe infection by the pathogenic bacteria *S. typhimurium* (Fig. 6, D to F). Although the detailed mechanisms of why *Fut2*$^{-/-}$ mice are susceptible to *Salmonella* infection remain unknown, one possibility is that fucosylated mucin produced by goblet cells blocks the attachment of *S. typhimurium* to the epithelium. Commensal microbes continuously stimulated goblet cells to release fucosylated mucin into the intestinal lumen (Fig. 2C). Indeed, in a previous in vitro study, H-type 2 antigens, which are synthesized by Fut2 in intestinal ECs, prevented the binding of *S. typhimurium* to fucosylated epithelia; this supports our present findings (*42*). Our findings suggest a protective role for ILC3-mediated mucus-associated fucosylated glycan against infection by pathogenic bacteria.

ILC3 play critical roles in regulation of immune responses during mucosal infection, especially

by producing IL-22, which promotes subsequent expression of the antimicrobial molecule RegIIIγ by ECs (*4*, *36*, *45*). In addition to this, our results describe a previously unknown biological role for ILC3 in the induction and maintenance of intestinal epithelial glycosylation, which leads to the creation of an antipathogenic bacterial platform in the intestine (Fig. 7). Furthermore, epithelial fucosylation contributes to the creation of a cohabitation niche for the establishment of normal commensal microbiota (*20*, *21*). Thus, ILC3-mediated control of epithelial-surface glycosylation might represent a general strategy for regulating the gut microenvironment. Targeted modification of these mechanisms has the potential to provide novel approaches for the control of intestinal infection and inflammation.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were purchased from CLEA Japan (Tokyo, Japan). *Fut2*$^{-/-}$ and *Il22*$^{-/-}$ mice (C57BL/6 background) were generated as described previously, and *Id2*$^{-/-}$ mice were kindly provided by Y. Yokota (*33*, *46*, *47*). *Fut2*$^{-/-}$ mice were crossed onto the BALB/c background for six generations. *Rag2*$^{-/-}$ mice were kindly provided by F. Alt. *Rag1*$^{-/-}$; *Rorc*$^{gfp/gfp}$, *Il6*$^{-/-}$, *Ltα*$^{-/-}$, *Tcrβ*$^{-/-}δ^{-/-}$, and *Igh6*$^{-/-}$ mice were purchased from The Jackson Laboratory. Antibiotic-treated mice were fed a cocktail of broad-spectrum antibiotics—namely, ampicillin (1 g/L; Sigma, Bandai, Japan), vancomycin (500 mg/L; Shionogi, Osaka, Japan), neomycin (1 g/L; Sigma), and metronidazole (1 g/L; Sigma)—or were given these antibiotics in their drinking water, for 4 weeks as previously described (*48*). These mice were maintained in the experimental animal facility at the University of Tokyo. GF and SFB or *L. murinus* gnotobiotic mice (BALB/c) were maintained in the GF animal facility at the Yakult Central Institute and at the University of Tokyo. In all experiments, littermates were used at 6 to 10 weeks of age.

Isolation of bacterial DNA

The isolation protocol for bacterial DNA was adapted from a previously described method (*49*), with some modifications. Bacterial samples in the duodenum and ileum were obtained from mice aged 8 weeks. After removal of PPs and intestinal contents, the intestinal tissues were washed three times with phosphate-buffered saline (PBS) for 10 s each time so as to collect bacteria embedded within the intestinal mucus for analysis of microbial composition. These bacteria-containing solutions were centrifuged, and the pellets were suspended in 500 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). Glass beads, Tris-phenol buffer, and 10% sodium dodecyl sulfate (SDS) were added to the bacterial suspensions, and the mixtures were vortexed vigorously for 10 s by using a FastPrep FP100 A (BIO 101). After incubation at 65°C for 10 min, the solutions were vortexed and incubated again at 65°C for 10 min. Bacterial DNA was then precipitated in isopropanol, pelleted by centrifugation, washed in 70% ethanol, and resuspended in TE buffer. Extracted bacterial DNA was subjected to 16*S* rRNA gene clone library (*50*).

16S rRNA gene clone library analyses

For 16*S* rRNA gene clone library analyses, bacterial 16*S* rRNA gene sequences were amplified by means of polymerase chain reaction (PCR) with the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers. Amplified 16*S* rDNA was ligated into the pCR4.0 TOPO vector (Invitrogen, Carlsbad, CA), and the products of these ligation reactions were then transformed into DH-5α–competent cells (TOYOBO, Osaka, Japan). Inserts were amplified and sequenced on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The 27F and 520R (5′-ACCGCGGCTGCTGGC-3′) primers and a BigDye Terminator cycle sequencing kit (Applied Biosystems) were used for sequencing. Bacterial sequences were identified by means of Basic Local Alignment Search Tool (BLAST) and Ribosomal Database Project searches (*50*).

Immunohistochemistry

Immunohistochemical analyses were performed as previously described, with some modifications (*51*). For whole-mount immunofluorescence staining, the mucus layer was removed by flushing the small intestine with PBS; then, the appropriate parts of the small intestine were fixed with 4% paraformaldehyde for 3 hours. After being washing with PBS, whole-mount tissues were stained for at least 3 hours at 4°C with 20 μg/mL UEA-1 conjugated to tetramethylrhodamine B isothiocyanate (UEA-1-TRITC; Vector Laboratories, Burlingame, CA) and 10 μg/mL wheat germ agglutinin (WGA) conjugated to Alexa Fluor 633 (Invitrogen). For whole-mount fluorescence in situ hybridization analysis, we modified the protocol previously described (*52*). After fixation with 4% paraformaldehyde, intestinal tissues were washed with 1 mL PBS and 100 μL hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, 0.1% SDS) containing 2 μg EUB338 probe (5′-GCTGCCTCCCGTAGGAGT-3′) conjugated to Alexa Fluor 488 (Invitrogen). After overnight incubation at 42°C, the tissues were washed with 1 mL PBS and stained for 3 hours with 10 μg/mL WGA conjugated to Alexa Fluor 633 in PBS. After being washed with PBS, all tissues were analyzed under a confocal laser-scanning microscope (TCS SP2; Leica Microsystems, Wetzlar, Germany).

Cell preparations

A standard protocol was used to prepare intestinal ECs (*53*). Tissues of the small intestine were extensively rinsed with PBS after removal of PPs. After the intestinal contents had been removed, the samples were opened longitudinally and cut into 1-cm pieces. These tissue pieces were mildly shaken in 1 mM EDTA/PBS for 10 min at 37°C. After passage through a 40-μm mesh filter, intestinal ECs were resuspended in minimum essential medium containing 20% fetal calf serum (FCS). Lamina propria (LP) cells were collected as previously described (*54*), with some modifications. Briefly, isolated small intestine was shaken for 40 min at 37°C in RPMI 1640 containing 10% FCS and 1 mM EDTA. Cell suspensions, including intestinal ECs and intraepithelial lymphocytes, were discarded, and the remaining tissues were further digested with continuous stirring for 60 min at 37°C with 2 mg/mL collagenase (Wako) in RPMI 1640 containing 10% FCS. After passage through a 190-μm mesh, the cell suspensions were subjected to Percoll (GE Healthcare) density gradients of 40 and 75%, and the interface between the layers was collected to retrieve LP cells. Stromal cells were identified as CD45$^-$ Viaprobe$^-$ cells. For fluorescence-activated cell-sorting (FACS) analysis of ILCs, isolated LP cells were further purified by magnetic-activated cell sorting so as to eliminate CD11b$^+$, CD11c$^+$, and CD19$^+$ cells. CD11b$^-$ CD11c$^-$ CD19$^-$ Viaprobe$^-$ CD45$^+$ LP cells were used to detect ILCs.

Antibodies and flow cytometry

For flow cytometric analysis, isolated intestinal ECs were stained with UEA-1-TRITC, anti-CD45–Pacific blue (PB; Biolegend, San Diego, CA), and Viaprobe (BD Biosciences, East Rutherford, NJ). Viaprobe$^-$ CD45$^-$ UEA-1$^+$ cells were identified as F-ECs. After blocking with anti-CD16/32 (FcγRII/III) (BD Biosciences), the following antibodies were used to stain spleen and LP cells: anti-CD45–PB (Biolegend), anti-CD11b–phycoerythrin (PE), anti-Foxp3-fluorescein isothiocyanate (FITC) (eBioscience, San Diego, CA), anti-CD11c–allophycocyanin (APC), anti-CD11b–FITC, anti-Gr-1-Alexa647, anti-CD3-APC, anti-B220-PE, anti-B220-APC, anti-IgA-FITC, anti-CD4-eFluor450, anti-CD90.2–FITC, anti-IL-17-PE, and anti-IFNγ-FITC (all from BD Biosciences), and Viaprobe. CD11b$^-$ CD11c$^-$ CD19$^-$ LP cells were purified by using anti-CD11b, anti-CD11c, and anti-CD19 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The results were obtained by using a FACSAria cell sorter (BD Biosciences) with FlowJo software (TreeStar, Ashland, Oregon).

Intracellular staining of Foxp3 and cytokines

Isolated LP cells were incubated for 4 hours at 37°C with 50 ng/mL phorbol myristate acetate (Sigma), 500 ng/mL ionomycin (Sigma), and GolgiPlug (BD Bioscience) in RPMI 1640 containing 10% FCS and penicillin and streptomycin. After incubation, cells were stained with antibodies against surface antigens for 30 min at 4°C. The cells were fixed and permeabilized with Cytofix/Cytoperm solution (BD Bioscience), and cytokines were stained with the fluorescence-conjugated cytokine antibodies. A Foxp3 staining buffer set (eBioscience) was used for intracellular staining of Foxp3.

Depletion of CD90$^+$ ILCs

Depletion of CD90$^+$ ILCs was performed as previously described, with some modifications (*36*). Two hundred and fifty micrograms of a mAb to CD90.2 or an isotype control rat IgG2b (BioXCell, West Lebanon, NH) was given by means of intraperitoneal injection a total of three times at

3-day intervals. Intestinal ECs and LP cells were collected 2 days after the final injection.

Hydrodynamic IL-22 gene delivery system

pLIVE control plasmid (Takara Bio, Shiga, Japan) or IL-22–expressing pLIVE vector (pLIVE-*mIl22*) was introduced into 8-week-old antibiotic-treated C57BL/6 or *Rorc*$^{gfp/gfp}$ mice. Ten micrograms per mouse of plasmid diluted in ~1.5 mL TransIT-EE Hydrodynamic Delivery Solution (Mirus Bio, Madison, WI) was injected via the tail vein within 7 to 10 s. To assess IL-22 expression, serum IL-22 was quantified by means of an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Generation of PP-null mice

mAb to IL-7R (A7R34) was kindly provided by S. Nishikawa. PP-null mice were generated by injecting 600 μg of mAb to IL-7R into pregnant mice on embryonic day 14 (*55*).

In vivo treatment with LTβR-Ig and antibody to IL-22

Neutralization antibody to IL-22 was purchased from eBioscience. Eight-week-old Rag-deficient mice were injected intraperitoneally with antibody to IL-22 a total of five times at 3-day intervals (on days 0, 3, 6, 9, and 12). Plasmid pMKIT-expressing LTβR-Ig and LTβR-Ig treatment was performed as described previously (*56*). Four-week-old C57BL/6 mice were injected intraperitoneally once a week for 3 weeks (on days 0, 7, 14, and 21) with LTβR-Ig fusion protein or control human IgG1 at a dose of 50 μg per mouse. Intestinal ECs were analyzed 3 days after the indicated injection time points.

Adoptive transfer of mixed BM

For mixed BM transfer experiments, *Rorc*$^{gfp/gfp}$ mice were irradiated with two doses of 550 rad each, 3 hours apart. BM cells (1×10^7) from *Rorc*$^{gfp/gfp}$ mice was mixed with BM cells (1×10^7) from C57BL/6 or *Ltα*$^{-/-}$ mice and intravenously injected into irradiated recipient mice. BM chimeric mice were used for experiments 8 weeks after the BM transfer.

Isolation of RNA and real-time reverse transcriptase PCR analysis

Intestinal ECs and subsets of LP cells were sorted with a FACSAria cell sorter (BD Biosciences). The sorted cells were lysed in TRIzol reagent (Invitrogen), and total RNA was extracted in accordance with the manufacturer's instructions. RNA was reverse-transcribed by using a SuperScript VILO cDNA Synthesis Kit (Invitrogen). The cDNA was subjected to real-time reverse transcriptase–PCR (rRT-PCR) by using Roche (Basel, Switzerland) universal probe/primer sets specific for *Ltα* (primer F: 5′-tccctcagaagcacttgacc-3′, R: 5′-gagttctgcttgctggggta-3′, probe No. 62), *Ltβ* (primer F: 5′-cctggtgaccctgttgttg-3′, R: 5′-tgctcctgagccaatgatct-3′, probe No. 76), *Il22* (primer F: 5′-tttcctgaccaaactcagca-3′, R: 5′-tctggatgttctggtcgtca-3′, probe No. 17), *Il22r1* (primer F: 5′;-tgctctgttatctgggctacaa-3′, R: 5′-tcaggacacgttggacgtt-3′, probe No. 9), *Il10rβ* (primer F: 5′-attcggagtgggtcaatgtc-3′, R: 5′-gcatctcaggaggtccaatg-3′, probe No. 29), *Fut2* (primer F: 5′-tgtgacttccaccatcatcc-3′, R: 5′-tctgacagggtttggagctt-3′, probe No. 67), and *Gapdh* (primer F: 5′-tgtccgtcgtggatctgac-3′, R: 5′-cctgcttcaccaccttcttg-3′, probe No. 80). RT-PCR analysis was performed with a Lightcycler II instrument (Roche Diagnostics) to measure the expression levels of specific genes.

Infection with S. typhimurium

Streptomycin-resistant wild-type *S. typhimurium* was isolated from *S. typhimurium* strain ATCC 14028. *Fut2*$^{-/-}$ (BALB/c background) and control littermate mice pretreated with 20 mg of streptomycin 24 hours before infection were given 1×10^8 colony-forming units of the isolated *S. typhimurium* via oral gavage. After 24 hours, the mice were dissected, and the cecal contents were collected. Isolated cecum was treated with PBS containing 0.1 mg mL^{-1} gentamicin at 4°C for 30 min so as to kill bacteria on the tissue surface. The cecum was then homogenized and serial dilutions plated in order to determine the number of *S. typhimurium*. Sections of proximal colon were prepared 48 hours after infection. Infiltration of inflammatory cells was confirmed with hematoxylin and eosin staining.

Statistical analysis

Statistical analysis was performed with an unpaired, two-tailed Student's *t*-test. P values <0.05 were considered statistically significant.

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ACKNOWLEDGMENTS

We thank M. Shimaoka, G. Eberl, M. Pasparakis, K. Honda, C. A. Hunter, C. O. Elson, and J. R. Mora for their critical and helpful comments and advice on this research. Y. Yokota and M. Yamamoto kindly provided Id2-deficient mice and LTβR-Ig, respectively. R. Curtis III and H. Matsui kindly provided several strains of *Salmonella typhimurium*. We thank Y. Akiyama for her technical support with the *S. typhimurium* infection model. S. Tanaka gave us helpful technical suggestions for performing flow cytometric analysis. The data presented in this paper are tabulated in the main paper and in the supplementary materials. Sequences of the bacterial 16S rRNA genes obtained from duodenal and ileal mucus bacteria have been deposited in the International Nucleotide Sequence Database (accession nos. AB470733 to AB470815). This work was supported by grants from the following sources: the Core Research for Evolutional Science and Technology Program of the Japan Science and Technology Agency (to H.K.); a Grant-in-Aid for Scientific Research on Priority Areas, Scientific Research (S) (to H.K.); Specially Promoted Research (230000-12 to C.S.); Scientific Research (B) (to J.K.); for the Leading-edge Research Infrastructure Program and the Young Researcher Overseas Visits Program for Vitalizing Brain Circulation (to Y.G., J.K., and H.K.); for the Leading-edge Research Infrastructure Program (to J.K. and H.K.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Global Center of Excellence (COE) Program "Center of Education and Research for Advanced Genome-based Medicine" (to H.K.); the Ministry of Health, Labor and Welfare of Japan (to J.K. and H.K.); the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (to J.K.); Mochida Memorial Foundation for Medical and Pharmaceutical Research (to J.K.); the National Institutes of Health (1R01DK098378 to I.I.); and by the Crohn's and Colitis Foundation of America (SRA#259540 to I.I.). The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1254009/suppl/DC1
Figs. S1 to S11
Table S1

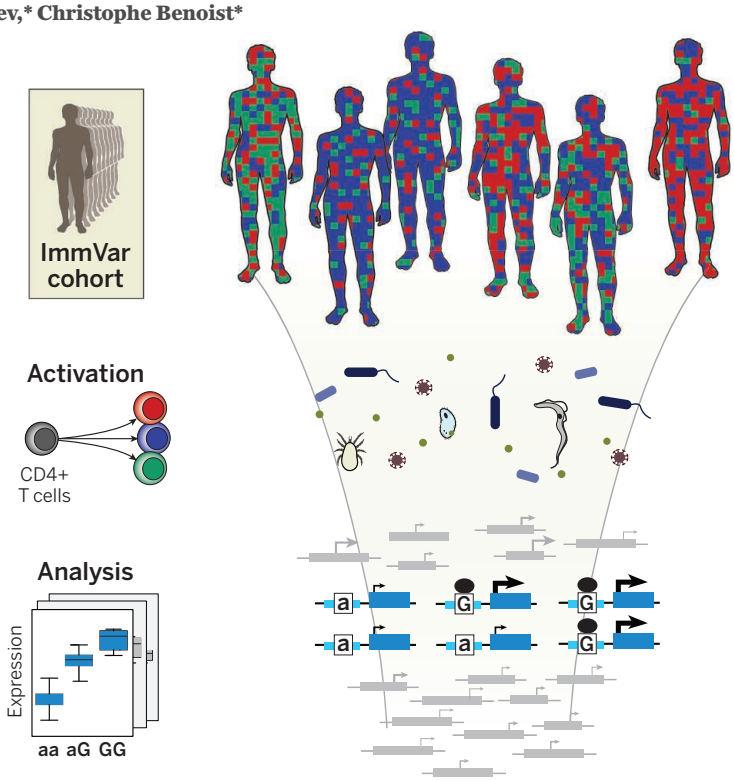
27 March 2014; accepted 25 July 2014
10.1126/science.1254009

RESEARCH ARTICLE SUMMARY

IMMUNOGENETICS

Intersection of population variation and autoimmunity genetics in human T cell activation

Chun Jimmie Ye, Ting Feng, Ho-Keun Kwon, Towfique Raj, Michael Wilson, Natasha Asinovski, Cristin McCabe, Michelle H. Lee, Irene Frohlich, Hyun-il Paik, Noah Zaitlen, Nir Hacohen, Barbara Stranger, Philip De Jager, Diane Mathis, Aviv Regev,* Christophe Benoist*



Characterizing the variation in T cell response. A total of 348 individuals from the ImmVar cohort donated blood for CD4⁺ T cell isolation and genotyping. The T cells were subsequently activated to mimic the recognition of the cognate antigen. The variability in adaptive immune response, which may have genetic and nongenetic components, was characterized by profiling the response of activated T cells. A computational analysis uncovered genetic drivers for this variation and pinpointed molecular mechanisms by which they may act.

INTRODUCTION: The activation of CD4⁺ T lymphocytes by antigen initiates adaptive immune responses, amplifying rare antigen-specific clones and leading to functional specialization of effector T cells, in particular through the spectrum of cytokines they produce. Pathogens have exerted selective pressure during recent human evolution and migrations, but selection for a type of response that is optimal against one microbe class could carry a price in less-effective responses to other microbes or in impaired self-tolerance and autoimmune disease. Little is known about interindividual variation in the responsiveness of human CD4⁺ T cells and how genetic variation affects the tone and intensity of T cell responses in human individuals.

RATIONALE: As the third arm of the ImmVar project, we performed a rigorously controlled analysis of the responses of human blood CD4⁺ T cells to activation in unbiased conditions or in a culture regimen that promotes differentiation to the T helper 17 cell (T_H17) phenotype. To permit an appreciation of the breadth of variation across humans, we investigated these responses in a cohort of 348 healthy human volunteers representing three different ancestries (African, Asian, and European). Responses to activation were evaluated by gene expression profiling, focused on the transcripts that best represent the response, its variability, and its functional consequences. Relating these data to dense single-nucleotide polymorphism genotypes from the participants, we identified the genetic contributions to this variation by using heritability analysis and fine-mapped previously unknown expression quantitative trait loci (eQTLs) that control these responses by using trans-ethnic meta-analysis.

RESULTS: We observed a high degree of interindividual variability, much of which was reproducible for a given subject. This variability followed complex patterns and did not reduce simply to dominant T_H1, 2, or 17 types. We identified 39 loci associated in cis with gene activation in T cells. These explained on average 25% of the repeatable variation, but a major element could not be ascribed to simple genetic effects, instead reflecting environmental influences, immunologic history, or complex integration of network regulation. Of activation-induced genes, cytokines showed the most variability, but with little or no cis genetic control, in contrast to cytokine receptors, which were less variable but for which several eQTLs were detected. Ancestry of the donors markedly influenced T cell responses, with stronger T_H17 being associated with African descent. We fine-mapped and validated experimentally a single-base variant that modulates binding of the transcription factor YY1 and hence the activity of an enhancer element controlling the autoimmune-associated *IL2RA* gene, affecting its activity in activated but not regulatory T cells.

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CONCLUSION: Echoing the linked ImmVar studies, we find that the relevant cell type and context are essential for discovering the genetic drivers of cell-specific responses and of connected autoinflammatory diseases. Our study lays the groundwork for further explorations into the relative contributions of genes and their environment on immunological processes, which should aid in our understanding of autoimmune disease and its genetic underpinnings. ■

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Cite this article as C. J. Ye *et al.*, *Science* **345**, 1254665 (2014). DOI: 10.1126/science.1254665

Published by AAAS

RESEARCH ARTICLE

IMMUNOGENETICS

Intersection of population variation and autoimmunity genetics in human T cell activation

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T lymphocyte activation by antigen conditions adaptive immune responses and immunopathologies, but we know little about its variation in humans and its genetic or environmental roots. We analyzed gene expression in CD4$^+$ T cells during unbiased activation or in T helper 17 (T_H17) conditions from 348 healthy participants representing European, Asian, and African ancestries. We observed interindividual variability, most marked for cytokine transcripts, with clear biases on the basis of ancestry, and following patterns more complex than simple $T_H1/2/17$ partitions. We identified 39 genetic loci specifically associated in cis with activated gene expression. We further fine-mapped and validated a single-base variant that modulates YY1 binding and the activity of an enhancer element controlling the autoimmune-associated *IL2RA* gene, affecting its activity in activated but not regulatory T cells. Thus, interindividual variability affects the fundamental immunologic process of T helper activation, with important connections to autoimmune disease.

Activation of T lymphocytes occurs through the recognition of the cognate antigen presented by molecules of the major histocompatibility complex (MHC) and is arguably the linchpin of the adaptive immune system. This process initiates the amplification of rare T cells specific for a given antigen within the naïve repertoire, which rapidly builds the cell cohorts required for an effective response. T cell amplification also coincides with phenotypic differentiation into various "flavors" of effector CD4$^+$ T cells, each of which is beneficial in distinct contexts of microbial challenge (*1*): T helper 1 (T_H1) cells producing interferon-γ (IFN-γ) and tumor necrosis factor–α (TNF-α) are most effective against viruses and intracellular microbes; T_H2 cells producing interleukin-5 (IL-5) and IL-4/13 are most effective in controlling parasites; T_H17 cells and the IL-17 family cytokines partake in antifungal and antibacterial defenses at mucosal interfaces; and follicular T_H cells help B cells to produce high-affinity immunoglobulins. Choices between effector phenotypes are themselves modulated by the cytokine network, such as the reinforcement of the T_H17 identity through IL-23. These pathways also drive major immunoinflammatory diseases. Pathogenic T_H1 or T_H17 cells have been implicated in rheumatoid arthritis, multiple sclerosis (MS), and inflammatory bowel disease (IBD), and T_H2-type responses in asthma and other atopic diseases (*1*).

Pathogens exert strong selective pressures during human evolution, including the migrations of the last tens of millennia (*2*). Optimal fitness, however, must balance pathogen clearance with the cost to the organism that may result from collateral tissue damage or autoimmune deviation (*3*, *4*). Akin to the diversification of MHC alleles, it is plausible that selection for responses to different types of pathogens has diversified the overall efficacy and/or functional bias of T cell activation in humans. However, selection for a T_H phenotype, advantageous in one environment, might lead to subpar responses to dominant pathogens in other geographic locales, or carry a penalty in terms of self/nonself discrimination and autoimmune diseases. Indeed, there is evidence that genetic variants that influence T cell activation can enhance susceptibility to immunologic diseases (*5*–*8*), such as the variants in the *IL2RA* locus, which encodes a key trophic cytokine receptor, associated with MS and type 1 diabetes (T1D) (*9*–*14*).

Little is known, however, about interindividual variation in the responsiveness of human CD4$^+$ T cells. Are some individuals inherently more responsive to T cell activation or more prone to mount T_H1- or T_H17-biased responses? Genetic variants in regulatory elements that affect transcript abundance can be mapped as expression quantitative trait loci (eQTLs) that associate individual genetic variation with resulting variation in gene expression (*15*, *16*). Although these can, in principle, facilitate the interpretation of disease-associated variants, most eQTL studies have been performed in cell types or states that are not directly relevant to pathogenic processes involving T lymphocytes.

An experimental and analytic strategy to dissect interindividual variation

We followed a stepwise approach to dissect the variation in the responses of primary T cells in 348 healthy participants of African (91), Asian (74), or European (183) ancestry from the Boston PhenoGenetic Project, which were also analyzed in other arms of the ImmVar study (*17*, *18*) (Fig. 1A and table S1). By design, we limited the age range (18 to 56 years) to constrain the effects of age on immune function and sampled the participants in a manner that minimizes variation related to circadian or hormonal secretion rhythms or food consumption (fig. S1). First, we established a rigorously standardized protocol to purify and activate, in a highly parallel mode, fresh CD4$^+$ T cells from human blood. Cells were activated via the T cell receptor (anti-CD3+CD28 beads—hereafter "α3+28") alone or in biasing conditions that favor differentiation to a T_H17 phenotype (supplementation with TGF-β, IL-6, IL-23, IL-1B, anti–IL-4, and anti-IFN-γ—hereafter "α3+28/T_H17") or with addition of IFN-β. These conditions allowed us to map interindividual variation in T cell transcriptional responses that mimic activation through the antigen-specific receptor in an unbiased setting or when coerced toward the T_H17 phenotype. Genome-wide expression profiling established the time points to sample (with cells from a pool of donors) and the extent of interindividual variability in these responses (with cells from a set of 15 donors). From these data, we selected the five most informative conditions (stimulus time point pairs) and a set of 236 transcripts (table S2) that best capture the responses and their variance across donors and activation states, as previously described (*19*), complemented with transcripts of known importance, including 16 key defining cytokines. We then used direct molecule counting (NanoString Technologies, Seattle, WA) to measure the 236-transcript gene set in CD4$^+$ T cells from all 348 donors in each of these five conditions. We estimated the stable interindividual variation from a subset of eight donors who were sampled twice and distinguished components attributable to cis genetic or to nongenetic effects (demographic, physical, and environmental) from the 183 donors of European ancestry. Leveraging the enhanced resolution offered by meta-analysis across population groups (*20*), we fine-mapped eQTLs that control these responses. On the basis of these results, we validated and dissected molecular interactions around

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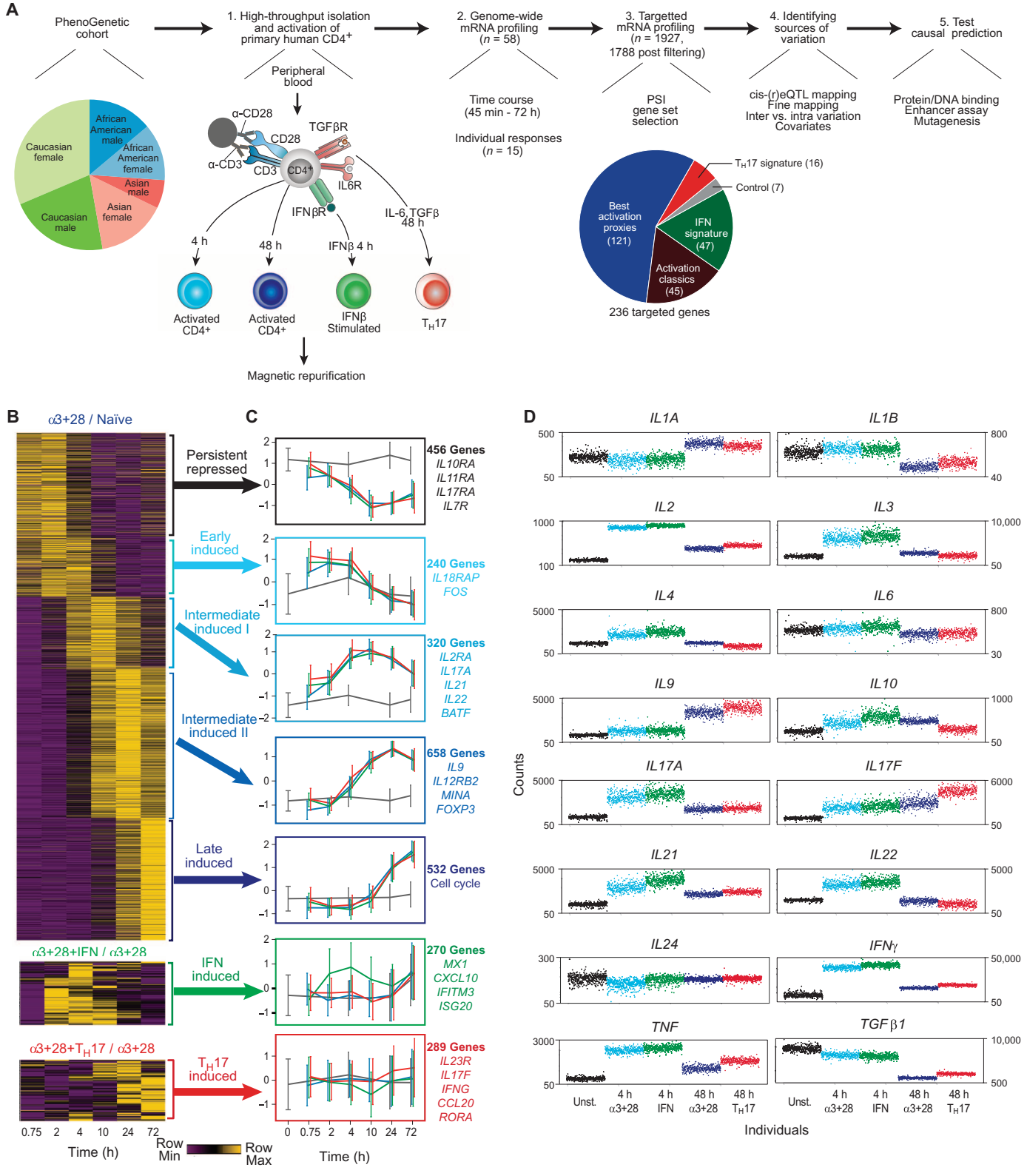

Fig. 1. A systematic approach to characterize the variation in T cell activation and response. (**A**) Overview of study, from (1) cell purification and activation in four conditions, (2) genome-wide or (3) signature expression profiling, (4) computational analysis to decompose expression variance and identify eQTLs, and (5) functional validation of potential causal variants. (**B**) Time course profiles of gene expression in unbiased α3+28 cells (fold change relative to 0-hour baseline; top), and response to IFN-β (fold change relative to unbiased α3+28; middle) or to T_H17 polarization cytokines (fold change relative to unbiased α3+28; bottom). Each row (gene) is normalized to its mean fold change. (**C**) Profiles of gene expression for seven gene clusters, for conditions outlined and color-coded as in (A). A sample of representative genes in each cluster is listed. (**D**) Expression of 16 cytokines across 348 individuals and 5 conditions [black, unstimulated; other color codes as in (A)].

a single-nucleotide polymorphism (SNP) that controls *IL2RA* expression upon T cell activation.

Interindividual variation in T cell responses, co-regulated cytokine clusters

Genome-wide expression profiling determined the signatures of dynamic T cell activation and differentiation responses to different stimuli in a pool of donors of different genders and ancestries (Fig. 1, B and C). After α3+28 activation, we observed successive waves of induction consistent with patterns of responses to T cell activation in vivo (*21*, *22*) (Fig. 1, B and C), affecting 1750 induced and 456 repressed genes (fold change >1.68; table S3), including proliferation and effector genes (*23*, *24*). In T_H17-biased conditions, few transcripts (289) were affected that were not already induced in unbiased α3+28 conditions (we did observe the expected overinduction of the *IL17* family and, more surprisingly, of *IFNG*, the prototypical T_H1 cytokine). The 270 genes induced in response to IFN-β peaked between 2 and 4 hours, including canonical IFN-responsive targets. From these data, we selected representative time points at 4 and 48 hours for α3+28, 4 hours for α3+28+IFN-β, and 48 hours for α3+28/T_H17 activations, and unstimulated controls harvested at 4 hours.

We determined the extent of interindividual variation in T cell responses across 348 individuals, with NanoString profiling of the 236-transcript set. For cytokine transcripts, there was a generally consistent pattern of responses across individuals [Fig. 1D; note the surprisingly early response of many cytokine genes (for example, *IL4*, *IL17A*, *IL22*, and *IFNG*) and the temporal disconnect between *IL17A* and *IL17F*]. There was, however, a spectrum in the extent of interindividual variability (Fig. 1D): large for *IL3* and *IL17A* (9.3- and 5.3-fold, respectively) and tight for *IL2* and *TNF* (2.4-fold; for comparison, the range for *ACTB* was 1.07). Indeed, a set of 14 transcripts, enriched for cytokines and chemokines (13 of 14, $P < 1 \times 10^{-7}$), showed an increase in variance relative to unstimulated baseline, contrary to the bulk of responsive genes whose variance tended to shrink upon activation (fig. S2). The range of variability in cytokine responses was largely independent of the proportion of $CD62L^{lo}$ "memory" cells in the starting population, despite a slight positive correlation between *IL17F* and *IFNG* responses at 48 hours and the proportion of $CD62L^{lo}$ cells (Pearson r = 0.07 and 0.12; fig. S3).

The intensity of initial T cell activation is an important determinant of the outcome of a response to viral pathogens (*25*). To assess differences in overall responsiveness, that is, whether individuals differ in the general intensity of T cell responses, we calculated a "response index" by averaging the centered expression values of 51 transcripts induced (fold change >1.68, table S4) at 48 hours of α3+28 activation. This index spanned a somewhat narrow range (1.69-fold across the 348 donors; fig. S1A) and was not or only minimally affected by participant gender or age (fig. S1, B to D). The response index correlated strongly with a similar index computed from cytokine transcripts, which ranged over 3.4-fold (fig. S1A). This variability in the activation response is significantly correlated [principal component 2 (PC2): Pearson $r = -0.52$, $P < 1 \times 10^{-16}$; PC3: Pearson $r = 0.36$, $P < 1 \times 10^{-12}$] with principal components computed over the entire expression matrix (fig. S1, E and F).

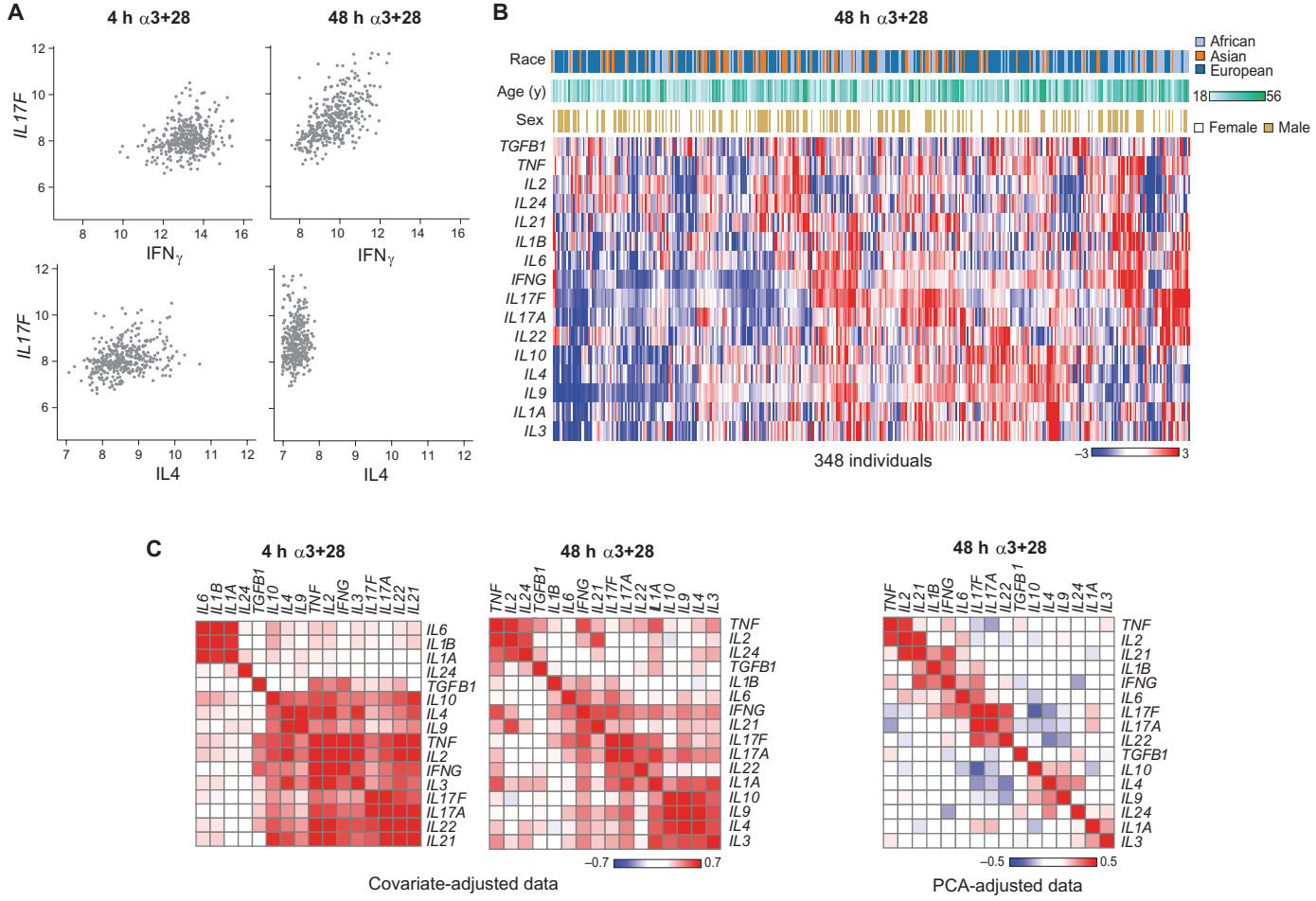

Fig. 2. Interindividual variation in T cell response. (**A**) Relation between expression values (log_2 scale) for pairs of T_H defining cytokines (IL-4, IL-17F, and IFN-γ) in unbiased 4-hour α3+28 and 48-hour α3+28 conditions; each point is an individual sample. (**B**) Heatmap of 16 cytokines clustered by expression across 348 individuals in 48-hour α3+28 with demographic covariates race, age, and sex. (**C**) Pairwise correlation between 16 cytokines in covariate-adjusted data during early activation (left), late activation (middle), and late activation with principal components analysis (PCA)–adjusted data to account for overall responsiveness (right).

We asked whether some individuals could be distinguished by "response types," skewed toward the cytokine signature of a particular T_H subtype. No simple "T_HX-type" individuals emerged from the expression of signature cytokines: The levels of *IL17F* and *IL4* were largely independent (Pearson $r = 0.07$, $P = 0.16$), whereas the levels of *IL17F* and *IFNG* were positively correlated at 48 hours (Pearson $r = 0.6$, $P < 1 \times 10^{-15}$) (Fig. 2A). This complexity in individual responses is exemplified by a cluster of donors characterized by higher *IL4* and *IL9* expression, but which included some high *IL17F* or *IFNG* responders as well (Fig. 2B). Some of the high *IFNG* responders had low *IL4* responses, as would be predicted from classic T_H1/T_H2 opposition, but this was certainly not the norm. These response-defining cytokines are highly repeatable between replicate draws from the same donors (fig. S4).

Some groups of cytokines are co-regulated in mouse T cells in response to particular pathogen challenges. We asked whether similar covariation patterns of cytokine transcripts exist across healthy humans, as affected by genetic and environmental variation. We calculated, for each condition, the pairwise correlation between 16 cytokine transcripts. Early in α3+28 activation, many classic T cell cytokines tended to be correlated, with only limited differentiation of T_H17 cytokines and a well-individualized cluster of inflammatory cytokines (*IL1A/B* and *IL6*; Fig. 2C, left). Clusters became more refined after 48 hours (Fig. 2C, middle), some of which were expected (*IL17A/F* and *IL22*), and others more surprising (for example, *IFNG* with *IL21*, or *IL4* with *IL9*, *IL10*, and *IL3*). Expression of most cytokines still exhibited some degree of positive correlation, perhaps indicative of general responsiveness. Indeed, the distinctions between cytokines became more apparent when the data were adjusted for eight principal components (Fig. 2C, right).

To test whether variation at early times may be predictive of later outcomes, we computed pairwise correlations between genes across time points (fig. S5) and found a positive correlation between the expression of the *IL17* family at 4 hours and *IL17F* expression at 48 hours and between early *IFNG* and later *IFNG* production, indicating that interindividual variation sets the type of response. This early bias was not an amplification of a predetermined state, because there was no correlation with the same in unstimulated samples. Other baseline transcripts were correlated to responsiveness (*26*), including the abundance of IFN-I–responsive transcripts (*DDX58*, *IFIH1*, and *STAT2*) predicting later *IL17F* induction, suggesting an interesting connection between tonic IFN-I signals and the ability to mount IL-17 responses (*26*).

Contributions of genetics and environment to repeatable variation in T cell response

To estimate the relative contributions of genetic and environmental factors, we first estimated for each gene its intraindividual repeatability with data from eight individuals whose cells were sampled on two different dates and stimulated in the same conditions (Fig. 3A). The average repeatability (*27*) (linear mixed model bootstrap $\overline{R} \pm SE_{\overline{R}}$, table S5, medium fill in Fig. 3A) was highest in late response (45 ± 2% in either 48-hour α3+28 or 48-hour T_H17) and lower in early response (20 ± 3% in 4-hour α3+28). Although there was no general trend between repeatability and the coefficient of variation, some of the most variable genes, including *IL17A/F*, *IL4*, *IFNG*, and *IL9*, were also among the most repeatable (fig. S6).

Next, we estimated the proportion of variance attributable to known covariates or genetic components in samples only from the 183 donors of European ancestry. The proportion of expression variance explained by physiological covariates together (for example, age, gender, height, weight, and blood pressure) was generally low (2.4 to 3.6%, adjusted $\overline{r}^2_{fixed} \pm SE_{\overline{r}^2_{fixed}}$, light fill in Fig. 3, A and B), with sex [10 genes, *t*-test false discovery rate (FDR) < 0.05] and age (53 genes, *F*-test FDR < 0.05) the only covariates significantly correlated with gene expression in 48-hour α3+28 (table S6).

The average proportion of variance explained by cis heritability (*28*, *29*) (permutation $\overline{h}^2_{cis} \pm SE_{\overline{h}^2_{cis}}$, dark fill in Fig. 3A) was higher in 48-hour activated conditions (9.2 ± 0.9%) than in unstimulated T cells (5.8%) and 4-hour activated conditions (~6.6%). Whereas the latter are consistent with previous estimates in whole tissue with identity by descent (*28*), the higher 48-hour estimates may reflect increased power to detect activation-specific eQTLs.

Repeatable variance attributable to common cis genetic and nongenetic factors varied widely between genes (Fig. 3, A and B). Cytokine transcripts were repeatable in their expression upon activation for the eight individuals tested, but common genetic variation did not contribute substantially to their variability across the full data set of 348 individuals (Fig. 3B). In contrast, repeatable variation in cytokine receptors, in particular *IL23R* and *IL2RA*, was significantly driven by cis genetic factors (~50%) in activated conditions. Overall, our results suggest that although the T cell response is generally reproducible, common cis genetic effects account for ~25% of repeatable variability; physiological covariates account for very little (~4%); and the rest may be due to established environmental effects, immunological history, or trans-genetic regulation.

Population differentiation in T cell response

When examining differences in T cell responses across the cohort of 348 donors with a linear model, ancestry differentiated the expression of 94 of 229 (41%) genes in the two 48-hour conditions (linear regression *F* test, FDR < 0.01, table S6), explaining a median 7% of the expression variance. We observed ordering in the mean expression of response genes, with a stronger trend of overexpression for donors of African ancestry, lower for European ancestry, and a mixed pattern for Asian ancestry (Fig. 3C). Differentially responsive genes include key indicators of T_H phenotypes, *IL17* family cytokines (overinduced in individuals of African ancestry), and *IFNG*, which showed an opposite pattern. We are cognizant that such differences can be artifactually generated by systematic bias in sampling, batch, or lifestyle confounders, but the differences observed likely have some genetic underpinning because sampling was from the same geographical area, over the same time period, at the same location, under a controlled protocol, and samples from different ancestries were randomly distributed across experimental batches. After adjusting the data for the first eight principal components to account for unknown confounders, the pattern was weakened but still present for many genes (ancestry explaining a median of 2.4 and 2.7% of expression variation in the 48-hour conditions; Fig. 3D and fig. S7). Even the most stringent estimates of population differentiation are higher than previous estimates (*17*, *30*–*33*), which may partially be due to enrichment of immune-related genes in our gene set, consistent with previous observations (*17*, *30*–*33*). Further, although the trend was present in unstimulated samples, it was amplified after 48-hour stimulation, indicating that the population differentiation required activation to be manifest.

In addition to genes with known population differentiation resulting from population-specific genetic polymorphisms [*UTS2* and *IFITM3* (*18*, *34*)], we detected population differentiation in transcripts encoding cytokines, chemokines, or their receptors (table S6 and Fig. 3C). Notably, *IL2RA* was one of the most statistically differentially expressed genes (*t* test, $P < 1 \times 10^{-16}$ in both 48-hour activation conditions, $P = 0.03$ after PCA adjustment): On average, activated T cells from Europeans expressed ~15% less *IL2RA* mRNA than individuals of African ancestry. Together, these results suggest that there is strong population differentiation in T cell responses even after stringent removal of the general response and may include a genetic component.

Genetic variants associated with expression variation during T cell activation and T_H17 polarization

To identify the genetic variants underpinning expression differences, we associated ~10 million imputed genotypes [1000 Genomes Project (*35*), minor allele frequency (MAF) > 0.05] from our cohort of 348 donors with gene expression levels in each condition. Almost half the genes in our gene set (112 of 222, excluding X- and Y-encoded genes; Fig. 4A and table S7) had a significant cis-eQTL within 1 Mb around the gene, in at least one state (permutation FDR < 0.01, table S8). Some cis-eQTLs were detected in all conditions (Fig. 4B), but 38 were state-specific (12 late, 2 early, 11 pan activation, and 13 other patterns; table S7). We did not detect any cis-eQTLs specific to T_H17 polarizing or IFN-β conditions beyond those detected in unbiased activation alone, although a few eQTLs (eight in T_H17, five in IFN-β) had larger effect sizes (table S7). Some of the condition-specific cis-eQTLs were related to transcript levels (Fig. 4, C and D), but this was not the case for many (Fig. 4, E and F)—*IL2RA*

was expressed before stimulation, but it only had a significant cis-eQTL in the late activation conditions (rs12251836, meta $P < 1 \times 10^{-16}$). The low-expression variant at rs12251836 was rare in African (MAF = 0.13) and Asian (MAF = 0.03) but common in European genomes (MAF = 0.41) (Fig. 4G).

We identified only six trans-eQTLs at genome-wide significance (permutation FDR < 0.2), including rs6498114, a variant in the *CIITA* locus, a gene known to control MHC class II expression, which fittingly correlated with *HLA-DRA* expression (table S9). When restricting to test for trans impact only those SNPs identified as having local cis effects, we identified six other significant signals, such as the association of the *IL23R* cis-eQTLs (rs2064689 and rs2863204) in the α3+28/T_H17 condition with the change in expression of *IL21* between 48-hour α3+28/T_H17 and unbiased stimulation (table S10), explaining 11 ± 8.7% of the variance (permutation FDR <0.2; mediation

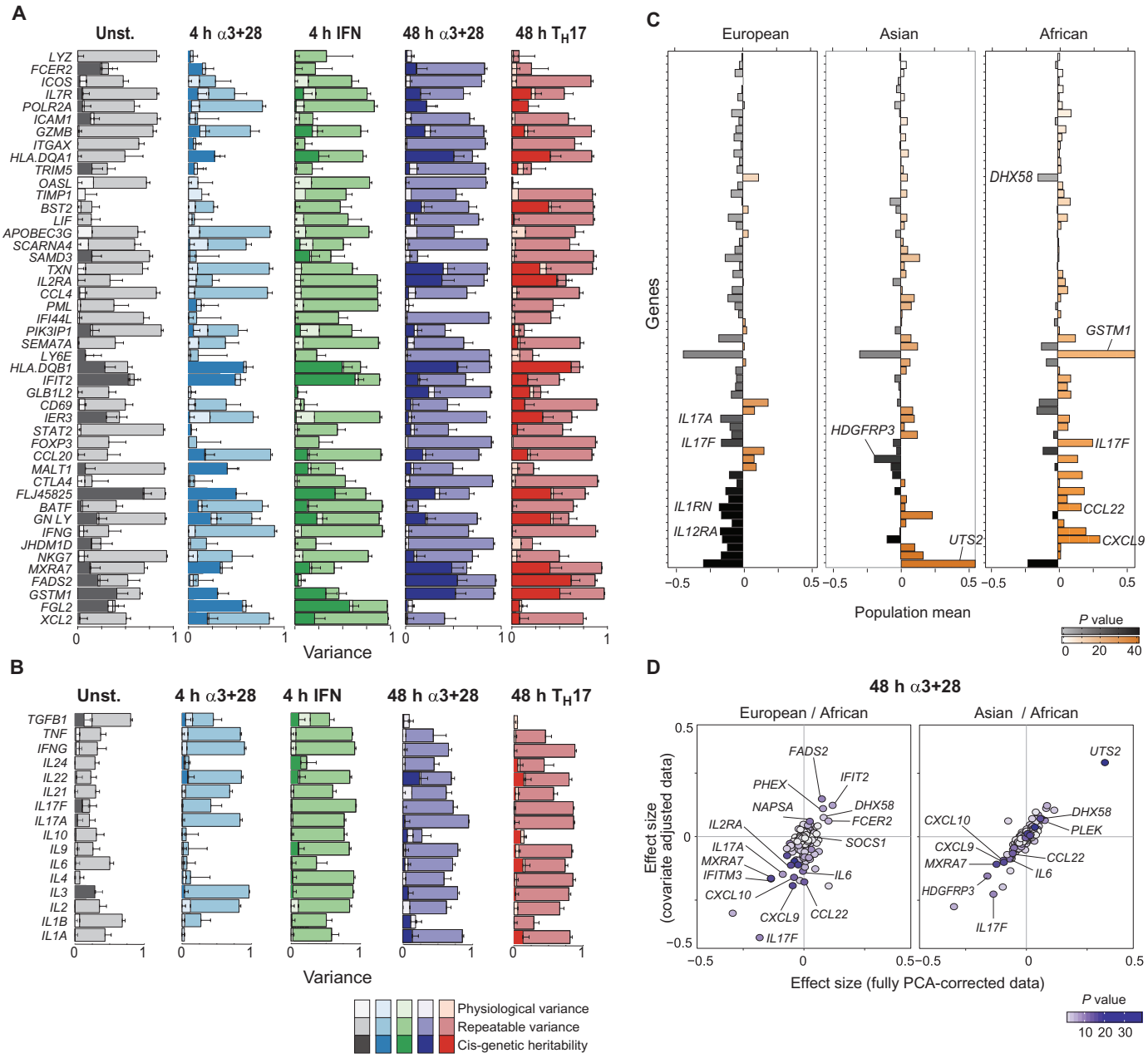

Fig. 3. Sources of variation across T cell activation conditions. (**A** and **B**) Top 56 non-cytokine genes (A) or top 16 key cytokines (B) ranked by maximum repeatability across all conditions estimated from eight recalled individuals. For each gene, repeatability (medium shading) and contributions of cis heritability (dark shading) and of physiological covariates [age, gender, body mass index (BMI), weight, height, diastolic, systolic, season, and month; light shading] to repeatable variation are estimated (European cohort only). Cis heritability is defined as the proportion of phenotypic variance explained by variants within 1 Mb of the gene estimated using linear mixed models (*29*). Physiological variance explained is the proportion of variance explained by all known covariates including gender, age, weight, height, BMI, and blood pressure estimated from multiple regression. Error bars indicate bootstrap SEs around each estimate. (**C**) Percent difference of average population expression (median) from overall average (median) that shows population differentiation in expression. (**D**) Fold change (effect size) in normalized expression for donors of European (left, n = 183) and Asian (right, n = 74) ancestry relative to donors of African ancestry (n = 91), comparing 48-hour α3+28 data adjusted for all PCs (x axis) or for experimental and physiological covariates only (y axis). Darker shading indicates more statistically significant population differentiated genes as determined by an omnibus F test.

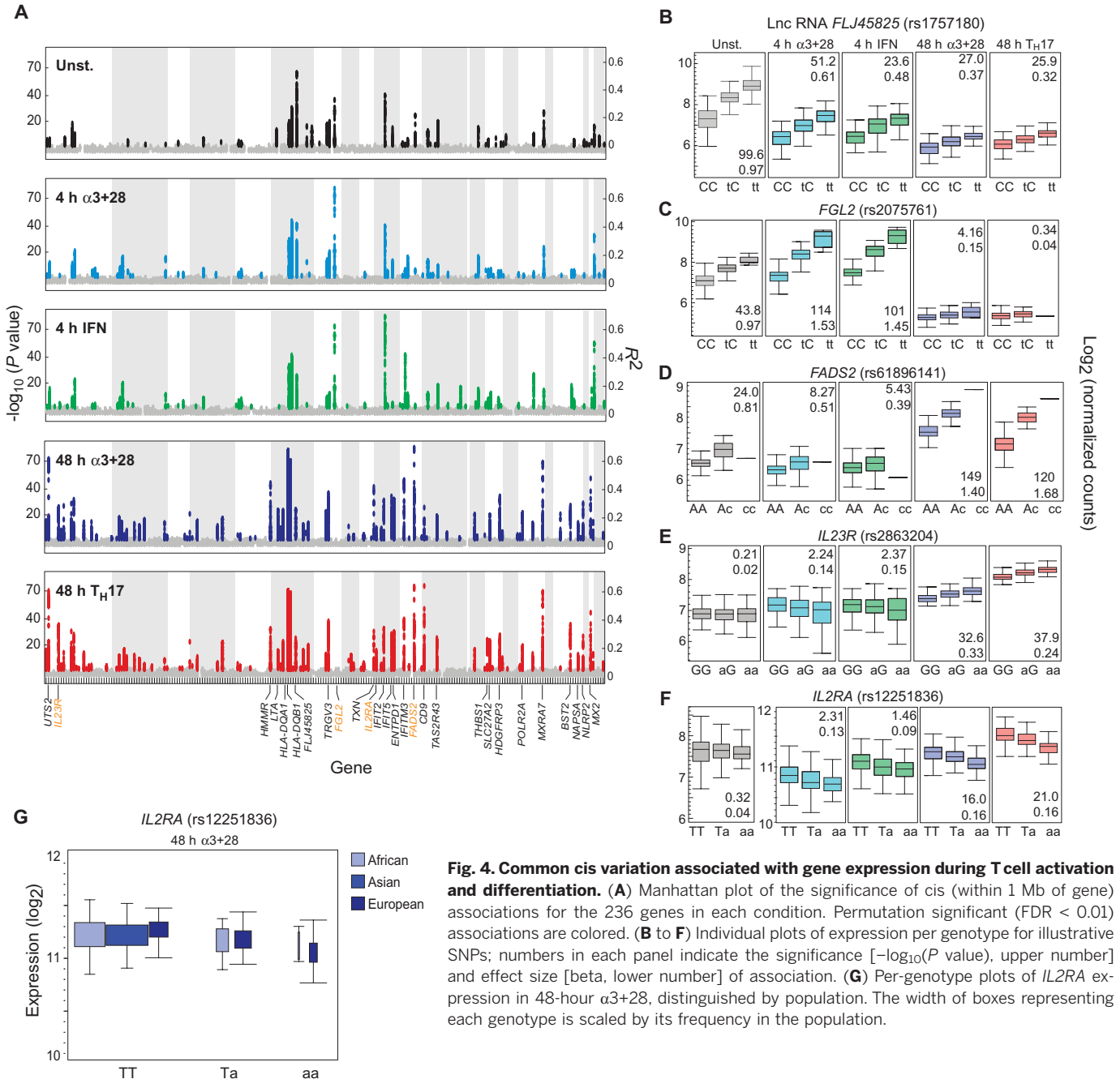

Fig. 4. Common cis variation associated with gene expression during T cell activation and differentiation. (**A**) Manhattan plot of the significance of cis (within 1 Mb of gene) associations for the 236 genes in each condition. Permutation significant (FDR < 0.01) associations are colored. (**B** to **F**) Individual plots of expression per genotype for illustrative SNPs; numbers in each panel indicate the significance [$-\log_{10}$(*P* value), upper number] and effect size [beta, lower number] of association. (**G**) Per-genotype plots of *IL2RA* expression in 48-hour α3+28, distinguished by population. The width of boxes representing each genotype is scaled by its frequency in the population.

analysis by conditioning on *IL23R* transcript levels excluded that the effect might be due to the nearby H3Q codon change), consistent with the amplification of IL-21 production by IL-23 signals during T_H17 differentiation (*36*, *37*).

Overlap of genetically variable T cell responses with genome-wide association study loci identifies potential disease-relevant variants

To assess the relevance of activation eQTLs to disease, we intersected our eQTLs with the genome-wide association study (GWAS) catalog and identified intriguing disease-associated variants, notably near *IL23R* and *IL2RA* (Fig. 5 and table S11), two key cytokine receptors implicated in a number of autoimmune diseases.

IL23R encodes the receptor for IL-23, partakes in establishing stable T_H17 differentiation (*38*), and is strongly associated with IBD (*39*, *40*). Here, rs2863204 was the variant most significantly associated with *IL23R* expression in 48-hour α3+28/T_H17 (Fig. 5A). It is not on the same haplotype as the IBD-associated nonsynonymous variant (rs11209026, R381Q) (*40*). However, meta-analysis of ulcerative colitis and Crohn's disease separately (*40*) revealed allelic heterogeneity in *IL23R* where an additional variant, tagged by rs12095335, is associated with Crohn's disease but not ulcerative colitis (Fig. 5B). When conditioned on rs2863204, rs12095335 appeared as a secondary association to *IL23R* mRNA levels (meta $P = 2 \times 10^{-4}$; Fig. 5C), suggesting that there may be at least two independent effects on *IL23R* expression in the region. The trans-association between these *IL23R* variants and *IL21* expression in the T_H17 condition further implies functional consequences for the region with regard to T_H17 differentiation.

Perhaps most intriguing was the discovery of SNPs associated with *IL2RA* expression. *IL2RA* encodes CD25, the high-affinity IL-2 receptor, and has intricately balanced functions, reflecting the divergent roles of IL-2 as a trophic factor for inhibitory T regulatory (T_{reg}) cells and in aiding the expansion of proinflammatory effector T cells

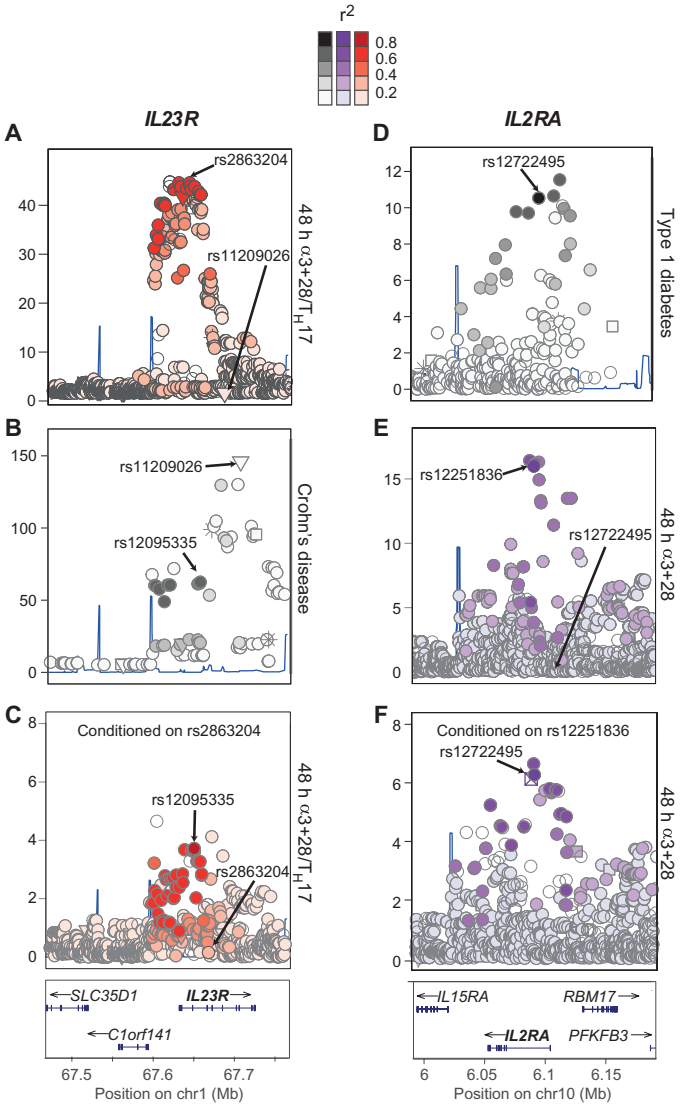

Fig. 5. Trans-ancestry fine mapping identifies activation-specific cis-eQTLs in GWAS regions. (**A**) In 48-hour α3+28/T_H17, rs2863204 (MAF = 0.53, European) is best associated with *IL23R* expression and is independent from rs11209026 (R381Q). (**B**) At *IL23R*, a coding variant (rs11209026, R381Q, MAF = 0.06, European) and a regulatory variant (rs12095335) are associated with Crohn's disease. (**C**) Conditioning on rs2863204 recovers rs12095335 (secondary Crohn's disease association) as a significant secondary association. (**D**) At the *IL2RA* locus, the best T1D association is a regulatory variant, rs12722495. (**E**) In 48-hour α3+28, rs12251836 (MAF = 0.41, European) is best associated with *IL2RA* expression and is independent from rs12722495. (**F**) Conditioning on rs12251836 recovers rs12722495 (primary T1D association). *x* axis defines genomic intervals local to each gene, and *y* axis shows the −$\log_{10}$ (*P* value) of association in GWAS (Crohn's disease, T1D) and eQTL analysis (48-hour α3+28 or 48-hour α3+28/T_H17).

or natural killer cells (*41*). Complementary susceptible and protective haplotypes in MS and T1D have been demonstrated (*9*, *12*, *42*). These map to the promoter region or first intron of *IL2RA* and have been diversely associated with titers of soluble IL-2 receptor, expression of CD25 at the cell surface, or IL-2 signaling efficacy (*12*, *43*, *44*). Three independent haplotypes have been reported to be associated with T1D. The strongest, a protective haplotype tagged by rs12722495 (Fig. 5D), leads to higher expression of *IL2RA* in steady-state $CD45RA^-$ memory and T_{reg} cells (*43*–*45*). Here, rs12251836, the most significant variant associated with *IL2RA* expression in 48-hour α3+28 activated cells, was unlinked to rs12722495, which showed little signal (Fig. 5E). However, by conditioning on rs12251836, we detected in our data a secondary association between rs12722495 and *IL2RA* expression in activated cells ($P = 5.3 \times 10^{-7}$, Fig. 5F). Together, these results point to functional effects of at least two independent associations in the *IL2RA* locus, which may have balancing effects. We surmise that the associated variant rs12251836 is mainly active in acutely triggered T effector cells, but not in T_{reg} cells, because *IL2RA* mRNA in unstimulated $CD4^+$ T cells would predominantly come from $CD25^{hi}$ T_{reg} cells, and this association was not apparent in unstimulated cells. On the other hand, the rs12722495 variant, weaker here but dominant in fresh $CD4^+$ blood T cells (*43*), might preferentially operate in chronically triggered cells such as T_{reg} cells. On balance, these two variants would then control the effectiveness of IL-2 signals in cells of opposite outcome in a disease context. The divergent GWAS results for T1D and MS may also result from this interplay.

A disease-related SNP affecting YY1 binding and activity of an enhancer motif in *IL2RA*

Given the overlap between the *IL2RA* eQTL and autoimmunity-associated GWAS SNPs, we investigated the functional effects of the top five candidate SNPs located in the 5′ flanking and first intron of *IL2RA* (Fig. 6A). We first tested for enhancer activity by introducing ~200–base pair (bp) fragments centered around each SNP (Fig. 6A) into a luciferase reporter vector, driven by SV40 or *IL2RA* minimal promoters, and transfecting them into Jurkat T cells [+/− phorbol 12-myristate 13-acetate (PMA) + ionomycin activation]. Four of the segments were essentially inactive (or even had slight inhibitory effects, fig. S8), but fragment IV, which encompasses rs12251836, showed enhancer activity ("836" for short in Fig. 6B). The A allele, associated with lower *IL2RA* expression, had lower enhancer activity than the T allele (Fig. 6B). The higher enhancer function of the T allele was dependent on activation of the Jurkat cells (Fig. 6C), suggesting that enhancer activity is boosted upon cell activation by interactions affected by the single base change at rs12251836.

We examined whether the rs12251836 SNP affects the binding of transcription factors (TFs). After incubation of nuclear extracts from primary human $CD4^+$ T cells, we detected protein binding to a 25-bp oligonucleotide encompassing $rs12251836^T$, but not to the $rs12251836^A$ counterpart (Fig. 6D). Furthermore, the formation of this DNA-protein complex in primary T cells depended on their stimulation (Fig. 6E), paralleling enhancer activity in the region. Searching for TF-binding motifs showed that the $rs12251836^T$ sequence has putative ($P < 1 \times 10^{-5}$) binding sites for YY1 and RUNX1 straddling the SNP, with a FOX-binding site immediately downstream (Fig. 6F) (*46*). The 836^A allele maintains the FOX-binding motif, but the predicted RUNX and YY1 sites are lost. To test these predicted interactions, we expressed epitope-tagged versions of these three TFs and assayed for binding to oligonucleotides spanning this segment. FOXP3 and RUNX1 bound the rs12251836 oligonucleotides equally for both alleles, but binding of YY1 was restricted largely to $rs12251836^T$ allele. The allele-specific binding of YY1 was also confirmed by electrophoretic mobility shift assay (EMSA) (fig. S8). Furthermore, transfection of a YY1 expression vector boosted the enhancer activity of fragment IV, but only with the plasmid carrying the $rs12251836^T$ variant (Fig. 6G). These results show that this variant is nested in an activation-augmented enhancer region and mediates differential *IL2RA* expression by disrupting a functional YY1-binding site.

Discussion

The exploration of how natural variation affects gene expression during human T lymphocyte activation and differentiation identified complex patterns of variability between individuals, more complex than simple T_H paradigms. Ancestry had a surprisingly profound effect on these response patterns.

By focusing on primary cells and profiling them in the activated cellular state that has the most likelihood to reveal regulatory variation related to autoimmune pathogenesis, we estimated higher cis heritability and identified novel genetic associations. This work represents the third arm of the ImmVar project, in which the same donors

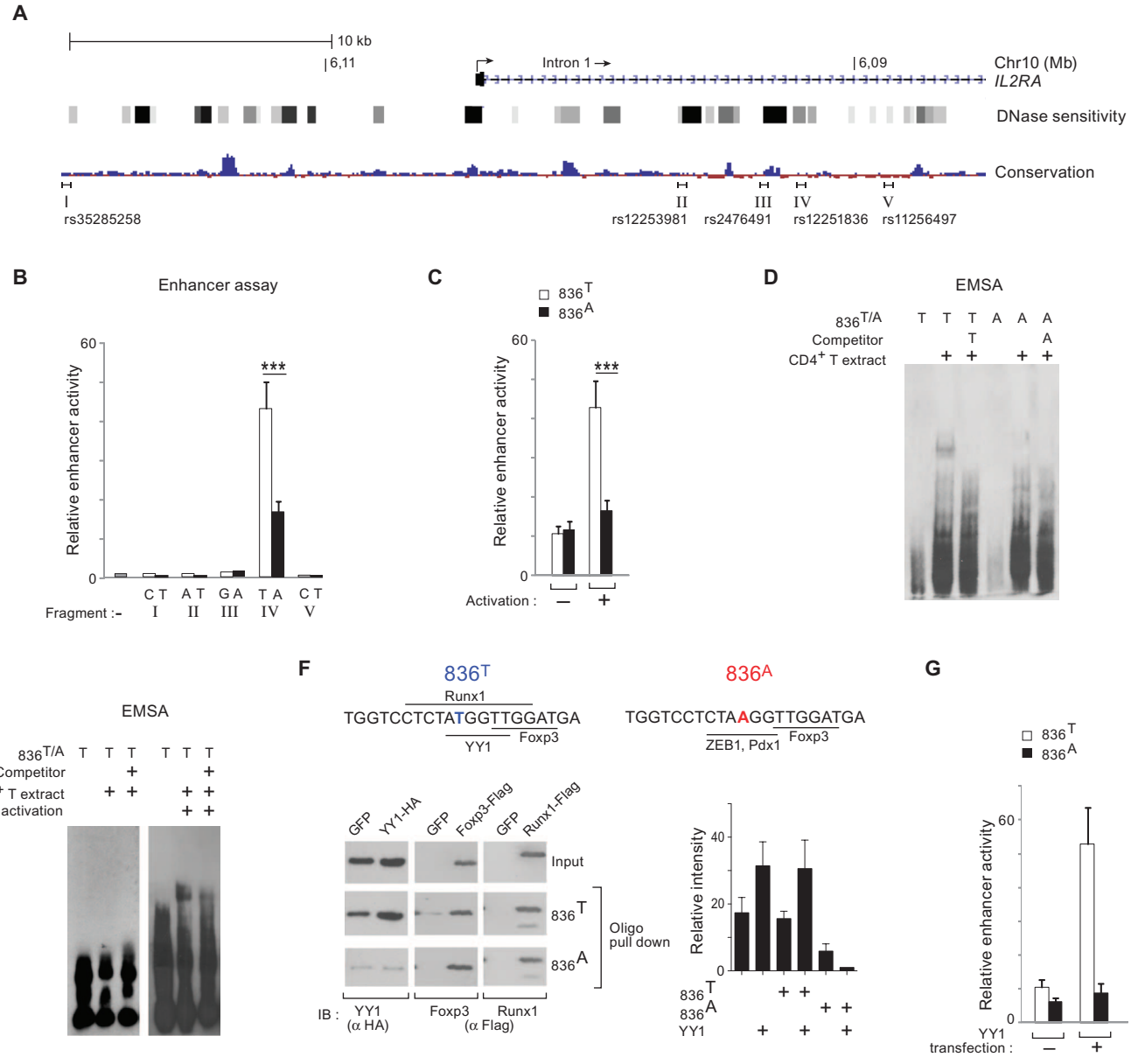

Fig. 6. Validation of rs12251836, a causal variant in the *IL2RA* locus. (**A**) Genomic locations of five top associated SNPs in the *IL2RA* locus, together with mammalian sequence conservation and deoxyribonuclease (DNase) hypersensitivity sites (in T_H1 cells). The ~200-bp fragments centered around each of the variants used in enhancer assays are marked (I to V). (**B**) Enhancer activity in activated Jurkat cells for both alleles (as marked) of the five selected fragments cloned into a minimal promoter vector (firefly luciferase, all values normalized to cotransfected *Renilla*). (**C**) Enhancer activity of fragment IV, as in (B), with or without PMA + ionomycin stimulation. (**D**) Protein binding to the two alleles of rs12251836 tested by EMSA with nuclear extract from Jurkat or human primary $CD4^+$ T cells—these gels were run for different times, and apparent sizes should not be compared. (**E**) Specific binding of YY1 to the rs12251836^T allele in nuclear extract of primary $CD4^+$ T cells, with or without prior 6-hour activation with α3+28. (**F**) Top: Predicted TF-binding sites around the two alleles of rs12251836. Bottom: Pull-down assay of the three main predicted TFs [transfected into human embryonic kidney 293 cells, hemagglutinin (HA)– or Flag-tagged] with oligonucleotides encompassing both rs12251836 alleles or an irrelevant control (EBNA) and detected by immunoblotting for the relevant tags. (**G**) Trans-activating activity of overexpressed YY1 in Jurkat cells also transfected with the enhancer reporter plasmids used in (B) (with either allele at rs12251836). Data are representative of three or more independent experiments; error bars indicate SD.

were profiled with respect to fresh immunocytes at baseline (*17*) and to activation responses in dendritic cells (*18*). Sixty-nine of the 112 eQTLs discovered here were not detected in baseline $CD4^+$ T cells of Raj *et al.* (*17*). These eQTLs were discovered for genes of direct importance in anti-infectious responses and autoimmunity (*IL23R* and *IL2RA*). Of the eQTLs for IFN-responsive genes, 11 of 15 overlapped with those detected in IFN-treated dendritic cells (*18*), reflecting the sharing of IFN-β response pathways. A comprehensive elucidation of the genetic control of immune responses will require similar analyses in an array of relevant cell types under relevant conditions of stimulation.

Are some individuals more prone to mount T_H-biased responses? Of the changes elicited by T cell triggering, cytokine transcripts showed the strongest change in interindividual variance. This diversification did not generally track along simple $T_H1/2/17$ models but adopted more-complex patterns. This observation is of importance for the relationship between T cell differentiation profiles and immune diseases and for the definition of biomarkers: Rather than correlating disease

or therapeutic outcome with levels of a single cytokine, it may be necessary to map individuals to a multidimensional outcome. Very few cis-eQTLs were detected for cytokine transcripts, but many were found for cytokine receptors. This contrast implies that genetic fluctuations are tolerated at cytokine receptor loci, whereas the cytokines themselves may be under more uniform genetic control but vary with environmental cues or the individual's immunological history. In addition, the interindividual variance exhibited a general "responsiveness": a widespread correlation between all cytokines, fine distinctions between clusters of co-regulated cytokines only becoming apparent over time in culture. Thus, individuals vary in the quantitative responsiveness of their T cells, as they do in the qualitative sense.

The study uncovered an unexpected but substantial population differentiation in overall immune response and in the expression of genes encoding key cytokines, with a trend toward higher activation in cells from donors of African ancestry. This differentiation was almost as pronounced as that of transcripts well recognized as being differentially expressed between human populations due to known genetic polymorphisms, such as *UTS2* or *GSTM1*. Suggestively, the IL-17 cytokines were in that group, such that these differences may have direct consequences on immunopathology. It is possible that some differences in responsiveness reflect disparate nutritional or environmental influences in addition to the genetic component, but many (34 of 73 in 48-hour $\alpha 3{+}28/T_H17$) involve cis genetic differences, for instance, the rs12251836 variant that controls *IL2RA* through differential binding of YY1. The low responder allele is a derived allele, present almost exclusively in donors of European descent but rare in African and Asian descendants. The lower responses to activation and the overrepresentation of the corresponding alleles in European-derived populations suggest that tolerance mechanisms to avoid immunopathology resulting from exuberant T cell responses may also have led to the evolution of protection from autoimmunity.

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ACKNOWLEDGMENTS

We are grateful to study participants in the PhenoGenetic project for their contribution. We thank the ImmVar participants, V. Kuchroo and D. Hafler for valuable discussions, K. Rothamel for processing of the microarray samples, and C. Laplace for graphics. This work was supported by U.S. National Institute of General Medical Sciences grant RC2 GM093080 (C.B.). T.R. was supported by an NIH F32 Fellowship (F32 AG043267). P.D.J. is a Harry Weaver Neuroscience Scholar Award Recipient of the National Multiple Sclerosis Society (JF2138A1). The gene expression data have been deposited at the Gene Expression Omnibus under accession no. 60236.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1254665/suppl/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S11
References (*47*–*55*)

11 April 2014; accepted 28 July 2014
10.1126/science.1254665

RESEARCH ARTICLES

PHOTOACCLIMATION

Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light

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Cyanobacteria are unique among bacteria in performing oxygenic photosynthesis, often together with nitrogen fixation and, thus, are major primary producers in many ecosystems. The cyanobacterium, *Leptolyngbya* sp. strain JSC-1, exhibits an extensive photoacclimative response to growth in far-red light that includes the synthesis of chlorophylls *d* and *f*. During far-red acclimation, transcript levels increase more than twofold for ~900 genes and decrease by more than half for ~2000 genes. Core subunits of photosystem I, photosystem II, and phycobilisomes are replaced by proteins encoded in a 21-gene cluster that includes a knotless red/far-red phytochrome and two response regulators. This acclimative response enhances light harvesting for wavelengths complementary to the growth light (λ = 700 to 750 nanometers) and enhances oxygen evolution in far-red light.

Cyanobacteria are unique among bacteria in performing oxygenic photosynthesis and are ecologically important primary producers (*1*). Marine cyanobacteria, mostly *Prochlorococcus*, *Synechococcus*, and *Trichodesmium* species, account for >25% of the net primary productivity in oceans, and terrestrial cyanobacteria also contribute substantially to global photosynthesis (*2*, *3*). The estimated cyanobacterial biomass in terrestrial, endolithic, and freshwater lake ecosystems is ~40% of that in oceans, but this estimate would increase if polar and subarctic soils, topsoils in subhumid climates, and shallow marine and freshwater benthic environments were included (*2*). Because approximately equal solar irradiance reaches Earth's surface in the wavelength ranges 600 to 700 nm and 700 to 800 nm (18.4% versus 14.9% of the total irradiance between 400 and 1100 nm), the capacity to use far-red light to perform oxygenic photosynthesis in terrestrial niches could thus have important consequences in natural and engineered systems.

Cyanobacteria mainly use three large, multisubunit complexes to harvest and convert light into stable, energy-rich compounds: photosystem (PS) I, PS II, and phycobilisomes (PBSs) (*1*, *4*–*7*). Cyanobacteria have evolved many mechanisms to maximize their photosynthetic efficiency in response to the incident irradiation. Examples include adjusting the total cellular chlorophyll (Chl) content and the ratio of PS II to PS I ("intensity adaptation") (*8*); state transitions that redistribute light energy transfer from PBSs to PS I and PS II (*9*, *10*); and nonphotochemical quenching by orange carotenoid-binding protein (*11*). Although cyanobacteria were once thought to have simple pigmentation (Chl *a*, β-carotene, and phycobiliproteins), it is now recognized that some cyanobacteria synthesize Chls *b*, *d*, or *f* (*12*), many functionally distinct carotenoids (*13*), and spectroscopically diverse phycobiliproteins (*7*). Limitation for iron and other nutrients causes changes in light-harvesting proteins (*14*–*16*).

One of the best-characterized acclimative responses in cyanobacteria, complementary chromatic acclimation (CCA; formerly "adaptation"), was discovered more than 100 years ago. Gaidukov (*17*, *18*) observed that *Oscillatoria sancta* is reddish brown when grown in green light but blue-green when grown in red light, and he correctly surmised that the color differences were due to altered pigment synthesis (fig. S1). CCA results from compositional remodeling of the peripheral rods of PBSs (*19*, *20*) and occurs through transcriptional and posttranscriptional regulation of specific phycobiliprotein genes. Genes encoding phycoerythrin and its associated bilin reductases, bilin lyases, and linker proteins are expressed in cells grown in green light, whereas those for "inducible" phycocyanin and associated proteins are expressed in cells grown in red light (*19*–*22*). Central regulatory elements controlling CCA include two response regulators (RcaF and RcaC) and a phytochrome-related sensor histidine kinase, RcaE (*19*, *20*, *23*). Because PBSs are primarily, but not exclusively, associated with energy transfer to PS II (*9*, *10*), CCA enhances the overall rate of photosynthesis when the incident irradiation is complementary to cell coloration.

Using a systems-biology approach—including genome sequencing, comparative genomics, transcription profiling (RNA-seq), biochemical and spectroscopic analyses, and proteomics—we show that *Leptolyngbya* sp. strain JSC-1 (hereafter, JSC-1) (*24*) remodels its photosynthetic apparatus in response to far-red illumination. These changes are probably controlled by a red/far-red–responsive phytochrome. JSC-1 specifically synthesizes both Chl *d* and Chl *f*, in addition to Chl *a*, when cells are grown with light wavelengths ≥700 nm. *Halomicronema hongdechloris*, a cyanobacterium isolated from a stromatolite from Shark Bay, Australia, also synthesizes Chl *f* when cells are grown in far-red light (*25*, *26*). However, JSC-1 additionally undergoes an extensive acclimative response, in which the expression of ≥40% of the genome changes more than twofold after a shift from white light to far-red light. The resulting changes in gene expression lead to the replacement of most of the core proteins of PS I and PS II and to structural remodeling of PBS core substructures. We have named this global acclimation response "far-red light photoacclimation" (FaRLiP), and we show here that FaRLiP substantially improves photosynthetic performance in far-red light.

The JSC-1 genome has a 21-gene cluster that encodes paralogs of most of the core subunits of PS I, PS II, and PBSs (fig. S2). Similar clusters occur in 12 other cyanobacteria, including members of all five sections of the taxon *Cyanobacteria* (*27*) (fig. S3). All but one of these clusters contain six genes encoding subunits of PS I: *psaA2*, *psaB2*, *psaL2*, *psaI2*, *psaF2*, and *psaJ2* (in the remaining strain, *psaF2* and *psaJ2* are adjacent and probably cotranscribed with *psaB3*). The *psaA2* and *psaB2* genes are considerably diverged from the *psaA1* (~77% identity, 85% similarity), *psaB1* (85% identity, 92% similarity), and *psaB3* (82% identity, 90% similarity) genes, respectively. The other *psa* genes in this cluster (*psaL2*, *psaI2*, *psaF2*, and *psaJ2*) are similarly distantly related to paralogous *psa* genes (*psaL1*, *psaL3*, *psaI1*, *psaF1*, and *psaJ1*) encoded elsewhere in the genome (see table S1 for a list of genes for subunits of PS I, PS II, PBSs, and related proteins). Transcriptional analyses (tables S1 and S2) showed that the *psaA2*, *psaB2*, *psaL2*, *psaI2*, *psaF2*, and *psaJ2* genes are not expressed in cells grown under standard growth conditions for many cyanobacteria (*28*). Note that this locus also includes genes for a knotless phytochrome (*rfpA*), a DNA-binding response regulator (*rfpB*) with two CheY receiver domains and a winged-helix DNA binding domain, and a *cheY*-like gene (*rfpC*), which form an apparent operon upstream from *psbA4* (fig. S2) (*rfp* stands for regulator of far-red photoacclimation). Phytochromes are widespread red/far-red photoreceptors (*29*), but orthologs of *rfpA* only occur in 12 other cyanobacteria, within photosynthesis gene clusters similar to that in JSC-1 (fig. S3). On the basis of phylogenetic analyses and conserved domain architecture, these photoreceptors form a distinctive subfamily among knotless phytochromes (Fig. 1). From these observations and biochemical studies described below, we

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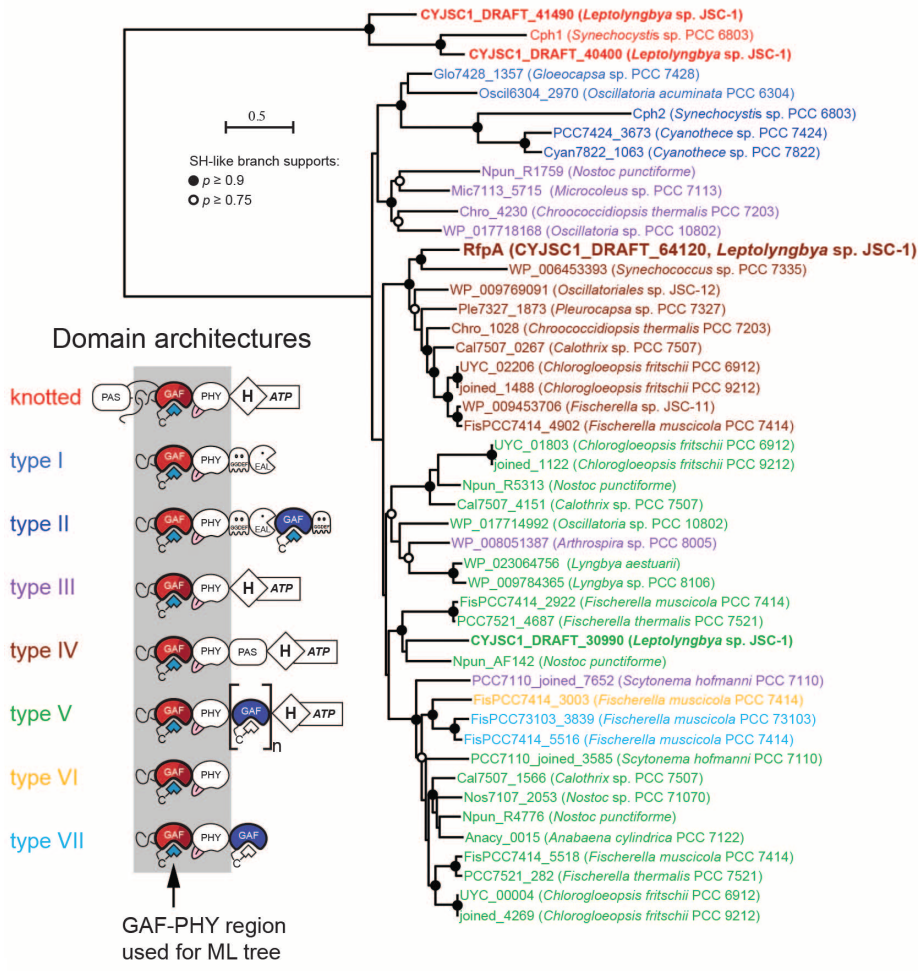

Fig. 1. RfpA forms a distinctive lineage of knotless phytochromes. The GAF-PHY domains of selected knotless phytochromes, including the products of 10 *rfpA* genes (fig. S3), were aligned with *Synechocystis* sp. PCC 6803 Cph1 and two closely related, knotted phytochromes from *Leptolyngbya* sp. JSC-1 as an outgroup (fig. S4). The resulting alignment was used to produce a maximum-likelihood (ML) phylogenetic tree; open and closed circles at nodes show the approximate statistical support for the branching pattern as indicated. The colors of the sequence names reflect seven domain architectures for knotless phytochromes (lower left), with phytochrome and related photosensory GAF domains highlighted in red and blue, respectively. RfpA orthologs have a conserved domain architecture (type IV) and form a distinct subfamily of knotless phytochromes, providing two independent lines of evidence that RfpA orthologs form a distinct lineage associated with FaRLiP gene clusters (fig. S3).

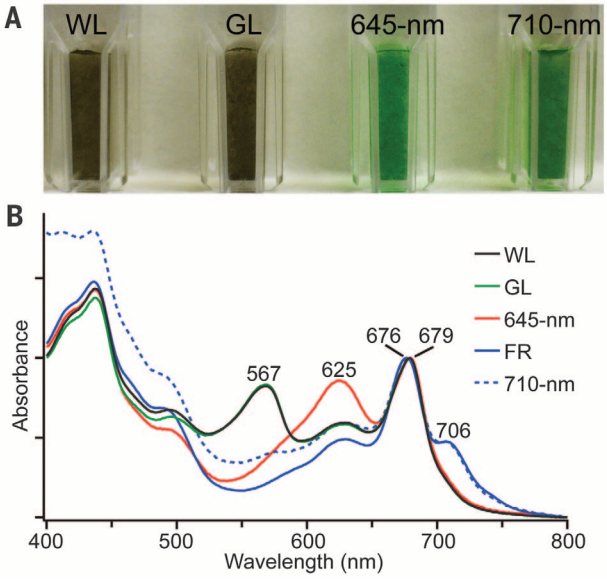

Fig. 2. JSC-1 cells have enhanced absorption at 700 to 750 nm when grown in far-red light. (**A**) Appearance of cells grown in WL, GL, 645-nm light, and 710-nm light. (**B**) Absorption spectra of strain JSC-1 cells grown in WL (black line), GL (green line), 645-nm light (red line), FR (solid blue line), and 710-nm light (dotted blue line).

hypothesize that the RfpA photoreceptor controls gene expression from this cluster.

Expression of *rfpA* or its GAF domain in an engineered, phycocyanobilin-producing strain of *Escherichia coli* (*28*, *30*) yielded red/far-red reversible proteins with nearly identical photochemical properties (figs. S5 and S6A). Like the knotless phytochrome NpR4776 from *Nostoc punctiforme*, recombinant RfpA-GAF holoprotein photoconverts between red-absorbing (P_r) and far-red–absorbing (P_{fr}) species under respective far-red or red illumination (fig. S6A). The P_r form of cyanobacterial phytochromes is initially synthesized in vivo (*31*, *32*). RfpA is converted to the P_{fr} form under a broad range of light wavelengths, but only far-red light ($\lambda \geq 700$ nm) specifically regenerates the P_r form (fig. S6B). Because genetic tools are not available to produce an *rfpA* mutant in JSC-1, the postulated role of RfpA in sensing far-red light and controlling expression of the 21-gene photosynthesis gene cluster cannot be verified by reverse genetics. However, RfpA specifically senses far-red light, exhibits higher transcript abundance in cells grown in far-red light, and is uniquely colocalized with genes that are only expressed in far-red light (figs. S2, S3, S5, and S6 and table S1). The JSC-1 genome contains other phytochromes and related photosensors, but transcript levels only increase in far-red light for the knotted phytochrome CYJSC1_DRAFT_40400 (Fig. 1 and table S2). *Calothrix* sp. PCC 7507 and *Synechococcus* sp. PCC 7335 have RfpA orthologs (fig. S3) but lack Cph1 orthologs. Thus, the distribution and photochemical properties of RfpA strongly support our proposal that RfpA controls the expression of the 21-gene cluster.

To examine the photobiology more closely, JSC-1 cells were grown under six light conditions (*28*): (i) white fluorescent light (WL), (ii)

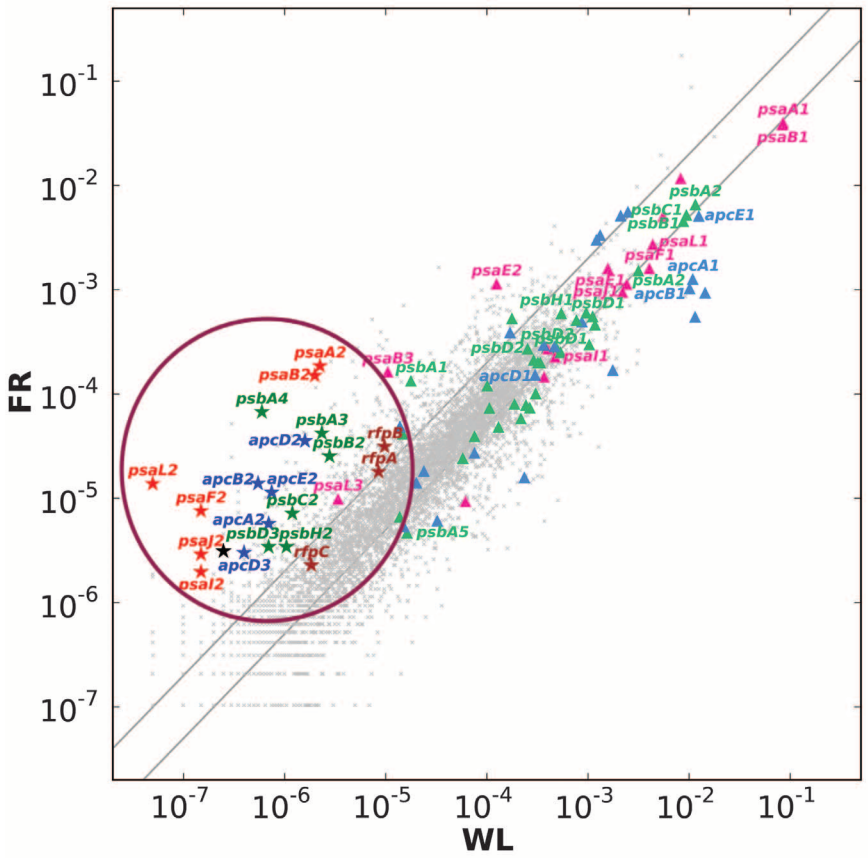

Fig. 3. Transcription of the 21-gene cluster in JSC-1 in far-red light is part of an extensive acclimative response. Scatter plot showing relative transcript levels for JSC-1 cells 24 hours after a shift from WL to FR. The circled stars indicate the relative transcript levels for the genes in the 21-gene cluster shown in fig. S2. The magenta, pale green, and aqua triangles indicate relative transcript levels for paralogous genes encoding subunits of PS I, PS II, and PBSs, respectively, that are preferentially expressed in WL. The parallel lines indicate a twofold increase or decrease by half in transcript abundance.

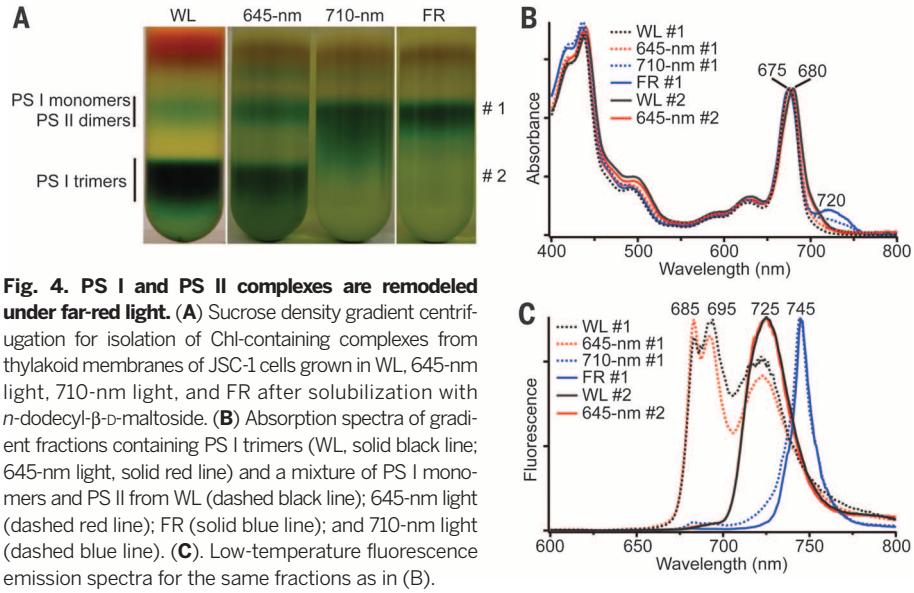

Fig. 4. PS I and PS II complexes are remodeled under far-red light. (**A**) Sucrose density gradient centrifugation for isolation of Chl-containing complexes from thylakoid membranes of JSC-1 cells grown in WL, 645-nm light, 710-nm light, and FR after solubilization with *n*-dodecyl-β-D-maltoside. (**B**) Absorption spectra of gradient fractions containing PS I trimers (WL, solid black line; 645-nm light, solid red line) and a mixture of PS I monomers and PS II from WL (dashed black line); 645-nm light (dashed red line); FR (solid blue line); and 710-nm light (dashed blue line). (**C**). Low-temperature fluorescence emission spectra for the same fractions as in (B).

green-filtered fluorescent light (GL), (iii) red-filtered fluorescent light (RL), (iv) 645-nm or (v) 710-nm light provided by light-emitting diodes (LEDs), and (vi) far-red light (FR) produced from filtered tungsten light (λ > 690 nm) (see fig. S7). The absorption spectra of cells grown in 710-nm light and FR (Fig. 2) showed that they had gained absorption at ~700 to 750 nm (λ_{max} ~706 nm) that was not present in cells grown in WL, GL, or 645-nm light. Low-temperature fluorescence emission spectra at 77 K (fig. S8) of cells grown under the first three listed conditions had emission maxima at 683 nm and 695 from PS II and a strong emission maximum at 725 nm from PS I. These spectra are typical of those for cells synthesizing Chl *a* and having a relatively high PS I:PS II ratio (*24*). JSC-1 cells grown in 710-nm light or FR have weak fluorescence emission at 683, 695, and 717 nm and strong emission at 745 nm, in which they resemble cells synthesizing Chl *f* (*25*, *26*).

Pigments extracted from these cells were subjected to reversed-phase high-performance liquid chromatography. Only Chl *a* (figs. S9 and S10, peak 3) and carotenoids were observed in pigment extracts from cells grown in WL or 645-nm light. Cells grown in FR or 710-nm light still produced Chl *a* as the major Chl, but they also synthesized a pigment with an absorption spectrum identical to that of Chl *f* (figs. S9 and S10, peak 2) (*33*, *34*). This pigment was confirmed to be Chl *f* by converting it to the corresponding pheophytin by removing the Mg^{2+} ion (figs. S11 and S12) and subsequent mass spectrometry (MS) and tandem MS (MS-MS) analyses (figs. S13 and S14). Another Chl, which was slightly more hydrophilic than Chl *f* (figs. S9 and S10, peak 1), was also detected. The absorption properties (figs. S10 and S12) of this Chl, as well as MS and MS-MS analyses (figs. S15 and S16) of the corresponding pheophytin, showed that it was Chl *d* (*33*, *34*). Therefore, JSC-1 synthesizes three Chls: Chl *a*, Chl *d*, and Chl *f*.

Preliminary reverse transcription polymerase chain reaction experiments confirmed that genes of the *psa2* operon (fig. S2) are transcribed in cells grown in FR (table S2). Cells were therefore grown in WL, transferred to FR for 24 hours, and transcription profiling (RNA-seq) was performed (*28*). Transcript levels for the photosynthesis-related genes in the 21-gene cluster (fig. S2) increased from 3-fold to 278-fold in cells grown in FR (Fig. 3 and table S1), and transcript levels increased at least twofold for ~900 genes (table S2). Transcript levels decreased by more than 50% for ~2000 genes in FR (table S2), including most of the paralogous genes encoding core subunits of photosynthetic complexes (Fig. 3 and table S1). The transcriptional changes during acclimation to FR are surprisingly extensive and exceed those for heterocyst (1036 genes) or hormogonia (1762 genes) differentiation in *Nostoc punctiforme* (*35*).

The transcription profiling data indicated that most core polypeptides of PS I, PS II, and PBSs should be replaced by products of the 21-gene cluster when cells are grown in FR. To verify that this was the case, we analyzed the proteins of photosynthetic complexes isolated from JSC-1 cells grown in WL, FR, 645-nm light, and 710-nm light. Fractions enriched in PS I and/or PS II were isolated on sucrose gradients after solubilization of thylakoid membranes with *n*-dodecyl-β-D-maltoside (Fig. 4A). For JSC-1 cells grown in WL and 645-nm light, two green fractions were observed: (i) an upper green fraction containing

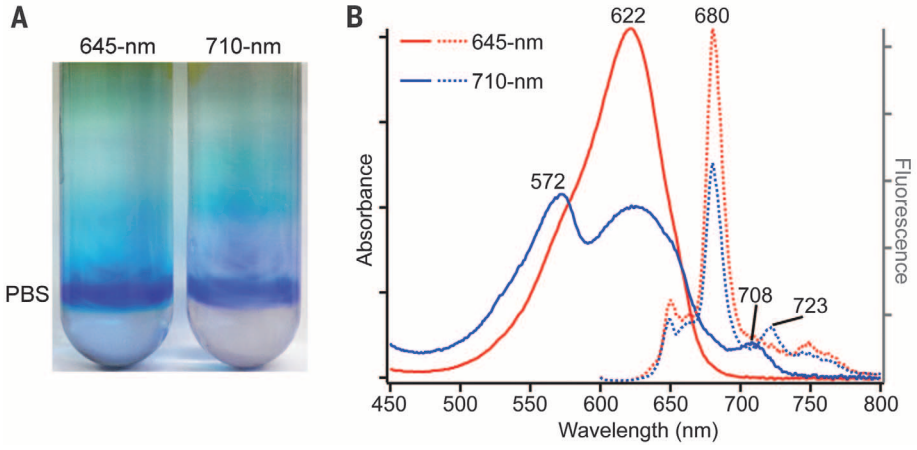

Fig. 5. Phycobilisomes isolated from cells grown in far-red light have enhanced absorption from 700 to 750 nm. (**A**) Sucrose gradients showing phycobilisomes isolated from cells grown in 645-nm light and 710-nm light. (**B**) Absorption spectra (solid lines) and fluorescence emission spectra (dashed lines) for PBS fractions from cells grown in 645-nm light (red lines) and 710-nm light (blue lines).

PS I monomers and PS II dimers, and (ii) a lower green fraction containing PS I trimers (Fig. 4A). Gradients prepared with solubilized membranes from JSC-1 cells grown in FR and 710-nm light differed dramatically. No PS I trimers were observed, and only a single fraction containing PS I monomers and PS II dimers was observed (Fig. 4A). The complexes derived from cells grown in WL or 645-nm light had absorption (Fig. 4B) and 77K fluorescence emission maxima (Fig. 4C) expected for PS I (725 nm) and PS II (685 and 695 nm) complexes containing Chl *a*. However, complexes isolated from cells grown in FR and 710-nm light had additional absorption features at ~720 nm (Fig. 4B) and had fluorescence emission maxima at 745 nm (Fig. 4C). These spectra demonstrate that both PS I and PS II complexes of cells grown in FR and 710-nm light contain Chl *f*.

Proteins associated with isolated photosynthetic complexes were analyzed by trypsin digestion and mass spectrometry of the resulting peptides

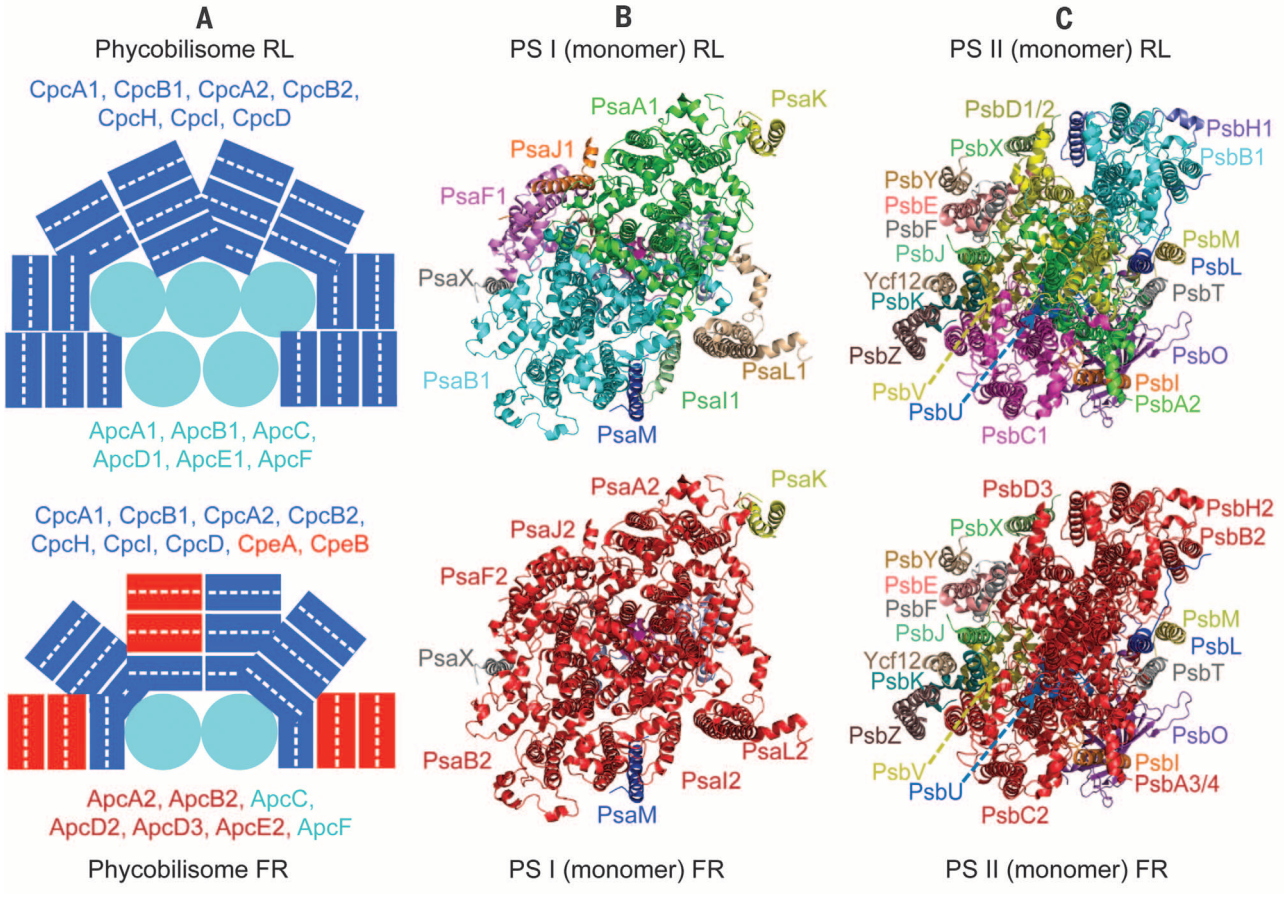

Fig. 6. Diagrams summarizing the remodeling of components of PBSs, PS I, and PS II, during FaRLiP. (**A**) Remodeling of PBS cores in FR. Except ApcF and ApcC (see table S1), all allophycocyanin-related components (shown in aqua) composing the PBS core substructure are replaced in FR. ApcE1 has four REP (linker) domains and should assemble a pentacylindrical substructure in cells grown in WL, GL, or RL (top). ApcE2 has only two REP (linker) domains and should assemble a bicylindrical core substructure in FR (*7*, *38*) (bottom). PBSs assembled in FR contain some phycoerythrin (table S1; shown as red disks; phycocyanin is shown in blue). This may occur because FR cannot efficiently photoconvert the CCA photoreceptor into its green-absorbing (Pg) form (*23*). Remodeling of PS I (**B**) and PS II (**C**) illustrated using the x-ray structures of PS I [Protein Data Bank (PDB) = 1JB0] (*47*) and PS II (PDB = 3BZ2) (*48*) from *Thermosynechococcus elongatus*. The view of PS I is from the lumenal side, so PsaC, PsaD, and PsaE are not visible. The view of PS II is from the cytoplasm-facing side. Each subunit is shown in a different color (top), and subunits replaced in FR are shown in red (bottom). Subunit Ycf12 of PS II is not encoded in the draft genome of JSC-1.

(table S1). This analysis demonstrated that the core polypeptides comprising complexes from cells grown in FR and 710-nm light were the products of those genes whose transcript levels strongly increased in cells grown under FR (e.g., *psaA2*, *psaB2*, *psaL2*, and *psaF2* and *psbB2*, *psbC2*, *psbD3*, *psbA3*, *psbA4*, and *psbH2*) (table S1). In contrast, the core proteins in complexes from cells grown in WL or 645-nm light contained products of paralogous genes with lowered transcript levels under FR (e.g., *psaA1*, *psaB1*, *psbB1*, and *apcE1*) (Fig. 3 and table S1). Thus, the proteins composing PS I and PS II differed in their responses to the growth light conditions.

We isolated PBSs from cells grown in 645-nm and 710-nm light to assess whether absorption differences accompanied polypeptide replacement in FR (Fig. 5A). PBSs isolated from cells grown in 710-nm light have an absorbance feature at 708 nm that is correlated with a low-temperature fluorescence emission peak at 723 nm, but PBSs isolated from cells grown in 645-nm light lack these features (Fig. 5B). The 708-nm absorption band is red-shifted nearly 40 nm compared with allophycocyanin-B (λ_{max} = 670 nm), previously the most red-shifted maximal absorption for characterized native phycobiliproteins (*36*). After denaturation of PBS proteins in 8.0 M urea at pH 3 (fig. S17), only phycocyanobilin and phycoerythrobilin were detected (*37*), which suggested that the long-wavelength absorption arises from phycocyanobilin and not from a new bilin chromophore. Even PBS ultrastructure changes in cells grown in FR. The ApcE1 core linker phycobiliprotein expressed in WL and 645-nm light has four linker-repeat (REP) domains and thus is predicted to assemble PBSs with "pentacylindrical" core substructures (*7*, *38*) (Fig. 6A, top). ApcE1 has a highly conserved cysteine residue at position 197 that ligates a phycocyanobilin chromophore via a thioether linkage (*7*). ApcE2, the core linker phycobiliprotein synthesized in FR, has only two REP domains and, thus, is predicted to produce PBSs with only two core cylinders (*7*, *38*) (Fig. 6A, bottom). ApcE2 has no cysteine residues in its N-terminal phycobiliprotein domain; thus, ApcE2 should bind phycocyanobilin noncovalently. Noncovalently bound phycocyanobilin would have one additional conjugated double bond, which should shift the absorption and fluorescence emission of ApcE2 to a longer wavelength, as shown by site-directed mutagenesis of *apcE* in *Synechococcus* sp. PCC 7002 (*39*). Such changes could extensively modify the energy transfer pathway(s) and efficiency in the cores of PBSs in cells grown in FR. These observations raise the possibility that other core PBS subunits (ApcA2, ApcB2, ApcD3, and ApcD4) could also bind phycocyanobilin noncovalently to increase absorption of FR light ($\lambda \geq$ 700 nm).

JSC-1 thus remodels its photosynthetic apparatus during FaRLiP by synthesizing Chl *d* and *f*, replacing the core subunits of PS I and PS II, and modifying the proteins of the PBS core as summarized in Fig. 6. Analogously to CCA, these changes produce photosynthetic complexes with absorption that is complementary to the incident irradiation between 700 and 750 nm. Additionally, JSC-1 alters relative transcript levels for >40% of the JSC-1 genome, which leads to extensive modification of cellular metabolism (table S2). The transcription changes and global replacement of core components of PS I, PS II, and PBSs during FaRLiP are distinct from the incorporation of PsbA variants in PS II complexes of *Synechocystis* sp. PC 6803 and other cyanobacteria in response to high light intensity or anoxic conditions (*40*) and from reported transcription changes for *psbD3* and *psbE2* of *Acaryochloris marina* cells grown in FR or WL at very low irradiance (*41*).

There is no overlap between the structural remodeling of photosynthetic complexes that occurs during CCA and FaRLiP, and it is noteworthy that JSC-1 performs both acclimation responses (see fig. S1) (*24*). We assume that PS I, PS II, and PBS subunits produced during FaRLiP have adapted through evolution to perform photosynthesis more efficiently when some Chl *a* molecules are replaced by Chl *f* (and Chl *d*) in cells growing in FR. Indeed, JSC-1 cells that have acclimated to 710-nm light have 40% greater oxygen evolution with far-red actinic light than cells acclimated to 645-nm light, although the two types of cells have identical light saturation behavior when the actinic light is WL (fig. S18). This enhanced photosynthetic performance in FR would be ecologically important whenever cells grow in light that is strongly filtered by Chl *a* absorbance—for example, in mats, stromatolites, cyanobacterial blooms, or the shade of plants. FaRLiP should also benefit organisms living in sandy soils, because far-red light penetrates deeper than visible wavelengths (*42*). The 730 nm:650 nm ratio shifts from ~0.84 at the soil surface to 2.8 at a depth of 6 mm (*43*), and this could be further enhanced by Chl *a* absorption filtering. Thus, FaRLiP could have a substantial impact on cyanobacterial photosynthesis in soil crusts (two organisms in fig. S3 are soil isolates).

Our results show that it is possible for cyanobacteria to retain paralogous copies of genes for functionally specialized photochemical reaction centers. This observation has important implications for the evolution of type 1 and type 2 reaction centers during the evolution of photosynthesis, as well as for the extension of Chl biosynthetic pathways (*44*). If type 1 reaction centers evolved first, a likely mechanism for the origin of type 2 reaction centers was gene duplication and functional divergence (*44*–*46*). As shown in this study, functionally distinctive and divergent PS I and PS II reaction centers are formed in strain JSC-1 during growth in FR. Finally, our findings could have important implications for introducing the capacity to utilize FR into plants.

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ACKNOWLEDGMENTS

This study was funded by grant MCB-1021725 from the NSF to D.A.B. The genome sequence of *Leptolyngbya* sp. strain JSC-1 was determined under the auspices of the U.S. Department of Energy's Office of Science, Biological, and Environmental Research

Program, and by the University of California, Lawrence Berkeley National Laboratory under contract no. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under contract no. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396. Spectroscopic characterization of RfpA and NpR4776-PCM was funded by a grant from the Chemical Sciences, Geosciences, and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy (DOE DE-FG02-09ER16117 to J.C.L.) The authors thank J. R. Miller for assistance in performing mass analyses on Chls *d* and *f*; B. Stanley and A. Stanley for advice and technical assistance in performing the tryptic peptide mass fingerprinting; and C. Praul and C. Price for assistance in performing RNA-seq profiling. The authors thank J. Lecomte, W. Schluchter, J. Golbeck, and A. Glazer for reading the manuscript and helpful suggestions. This whole-genome shotgun project for *Leptolyngbya* sp. strain JSC-1 (alternative name, *Marsacia ferruginose*; IMG taxon ID 2022827000; GOLD ID = Gi02032) has been deposited at DDBJ/EMBL/GenBank under the accession JMKF00000000; the version described in this paper is version JMKF01000000. RNA-seq data were deposited in the NCBI Sequence Read Archive (SRA) under accession number SRP041154.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/full/345/6202/1312/suppl/DC1
Materials and Methods
Figs. S1 to S18
Tables S1 and S2
References (*49*–*60*)

4 June 2014; accepted 31 July 2014
Published online 21 August 2014;
10.1126/science.1256963

PLANETARY DYNAMICS

Chaotic dynamics of stellar spin in binaries and the production of misaligned hot Jupiters

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Many exoplanetary systems containing hot Jupiters are observed to have highly misaligned orbital axes relative to the stellar spin axes. Kozai-Lidov oscillations of orbital eccentricity and inclination induced by a binary companion, in conjunction with tidal dissipation, constitute a major channel for the production of hot Jupiters. We demonstrate that gravitational interaction between the planet and its oblate host star can lead to chaotic evolution of the stellar spin axis during Kozai cycles. As parameters such as the planet mass and stellar rotation period are varied, periodic islands can appear in an ocean of chaos, in a manner reminiscent of other dynamical systems. In the presence of tidal dissipation, the complex spin evolution can leave an imprint on the final spin-orbit misalignment angles.

About 1% of solar-type stars host giant planets with periods of ~3 days (*1*). These "hot Jupiters" could not have formed in situ, given the large stellar tidal gravity and radiation fields close to their host stars. Instead, they are thought to have formed beyond a few astronomical units (AU) and migrated inward. However, the physical mechanisms of the migration remain unclear. In the past few years, high stellar obliquities have been observed in many hot Jupiter systems; that is, the spin axis of the host star and the planetary orbital angular momentum axis are misaligned (*2*–*7*). Planet migration in protoplanetary disks (*8*, *9*) is usually expected to produce aligned orbital and spin axes [however, see (*10*–*14*)], so the observed misalignment suggests that other formation channels may be required, such as strong planet-planet scatterings (*15*, *16*), secular interactions or chaos among multiple planets (*17*, *18*), and the Kozai-Lidov effect induced by a distant companion (*19*–*22*). Other observations suggest that multiple formation channels of hot Jupiters may be required (*23*–*25*).

In the "Kozai + tide" scenario, a giant planet initially orbits its host star at a few AU and experiences secular gravitational perturbations from a distant companion (a star or planet). When the companion's orbit is sufficiently inclined relative to the planetary orbit, the planet's eccentricity undergoes excursions to large values while the orbital axis precesses with varying inclination. At periastron, tidal dissipation in the planet reduces the orbital energy, leading to inward migration and circularization of the planet's orbit.

As the planet approaches the star in a Kozai cycle, the planet-star interaction torque due to the rotation-induced stellar quadrupole makes the stellar spin axis and the planetary orbital angular momentum axis precess around each other. Although the equations for such precession in the context of triple systems are known (*21*, *26*), previous work on the "Kozai + tide" migration either neglected such spin-orbit coupling or included it without systematically examining the spin dynamics or exploring its consequences for various relevant parameter regimes (*19*–*22*, *27*). However, the stellar spin has the potential to undergo rich evolution during the Kozai migration, which may leave its traces in the spin-orbit misalignments in hot Jupiter systems. Indeed, there are several examples of chaotic spin-orbit resonances in the solar system. For instance, Saturn's satellite Hyperion experiences chaotic spin evolution due to resonances between spin and orbital precession periods (*28*). The rotation axis of Mars also undergoes chaotic variation as a result of resonances between the spin precession and a combination of orbital precession frequencies (*29*, *30*).

We demonstrate here that gravitational interaction between the stellar spin and the planetary orbit can indeed induce a variety of dynamical behavior for the stellar spin evolution during Kozai cycles, including strongly chaotic behavior (with Lyapunov times as short as a few million years) and perfectly regular behavior in which the stellar spin stays aligned with the orbital axis at all times. We show that in the presence of tidal dissipation, the memory of chaotic spin evolution can be preserved, leaving an imprint on the final spin-orbit misalignment angles.

Kozai cycles and spin-orbit coupling

We consider a planet of mass $M_{\rm p}$ initially in a nearly circular orbit around a star of mass $M_\star$ at a semimajor axis a, with a distant binary companion of mass $M_{\rm b}$, semimajor axis $a_{\rm b}$, and eccentricity $e_{\rm b}$, which we set to 0. In that case, if the planet's initial orbital inclination relative to the binary axis, denoted by $\theta_{\rm lb}^0$, falls within the range $\{40°, 140°\}$, the distant companion induces cyclic variations in planetary orbit inclination and eccentricity, with a maximum eccentricity of $e_{\max} \approx \sqrt{1-(5/3)\cos^2\theta_{\rm lb}^0}$ (*31*, *32*). These Kozai cycles occur at a characteristic rate given by

$$t_{\rm k}^{-1} = n\left(\frac{M_{\rm b}}{M_\star}\right)\left(\frac{a}{a_{\rm b}}\right)^3 = \left(\frac{2\pi}{10^6\ \text{years}}\right)\left(\frac{M_{\rm b}}{M_\star}\right)\left(\frac{M_\star}{M_\odot}\right)^{1/2} \times \left(\frac{a}{1\ \text{AU}}\right)^{3/2}\left(\frac{a_{\rm b}}{100\ \text{AU}}\right)^{-3} \quad (1)$$

where $M_\odot$ is the mass of the Sun and $n = 2\pi/P$ (where P is the orbital period) is the mean motion of the planet. Note, however, that the presence of short-range forces, such as General Relativity and tidal distortions, tends to reduce the maximum attainable eccentricity, so that the actual $e_{\max}$ may be smaller than the "pure" (i.e., without short-range forces) Kozai value given above (*19*, *20*, *33*). Along with the eccentricity and inclination variations, the planetary orbital angular momentum vector precesses around the binary axis ($\hat{\mathbf{L}}_{\rm b}$) at a rate that, in the absence of tidal dissipation, is approximately given by

$$\Omega_{\rm pl} \approx \frac{3}{4}t_{\rm k}^{-1}\cos\theta_{\rm lb}^0\sqrt{1-e_0^2}\left[1-2\left(\frac{1-e_0^2}{1-e^2}\right)\frac{\sin^2\theta_{\rm lb}^0}{\sin^2\theta_{\rm lb}}\right] \quad (2)$$

(*34*), where e_0 is the initial eccentricity. Because of the rotation-induced stellar quadrupole, the

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planet induces precession in the stellar spin orientation, governed by the equation

$$\frac{d\hat{\mathbf{S}}}{dt} = \Omega_{\rm ps}\hat{\mathbf{L}} \times \hat{\mathbf{S}} \quad (3)$$

where $\hat{\mathbf{S}}$ and $\hat{\mathbf{L}}$ are unit vectors along the stellar spin axis and the planetary orbital angular momentum axis, respectively, and the precession frequency $\Omega_{\rm ps}$ is given by

$$\begin{aligned}\Omega_{\rm ps} &= -\frac{3GM_{\rm p}(I_3 - I_1)}{2a^3(1-e^2)^{3/2}}\frac{\cos\theta_{\rm sl}}{S} \\ &= -2.38\times10^{-8}\left(\frac{2\pi}{\rm years}\right)\frac{1}{(1-e^2)^{3/2}} \\ &\times\left(\frac{2k_{\rm q}}{k_\star}\right)\left(\frac{10^3M_{\rm p}}{M_\star}\right)\left(\frac{M_\star}{M_\odot}\right)^{1/2} \\ &\times\left(\frac{\hat{\Omega}_\star}{0.1}\right)\left(\frac{a}{1\ {\rm AU}}\right)^{-3}\left(\frac{R_\star}{R_\odot}\right)^{3/2}\cos\theta_{\rm sl} \quad (4)\end{aligned}$$

where I_3 and I_1 are principal moments of inertia of the star, S is its spin angular momentum, $\hat{\Omega}_\star \equiv \Omega_\star/\sqrt{GM_\star/R_\star^3}$ is its spin frequency in units of the breakup frequency, $R_\star$ is the stellar radius, $R_\odot$ is the radius of the Sun, and $\theta_{\rm sl}$ is the angle between the stellar spin axis and the planet angular momentum axis. We define $(I_3 - I_1) \equiv k_{\rm q}M_\star R_\star^3\hat{\Omega}_\star^2$ and $S \equiv k_\star M_\star R_\star^2\Omega_\star$. For a solar-type star, $k_{\rm q} \approx 0.05$ and $k_\star \approx 0.1$ (*35*). The stellar quadrupole also affects the planet's orbit by introducing additional periastron advance, at a rate on the order of $-\Omega_{\rm ps}S/(L\cos\theta_{\rm sl})$ (where $L \equiv M_{\rm p}\sqrt{GM_\star a(1-e^2)}$ is the orbital angular momentum), and making $\hat{\mathbf{L}}$ precess around $\hat{\mathbf{S}}$ at the rate $(S/L)\Omega_{\rm ps}$ (*34*).

During the Kozai cycle, orbital eccentricity varies widely from 0 to $e_{\rm max}$, and thus $\Omega_{\rm ps}$ and $\Omega_{\rm pl}$ change from $\Omega_{\rm ps,0}$ and $\Omega_{\rm pl,0}$ to $\Omega_{\rm ps,max}$ and $\Omega_{\rm pl,max}$, respectively. However, $\Omega_{\rm ps}$ is more sensitive than $\Omega_{\rm pl}$ to eccentricity variation and attains a larger range of values. We therefore expect three qualitatively different regimes for the spin evolution.

Regime I, $|\Omega_{\rm ps,max}| \lesssim |\Omega_{\rm pl,max}|$ ("nonadiabatic"): $|\Omega_{\rm ps}|$ is always smaller than $|\Omega_{\rm pl}|$. We expect $\hat{\mathbf{S}}$ to effectively precess around $\hat{\mathbf{L}}_{\rm b}$, the binary angular momentum axis (about which $\hat{\mathbf{L}}$ is precessing), maintaining an approximately constant angle $\theta_{\rm sb}$.

Regime II, $|\Omega_{\rm ps,max}| \gtrsim |\Omega_{\rm pl,max}|$ and $|\Omega_{\rm ps,0}| \lesssim |\Omega_{\rm pl,0}|$ ("transadiabatic"): A secular resonance occurs when the stellar precession rate approximately matches the orbital precession rate ($|\Omega_{\rm ps}| \approx |\Omega_{\rm pl}|$). As the eccentricity varies from 0 to $e_{\rm max}$ during the Kozai cycle, the system transitions from nonadiabatic to adiabatic. We expect this resonance crossing to lead to complex and potentially chaotic spin evolution.

Regime III, $|\Omega_{\rm ps,0}| \gtrsim |\Omega_{\rm pl,0}|$ ("adiabatic"): $|\Omega_{\rm ps}|$ is always larger than $|\Omega_{\rm pl}|$. We expect the spin axis to follow $\hat{\mathbf{L}}$ adiabatically, maintaining an approximately constant spin-orbit misalignment angle $\theta_{\rm sl}$.

For a given planet semimajor axis a and binary semimajor axis $a_{\rm b}$, the division between different regimes depends on the product of planet mass and stellar spin (fig. S1). In particular, systems with low $M_{\rm p}$ and $\Omega_\star$ lie in regime I, whereas those with large $M_{\rm p}$ and $\Omega_\star$ lie in regimes II and III.

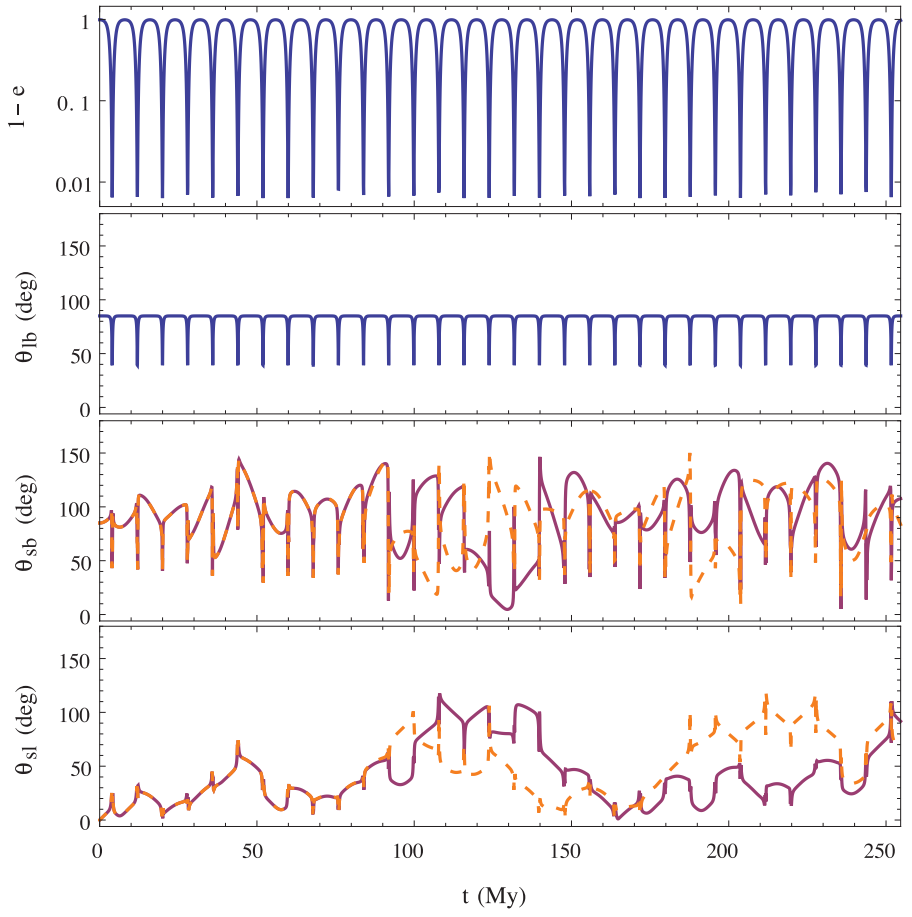

Fig. 1. Sample evolution curves for the "pure" Kozai system, demonstrating how the stellar spin evolves through Kozai cycles. The parameters for this run of 250 million years (My) are a = 1 AU, $a_{\rm b}$ = 200 AU, $e_{\rm b}$ = 0, $M_\star = M_{\rm b} = 1M_\odot$, $\hat{\Omega}_\star$ = 0.05, $M_{\rm p} = 4.6M_{\rm J}$, and initial e_0 = 0.01, $\theta_{\rm lb}^0$ = 85°. The spin's erratic evolution is suggestive of chaos; we therefore plot a "real" trajectory (red solid lines) and a "shadow" trajectory (orange dashed lines), used to evaluate the degree of chaotic behavior. The trajectories are initialized such that the "real" trajectory starts with $\hat{\mathbf{S}}$ parallel to $\hat{\mathbf{L}}$, whereas the "shadow" trajectory starts with $\hat{\mathbf{S}}$ misaligned by 0.000001° with respect to $\hat{\mathbf{L}}$. This figure corresponds to the orange scatterplot of Fig. 2B and the orange curve of Fig. 3A. The spin evolution is highly chaotic.

Numerical exploration

We first studied the evolution of stellar spin in "pure" Kozai cycles by integrating Eq. 3 together with the evolution equations for the planet's orbital elements, driven by the quadrupole potential from the binary companion (*34*), but excluding all short-range forces. Although at the octupole level the companion may induce chaotic behavior in the planet orbit (*36*–*39*), the effect is negligible if $ae_{\rm b}/[a_{\rm b}(1-e_{\rm b}^2)] \ll 0.01$ and is completely suppressed for $e_{\rm b} = 0$. To isolate the dynamics of stellar spin evolution, we exclude the precession of $\hat{\mathbf{L}}$ around $\hat{\mathbf{S}}$ and all other short-range forces; thus, although the planet's orbit influences the stellar spin, the stellar spin does not affect the orbit in any way. We consider different combinations of planet mass and stellar rotation rate to illustrate the different regimes described above (we set $M_\star = M_{\rm b} = 1M_\odot$ and $R_\star = 1R_\odot$ in all the examples shown below). We present four "canonical" cases that encapsulate the range of the observed spin dynamics, including a sample trajectory in the transadiabatic regime (regime II) (Fig. 1).

We find excellent agreement with the qualitative arguments outlined above. In the nonadiabatic regime I (Fig. 2A), the spin evolution is regular and periodic. Although we do not plot the spin-binary misalignment angle ($\theta_{\rm sb}$), it indeed stays constant. For trajectories that start with high initial misalignment of $\hat{\mathbf{S}}$ and $\hat{\mathbf{L}}$, the adiabatic regime III (Fig. 2D) is difficult to access because of the $\cos\theta_{\rm sl}$ factor in the spin precession frequency. Those trajectories that start with low initial $\theta_{\rm sl}$ (or with $\theta_{\rm sl}^0$ close to 180°) maintain that angle, as expected. In the transadiabatic regime II, two different types of behavior are observed. For most parameters that fall within this regime, the spin evolution is strongly chaotic, as indicated by the large degree of scatter that fills up the phase space (Fig. 2, B and C). However, periodic islands exist in the middle of this chaos,

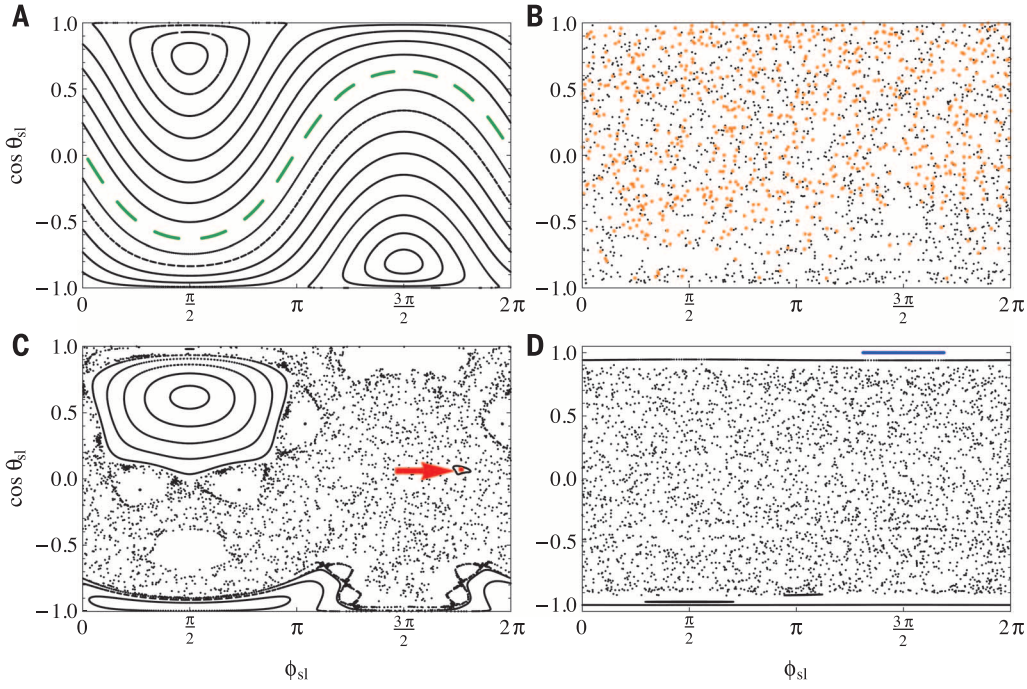

Fig. 2. Surfaces of section of the angle (θ_{sl}) between $\hat{\mathbf{S}}$ and $\hat{\mathbf{L}}$ versus the precessional phase (ϕ_{sl}) of $\hat{\mathbf{S}}$ around $\hat{\mathbf{L}}$ for the "pure" Kozai system, demonstrating the extent of chaos in stellar spin evolution. In all of these sample cases, a = 1 AU, a_b = 200 AU, e_b = 0, $M_\star = M_b = 1M_\odot$, and e_0 = 0.01, θ^0_{lb} = 85°. Each panel is composed of multiple unique trajectories corresponding to different initial values of θ^0_{sl} (with the initial spin-binary angle θ^0_{sb} ranging from 0 to π, and assuming $\hat{\mathbf{S}}$ to be initially in the same plane as $\hat{\mathbf{L}}$ and $\hat{\mathbf{L}}_b$). Each case is evolved for 12.7 billion years, corresponding to ~1500 Kozai cycles. Each point in a trajectory is recorded at the argument of pericenter $\omega = \pi/2$ ($+2\pi n$, with n an integer), corresponding to every other eccentricity maximum (fig. S2). (**A**) Regime I (nonadiabatic); $\hat{\Omega}_\star$ = 0.003, $M_p = 1M_J$. We show 18 unique trajectories, with θ^0_{sb} ranging from 5° to 175°; the green line corresponds to θ^0_{sl} = 0. The "equilibrium" states at (θ_{sl}, ϕ_{sl}) ≈ (40°, 90°) and (40°, 270°) correspond to $\hat{\mathbf{S}}$ parallel and antiparallel to $\hat{\mathbf{L}}_b$. (**B**) Regime II (transadiabatic); $\hat{\Omega}_\star$ = 0.05, $M_p = 4.6M_J$. The orange dots show θ^0_{sl} = 0; the black dots are a composite of several different values of θ^0_{sl}. (**C**) Regime II (transadiabatic); $\hat{\Omega}_\star$ = 0.03, $M_p = 1.025M_J$. There are 11 periodic or quasi-periodic trajectories and a composite chaotic region. An arrow points to the red dot at (cos θ_{sl}, ϕ_{sl}) ≈ (0.06, 1.8π), which corresponds to a periodic island with θ^0_{sl} = 0. (**D**) Regime III (onset of adiabaticity); $\hat{\Omega}_\star$ = 0.05, $M_p = 20M_J$. Five quasi-periodic trajectories and a composite chaotic region are shown. The blue line corresponds to θ^0_{sl} = 0. Note that although both the orange and red cases are found in regime II, the orange one is highly chaotic, and the red resides in a periodic island.

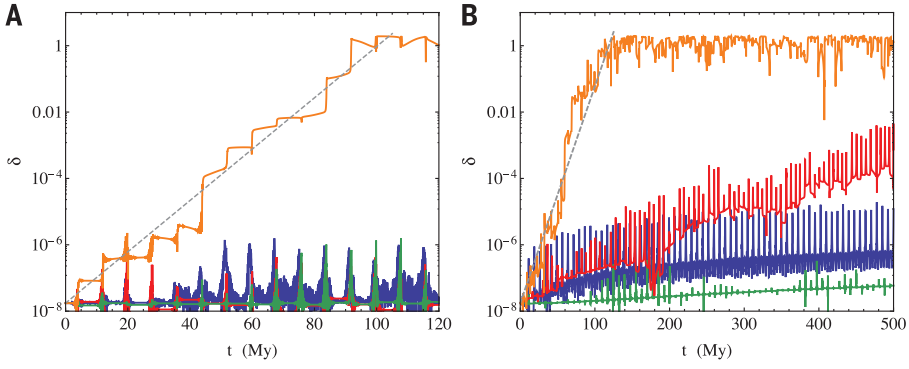

Fig. 3. Distance between two phase-space trajectories starting at slightly different initial spin orientations. (**A**) The "pure" Kozai system. The "real" trajectory starts with $\hat{\mathbf{S}}$ parallel to $\hat{\mathbf{L}}$, and the "shadow" trajectory starts with $\hat{\mathbf{S}}$ misaligned by 0.000001° with respect to $\hat{\mathbf{L}}$, for each of the sample θ^0_{sl} = 0 cases depicted in Fig. 2. The phase-space distance is calculated as $\delta = |\hat{\mathbf{S}}_{real} - \hat{\mathbf{S}}_{shadow}|$ and therefore has a maximum value of 2. The lines are color-coded to correspond to each of the cases of Fig. 2. The gray dashed line demonstrates that for the chaotic orange curve, $\delta \propto e^{\lambda t}$, with $\lambda \approx 0.18$ My^{-1}. (**B**) Same as (A), but including orbit precession due to stellar quadrupole and periastron advances due to General Relativity, stellar quadrupole, planet oblateness, and static tides in the planet. The orange curve shows chaotic growth with $\lambda \approx 0.15$ My^{-1}. The red curve, which is periodic in (A), is mildly chaotic here, with $\lambda \approx 0.02$ My^{-1}.

in which the stellar spin behavior is regular (Fig. 2C and fig. S3).

Because the stellar spin and planet orbital axes in real physical systems typically start out aligned, we focused on the trajectories with $\theta^0_{sl} = 0$. To assess the degree of chaos in each of the sample cases (Fig. 2), we evolved a "shadow" trajectory in addition to the real one (Fig. 1), with initial conditions very close to the original ones, and monitored how quickly the two trajectories diverged, particularly in the spin direction. As expected, three out of our four sample cases did not exhibit chaos; the fourth, in the transadiabatic regime, was strongly chaotic, with a Lyapunov time of $\lambda^{-1} \approx 5.6$ million years, corresponding to only ~1 Kozai cycle (Fig. 3A).

Next, we included the precession of $\hat{\mathbf{L}}$ about $\hat{\mathbf{S}}$ and other short-range forces (periastron advances due to General Relativity, the stellar quadrupole, and the planet's rotational bulge and tidal distortion) (*19*, *20*) in our calculations. We found that including these short-range forces for our four sample cases (Fig. 3B) does not change our general conclusion that chaotic evolution occurs in the transadiabatic regime, although it can shift the locations (in the parameter space) of periodic islands.

Clearly, the stellar spin behavior in the transadiabatic regime is very complex: highly chaotic for certain parameters, more regular for others. To explore this diversity further, we constructed a "bifurcation" diagram (Fig. 4) with which we could examine the degree of chaos over a large range of parameter values (particularly the planet mass). Visualized in this way, the topology of the chaos is more obvious: Most of the mass bins are highly chaotic, but they are interspersed with individual, isolated quasiperiodic islands. To better understand this complex topology, we have developed a simpler analytical toy model that captures many of the features of this system (*34*).

Widespread chaos in dynamical systems is typically driven by overlapping resonances (*40*). Repeated secular spin-orbit resonance crossings ($|\Omega_{ps}| \sim |\Omega_{pl}|$) during Kozai cycles play an important role in producing the observed chaotic spin behavior. On the other hand, Kozai cycles themselves result from the near-1:1 resonance ($\dot{\varpi} = \dot{\Omega}$) between the longitude of the periapse ϖ and the longitude of the ascending node Ω of the planet's orbit. The back-reaction of the stellar spin on the orbit can naturally couple these two

resonances. We suggest that all these effects are important in the development of the chaotic stellar spin evolution.

Tidal dissipation and memory of chaotic evolution

Having explored the variety of behaviors exhibited by stellar spin during Kozai cycles, we now assess the impact of this evolution on the production of hot Jupiters, particularly on their final stellar spin-orbit misalignment angles, by adding tidal dissipation to our equations. We used the standard weak friction model of tidal dissipation in giant planets with constant tidal lag time (*41*, *42*). To ensure that all our runs led to circularized planets and a final θ_{sl} within about 10^{10} years, we enhanced tidal dissipation by a factor of 14 (Fig. 5A) and 1400 (Fig. 5B) relative to the fiducial value for Jupiter (*34*, *43*). As long as the tidal evolution time scale of the orbit is much longer than the Lyapunov time for the chaotic spin evolution, we would not expect this enhancement to have a major qualitative effect on the final observed spin-orbit misalignment angle.

We found that tidal dissipation leads to a gradual decrease in the proto–hot Jupiter's semimajor axis and eventual circularization close to the host star (fig. S4). As the planet's orbit decays, Kozai cycles become suppressed by short-range forces. Also, as the semimajor axis decays, $|\Omega_{ps}/\Omega_{pl}|$ increases. Thus, even if we choose initial conditions that lie squarely in the nonadiabatic regime (regime I), as a decreases, all trajectories will eventually go through the $|\Omega_{ps}| = |\Omega_{pl}|$ secular resonance and end up fully adiabatic. At that point, the spin-orbit misalignment angle freezes out to some final, constant value θ_{sl}^{f}.

In all of the numerical examples of nondissipative evolution discussed above, we have held the value of the stellar spin rate $\Omega_\star$ constant. However, because the divisions between different spin evolution regimes depend on $\Omega_\star$, stellar spin-down can potentially have a substantial effect on the degree of chaos in the system. Isolated solar-type stars spin down via magnetic braking associated with the stellar wind (*44*). For simplicity, we use the empirical Skumanich law (*45*) to add stellar spin-down to our evolution equations, starting with an initial spin period of 2.3 days; the final spin period (at t = 5 billion years) is 28 days.

To assess the influence of chaotic stellar spin evolution on the final distribution of spin-orbit misalignment angles, we created a different kind of "bifurcation" diagram (Fig. 5). As in the nondissipative case (Fig. 4), we considered a range of planet masses. For each M_p, we took a set of initial conditions that were identical in all but the initial

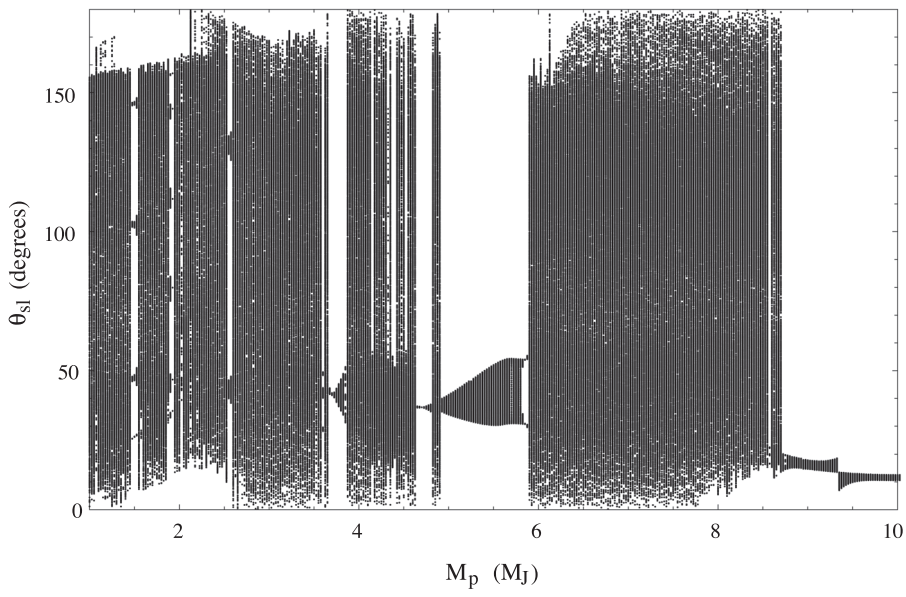

Fig. 4. "Bifurcation" diagram of spin-orbit misalignment angle versus planet mass, including all short-range effects. The procedure described in Fig. 2 is carried out for each value of planet mass; the spin-orbit misalignment angle is recorded at every other eccentricity maximum for ~1500 Kozai cycles. The parameters for this plot are a = 1 AU, a_b = 200 AU, e_0 = 0.01, θ_{lb}^{0} = 85°, $\hat{\Omega}_\star$ = 0.03. A high degree of scatter in a single mass bin indicates highly chaotic behavior. Note that multiple quasi-periodic islands appear in the middle of highly chaotic regions.

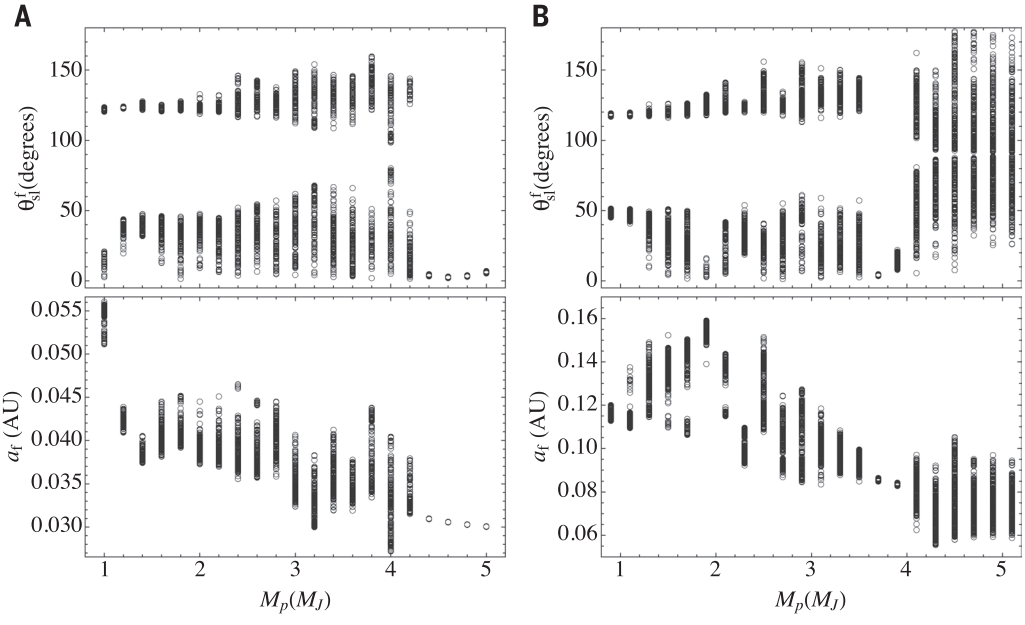

Fig. 5. Two "bifurcation" diagrams of the final spin-orbit misalignment angle (top row) and semimajor axis (bottom row) versus planet mass for a small range of initial planet-binary inclinations, including the effects of tidal dissipation and stellar spin-down. Parameters: a_b = 200 AU, e_0 = 0.01, $\hat{\Omega}_{\star,0}$ = 0.05. Each data point represents the outcome of a single complete run, starting with a_0 = 1.5 AU (**A**) and a_0 = 1 AU (**B**) and ending when the planet has sufficiently circularized (final eccentricity $e_f \leq 0.1$) and the final spin-orbit angle θ_{sl}^{f} is attained. For each run, we randomly select an initial inclination θ_{sl}^{0} from the range 86.99° to 87.01° (A) and 84.95° to 85.05° (B). Each mass bin contains ~200 points. The degree of scatter in θ_{sl}^{f} generally increases with increasing M_p but drops sharply in the adiabatic regime [for $M_p \gtrsim 4.4 M_J$ in (A)]. Quasi-periodic islands are still present [e.g., at ~$3.8 M_J$ in (B)].

orbit-binary misalignment angle θ_{lb}, which we randomly chose from a very small range: $\theta^0_{lb} \in \{86.99°, 87.01°\}$ (Fig. 5A) and $\theta^0_{lb} \in \{84.95°, 85.05°\}$ (Fig. 5B). We evolved these trajectories until the hot Jupiter circularized and θ_{sl} reached its final value. We found that the scatter in θ^f_{sl} depends on the planet mass. The scatter generally increases with increasing M_p but drops sharply in the adiabatic regime [for $M_p \gtrsim 4.4 M_J$ (where M_J is the mass of Jupiter) in Fig. 5A]. There also exist quasi-periodic islands where θ^f_{sl} has a rather small spread. Also, a range of misalignment angles around 90° appears to be excluded, with this range decreasing with increasing planet mass. Given the very small range of initial conditions, the evolution of any regular, nonchaotic system should result in only one final misalignment angle. Therefore, we suggest that this bimodality is the result of the system passing through the $|\Omega_{ps}| \approx |\Omega_{pl}|$ secular resonance and of the complex and possibly chaotic dynamics that occur during that time. We tentatively attribute the decrease of bimodality with increasing mass to an increase in chaotic behavior. The final semimajor axis a_f also exhibits "chaotic" spreads and periodic islands. Thus, in effect, the final distributions of θ^f_{sl} and a_f carry an imprint of the spin's past chaotic evolution.

As a final step, we ran a "mini" population synthesis calculation for fixed values of a_0 and a_b and a broader range of initial orbital inclinations (Fig. 6). A sharp contrast exists between the distributions of final spin-orbit misalignment angles at low M_p and high M_p. At low M_p, a bimodal distribution of θ^f_{sl} is produced [this bimodality has been found in some previous population synthesis calculations (*20*, *21*)]. At high M_p, the evolution is mostly adiabatic, producing very little spin-orbit misalignment. This is a clear signature of the complex spin evolution in the observed stellar obliquity. Other factors, such as the stellar spin-down rate and planetary tidal dissipation rate, can also affect the final misalignment distribution.

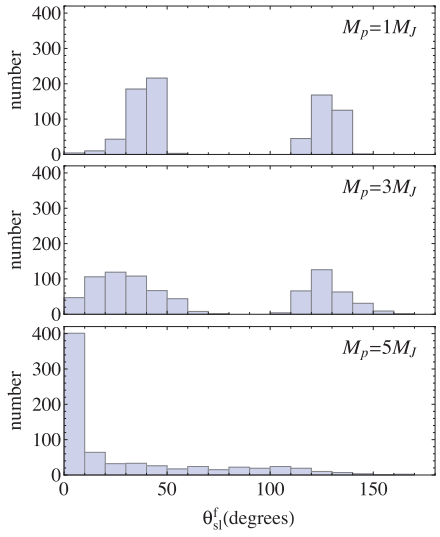

Fig. 6. Distribution of the final spin-orbit misalignment angles as a function of planet mass, including the effects of tidal dissipation and stellar spin-down, for initial planet-binary inclinations θ^0_{lb} in the range 85° to 89°. Parameters: a_0 = 1.5 AU, a_b = 200 AU, e_0 = 0.01, $\hat{\Omega}_{\star,0}$ = 0.05. Each evolutionary trajectory is integrated until it has sufficiently circularized ($e_f \le 0.1$), for a maximum of 5 billion years. If by the end of 5 billion years the planet is not circularized, it is discarded. Note that the bimodality featured in Fig. 5 is still present here, despite the wider range of initial inclinations. At $M_p = 5M_J$, the evolution is mostly adiabatic, and therefore it is difficult to generate misalignment.

Discussion

The discovery of spin-orbit misalignment in close-in exoplanetary systems in the past few years was a major surprise in planetary astrophysics. Much of the recent theoretical work has focused on the nontrivial evolution of the planetary orbit (such as orbital flip) due to few-body gravitational interactions (*23*, *37*, *38*). However, as we have shown here, the spin axis of the host star can undergo rather complex and chaotic evolution, depending on the planetary mass and the stellar rotation rate. In many cases, the variation of the stellar spin axis relative to the binary axis is much larger than the variation of the orbital axis. Therefore, to predict the final spin-orbit misalignments of hot Jupiter systems in any high-eccentricity migration scenario, it is important to properly account for the complex behavior of stellar spin evolution.

In the above, we have focused on the Kozai-Lidov mechanism for the formation of hot Jupiters, but similar consideration can be applied to the formation of short-period stellar binaries (*20*). Indeed, spin-orbit misalignment angles have been measured for a number of close-in stellar binaries (*46*–*48*). Because of the much larger stellar spin precession rate in stellar binaries relative to star-planet systems, the stellar spin evolution is expected to be largely in the adiabatic regime (depending on various parameters; fig. S1), in which case the observed spin-orbit misalignment angles in close binaries would reflect their initial values at formation.

It is a curious fact that the stellar spin axis in a wide binary (~100 AU apart) can exhibit such a rich, complex evolution. This is made possible by a tiny planet (~10^{-3} of the stellar mass) that serves as a link between the two stars: The planet is "forced" by the distant companion into a close-in orbit, and it "forces" the spin axis of its host star into wild precession and wandering.

The "binary + planet + spin" system studied here exhibits many intriguing dynamical properties. Although we have provided a qualitative understanding for the emergence of chaos in this system in terms of secular resonance crossing, much remains to be understood theoretically. Most remarkable is the appearance of periodic islands as the system parameters (planet mass and stellar spin) vary—a feature reminiscent of some well-known chaotic systems (*49*, *50*).

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ACKNOWLEDGMENTS

We thank K. Batygin, D. Fabrycky, M. Holman, and D. Muñoz for useful discussions. Supported by NSF Graduate Research Fellowship Program grant DGE-1144153 (K.R.A.), NSF grants AST-1008245 and AST-1211061, and NASA grants NNX12AF85G and NNX14AG94G.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1317/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S7

3 April 2014; accepted 30 July 2014
10.1126/science.1254358

REPORTS

LOW-DENSITY MATERIALS

Strong, lightweight, and recoverable three-dimensional ceramic nanolattices

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Ceramics have some of the highest strength- and stiffness-to-weight ratios of any material but are suboptimal for use as structural materials because of their brittleness and sensitivity to flaws. We demonstrate the creation of structural metamaterials composed of nanoscale ceramics that are simultaneously ultralight, strong, and energy-absorbing and can recover their original shape after compressions in excess of 50% strain. Hollow-tube alumina nanolattices were fabricated using two-photon lithography, atomic layer deposition, and oxygen plasma etching. Structures were made with wall thicknesses of 5 to 60 nanometers and densities of 6.3 to 258 kilograms per cubic meter. Compression experiments revealed that optimizing the wall thickness-to-radius ratio of the tubes can suppress brittle fracture in the constituent solid in favor of elastic shell buckling, resulting in ductile-like deformation and recoverability.

The ability to decouple properties such as strength and stiffness from density requires the use of advanced processing techniques combined with materials optimized for superior mechanical performance per unit weight. Many monolithic materials with high strength-to-weight (σ_{ys}/ρ) and stiffness-to-weight (E/ρ) ratios—such as technical ceramics, diamond, and metallic glasses—have excellent potential for use as strong and lightweight structural materials but are suboptimal because of their low toughness and brittle, flaw-sensitive nature. Some of these materials exhibit size effects in mechanical properties when reduced to nanoscale dimensions, such as improved strength (*1*, *2*), flaw tolerance (*3*), and enhanced ductility (*4*, *5*). Architected lightweight structures made from high-strength nanoceramics (*1*, *6*, *7*) and nanoceramic composites (*8*) have been reported to have enhanced strengths and stiffnesses, but they still suffer from brittle, catastrophic failure. Efforts to toughen fully dense brittle materials have focused primarily on using microstructural features to impede crack motion (*9*–*11*) and on forming composites (*12*, *13*), but these approaches have seen limited success in lightweight structures. Many natural hard materials such as sea sponge skeletons (*14*) and diatom shells (*15*) are simultaneously stiff, tough, and lightweight, a combination of properties that is thought to be attained by a hierarchical design of components within their bodies (*16*).

Lightweight structures that are both strong and tough may be engineered by utilizing such hierarchical design principles. The yield strength and stiffness of cellular structures scale as $\sigma_y \propto \sigma_{ys}\bar{\rho}^n$ and $E \propto E_s\bar{\rho}^m$, where $\bar{\rho}$ is the relative density, σ_{ys} and E_s are the yield strength and stiffness of the parent solid, and exponents n and m are functions of the architecture (*17*). Cellular geometries that typically lead to the highest strength are stretching-dominated, meaning that they have no intrinsic mechanisms that allow for bending of the individual truss members (*18*, *19*). The yield strength and stiffness of an ideal stretching-dominated structure scale linearly with relative density as $\sigma_y \sim \bar{\rho}$ and $E \sim \bar{\rho}$ (*20*). This is in contrast to architectures that are either periodic and bending-dominated, whose modulus scales as $E \sim \bar{\rho}^2$, or stochastic, with $E \sim \bar{\rho}^3$ scaling (*21*).

We created a strong, stiff, and energy-absorbing hollow-tube nanolattice with an octet-truss geometry (Fig. 1) that consists solely of a brittle ceramic, aluminum oxide (alumina), and exhibits nearly full recoverability after compressions in excess of 50% strain. Nanomechanical experiments reveal that the Young's modulus of our nanolattices scales with relative density as $E \sim \bar{\rho}^{1.61}$, and failure strength scales as $\sigma_f \sim \bar{\rho}^{1.76}$, which differ from the analytical scaling for both stretching- and bending-dominated structures because of the hollow tubes and nodes.

Creation of ceramic nanolattices begins with the design and writing of a three-dimensional (3D) polymer scaffold using two-photon lithography direct laser writing. A thin alumina film is then deposited onto the polymer scaffold by atomic layer deposition (ALD), so that it coats the entire surface. The outermost walls of the coated structure are then removed by focused ion beam milling (FIB), and the internal polymer is etched away in O_2 plasma. The resulting 3D freestanding ceramic nanolattice consists of a network of hollow tubes, as shown in Fig. 1. This fabrication

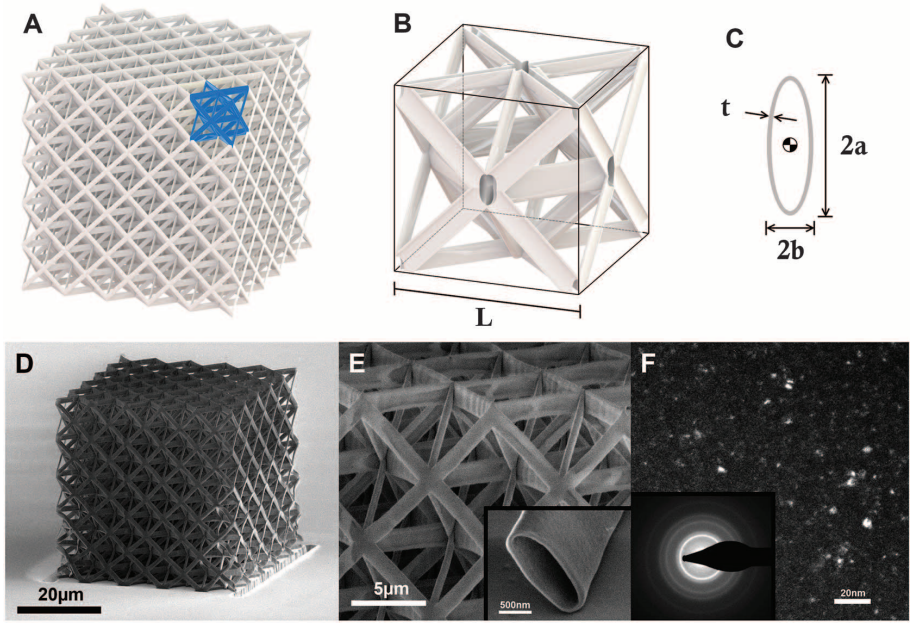

Fig. 1. Architecture, design, and microstructure of alumina nanolattices. (**A**) CAD image of the octet-truss design used in the study. The blue section represents a single unit cell. (**B**) Cutaway of hollow octet-truss unit cell. (**C**) Hollow elliptical cross section of a nanolattice tube. (**D**) SEM image of alumina octet-truss nanolattice. (**E**) Zoomed-in section of the alumina octet-truss nanolattice. The inset shows an isolated hollow tube. (**F**) TEM dark-field image with diffraction grating of the alumina nanolattice tube wall.

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method enables the creation of 3D structures with numerous geometries (*8*, *22*). Further fabrication details and a schematic of the deposition process can be found in (*23*) and are shown in fig. S1.

Nanolattices in this work were designed with relative densities spanning $\bar{\rho}$ = 0.21 to 8.6%. Using a reported value for the density of ALD alumina, ρ_s = 2900 kg/m^3 (*24*), the absolute densities of nanolattices were calculated to be ρ = 6.1 to 249 kg/m^3, which places the lightest ones into the ultralight regime, defined as materials with densities ≤10 kg/m^3 (*21*). This density range is comparable to that of aerogels (*25*) and other ultralight materials (*7*, *21*). In this work, nanolattices were designed to have tube wall thicknesses t of 5 to 60 nm, tube major axis a of 0.45 to 1.38 μm, and unit cell widths L of 5 to 15 μm (Fig. 1, B and C), spanning length scales that can be controlled across four orders of magnitude. Transmission electron microscopy (TEM) analysis revealed ALD alumina to contain 2- to 10-nm nanocrystalline precipitates intermixed in an amorphous matrix (Fig. 1F). A list of the parameters and relative densities is provided in Table S1.

Monotonic and cyclical uniaxial compression experiments were performed on nanolattices in a G200 XP Nanoindenter (Agilent Technologies). In the first set of experiments, structures were compressed uniaxially to ~50% strain at a rate of 10^{-3} s^{-1} to determine their yield stress and overall deformation characteristics (Figs. 2; 3, A to D; and 4, B and D; and fig. S2A). In the second set of experiments, structures were cyclically loaded and unloaded three times to ~70% of their failure load, and unloading slopes from each cycle were averaged to estimate Young's modulus (Fig. 4, A and C, and fig. S2B). Unloading rather than loading moduli were used to mitigate the possible effects of loading imperfections such as misalignment and partial initial contact (fig. S2B). Additional samples were compressed in an in situ nanomechanical instrument, InSEM (Nanomechanics Inc.), to observe local and global deformation characteristics and to investigate the failure modes that occurred during deformation (movies S1 to S3). Stress-strain data and still frames of the in situ compression experiments are shown in Fig. 2.

Two distinct deformation signatures were observed during nanolattice compressions. These are best characterized using the thickness-to-radius ratio of the tubes, t/a, as a figure of merit. Structures with $t/a \geq 0.03$, referred to as thick-walled, demonstrate linear elastic loading followed by catastrophic brittle failure (Fig. 3, A, B, E, and F). An example of a typical deformation and corresponding stress-strain data are shown in Fig. 2, F to J, and movie S3. Compressive stress-strain data for thick-walled structures show large strain bursts, with burst magnitude increasing at greater t/a; structures with t/a = 0.032 have bursts of ~10% strain (Fig. 3B), whereas structures with t/a = 0.067 show bursts of ~80% strain (Fig. 3A). This observed increase in burst magnitude is probably driven by greater elastic strain energy stored in thicker-walled structures during deformation. Each strain burst corresponds to a discrete brittle failure event, which leads to permanent damage of the structure (Figs. 2J and 3, E and F). This type of catastrophic failure has been observed in previous experiments on hollow ceramic nanolattices (*6*) and ceramic composites (*8*) and is generally typical of ceramic foams (*17*).

Thin-walled nanolattices, defined as those with $t/a \leq 0.02$, did not exhibit catastrophic failure or discrete strain bursts. Samples in this regime first deformed elastically, where stress increased linearly with strain, followed by a ductile-like, controlled deformation, with stress plateauing after yielding (Fig. 3, C and D). An example of a typical deformation and corresponding stress-strain data are shown in Fig. 2, A to E, and movie S1. As the t/a of the samples decreased, the serrated burst behavior seen in the thick-walled structures was suppressed, and stress-strain data became smooth (Fig. 3, C and D). After yielding, all ensuing deformation was accommodated through wrinkling and local buckling of the tube walls (Fig. 2, D and E, and movie S1). All thin-walled ceramic nanolattices exhibited notable recovery after deformation, with some recovering up to ~98% of their original height after compression to 50% strain (Figs. 2E and 3H) and others recovering by ~80% after compression to 85% strain (fig. S4). Structures with smaller unit cells demonstrated greater recoverability, each recovering to at least 95% of its original height. Nanolattices with larger unit cells recovered less on average, but all recovered to at least 75% of their original height (fig. S3). SEM images of post-deformed structures revealed localized cracking on and around the nodes (Fig. 3J), implying that the failure of ALD alumina remained brittle and that the observed deformability and recoverability probably emerged from structural effects.

Nanolattices with $0.02 \leq t/a \leq 0.03$ exhibited a combination of the two described deformation signatures. In these samples, both brittle and ductile-like deformation took place; several minor strain bursts were present, and marginal recovery occurred after compression to 50% strain (Fig. 3, C, G, and I, and movie S2). The in situ deformation movie S2 shows that each strain burst correlates with discrete local brittle fracture events in the tubes, and post-yield ductile-like behavior corresponds to buckling and wrinkling of the tube walls. The transition between these two deformation modes is probably driven by an energetic competition between elastic and brittle failure.

Three competing failure mechanisms exist for hollow-tube lattice structures: fracture of the tube wall, Euler (beam) buckling of a truss member, and local (shell) buckling of the tube wall (*26*). We define a failure mechanism (or failure mode) here to be any event that causes a loss of structural integrity of the nanolattice. Different combinations of these mechanisms can occur during

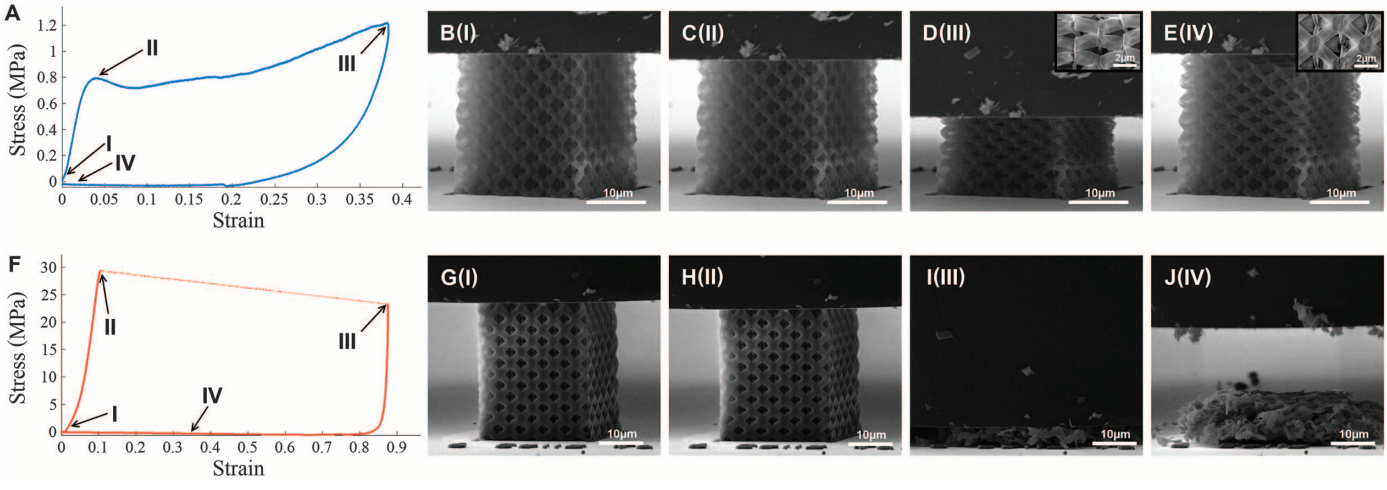

Fig. 2. Compression experiments on thick- and thin-walled nanolattices. (**A** to **E**) Mechanical data and still frames from the compression test on a thin-walled (L = 5 μm, a = 650 nm, t = 10 nm) nanolattice demonstrating the slow, ductile-like deformation, local shell buckling, and recovery of the structure after compression. (**F** to **J**) Mechanical data and still frames from the compression test on a thick-walled (L = 5 μm, a = 790 nm, t = 50 nm) nanolattice showing catastrophic brittle failure and no post-compression recovery.

deformation, depending on the stress state that arises in the beams during loading. Elastic deformation and potentially recoverability will occur in a structure when the stress necessary to initiate these processes is below the critical stress required for fracture. The condition for elastic deformation can be determined by calculating the transition point between two pairs of failure modes: shell buckling versus fracture, and Euler buckling versus fracture. Equating the stresses necessary to initiate each individual failure mechanism, we obtain an expression for the critical transition point between fracture and elastic failure [see (*23*) for the full derivation]

$$\left(\frac{t}{a}\right)_{crit} = \frac{\sigma_{fs}}{E}\sqrt{3(1-\nu^2)} \quad (1)$$

$$\left(\frac{a}{L}\right)_{crit} = \frac{3}{\pi}\sqrt{\frac{2\sigma_{fs}}{5E}} \quad (2)$$

Both of these functions depend on the constituent material properties: Young's modulus (E), fracture strength (σ_{fs}), and Poisson's ratio (ν). Equation 1 represents the critical ratio between the wall thickness (t) and the major radius (a) that is necessary to induce a transition between local buckling and fracture in the tubes. Equation 2 represents the critical ratio between the major radius (a) and length (L) of the tubes that describes a similar transition from Euler buckling to fracture.

Using mechanical property data reported for 75-nm-thick ALD alumina, E = 164 GPa, σ_{fs} = 1.57 to 2.56 GPa, ν = 0.24 (*27*), and Eqs. 1 and 2, the critical thickness-to-radius ratio that induces a transition from yielding to shell buckling in the nanolattices was calculated to be between $(t/a)_{crit} \approx$ 0.0161 and 0.0262, and the critical radius-to-length ratio that denotes transition from yielding to Euler buckling was between $(a/L)_{crit} \approx$ 0.0591 and 0.0755. The property space of all nanolattices studied here, along with their t/a, a/L, and predicted failure modes, are shown in table S1. The experimentally observed deformation behavior of each sample is also noted in the table.

The radius-to-length ratios, a/L, for nanolattices studied here ranged from 0.0750 to 0.180. All of these values are greater than or equal to $(a/L)_{crit}$ predicted by Eq. 2, which means that in an ideal structure, the beams will fracture before the Euler buckling condition is met. This prediction is consistent with our experimental results; no Euler buckling was observed in our in situ compression experiments (Fig. 2 and movies S1 to S3). This model is not capable of capturing local-scale stress concentrations, nor does it account for structures with a high degree of misalignment or pre-bending of the beams, which have been reported to reduce the critical load required to initiate buckling (*6*). The a/L values of the nanolattices are close to $(a/L)_{crit}$, suggesting that Euler buckling may occur in the samples with a large degree of misaligned or pre-bent beams, but it is not observed experimentally to be a dominant deformation mechanism.

The thickness-to-diameter ratios, t/a, of the nanolattices ranged from 0.0059 to 0.0862, which overlaps the range of $(t/a)_{crit}$ predicted by Eq. 1. For thick-walled structures, whose $t/a \geq 0.030 > (t/a)_{crit}$, the model predicts that failure of the beams is dominated by brittle fracture within the alumina tubes. Fractured segments of tubes are unable to carry any load, so every failure event will cause a strain burst whose magnitude depends on the amount of strain energy stored in the system before failure. These predictions are corroborated by experimental stress-strain data for the thick-walled structures (Figs. 2, I and J, and 3, E and F, and movie S3).

Failure in the thin-walled structures, whose $t/a \leq (t/a)_{crit} \leq 0.020$, is predicted to occur primarily via shell buckling, which is an elastic failure mode. This type of failure corresponds to a plateau in the stress-strain data caused by a gradual drop in load-carrying capacity of the beams (*28*), in contrast to the immediate drop in load-carrying capacity associated with fracture. Bending of an isolated thin-walled hollow beam often leads to shell buckling bifurcation, which can cause a jump in displacement (*29*). In a truss structure, the interactions and nodal support among all the beams delay the onset of bifurcation and allow the beams to gradually settle into a new mode. Shell buckling in thin-walled nanolattices is manifested as wrinkling

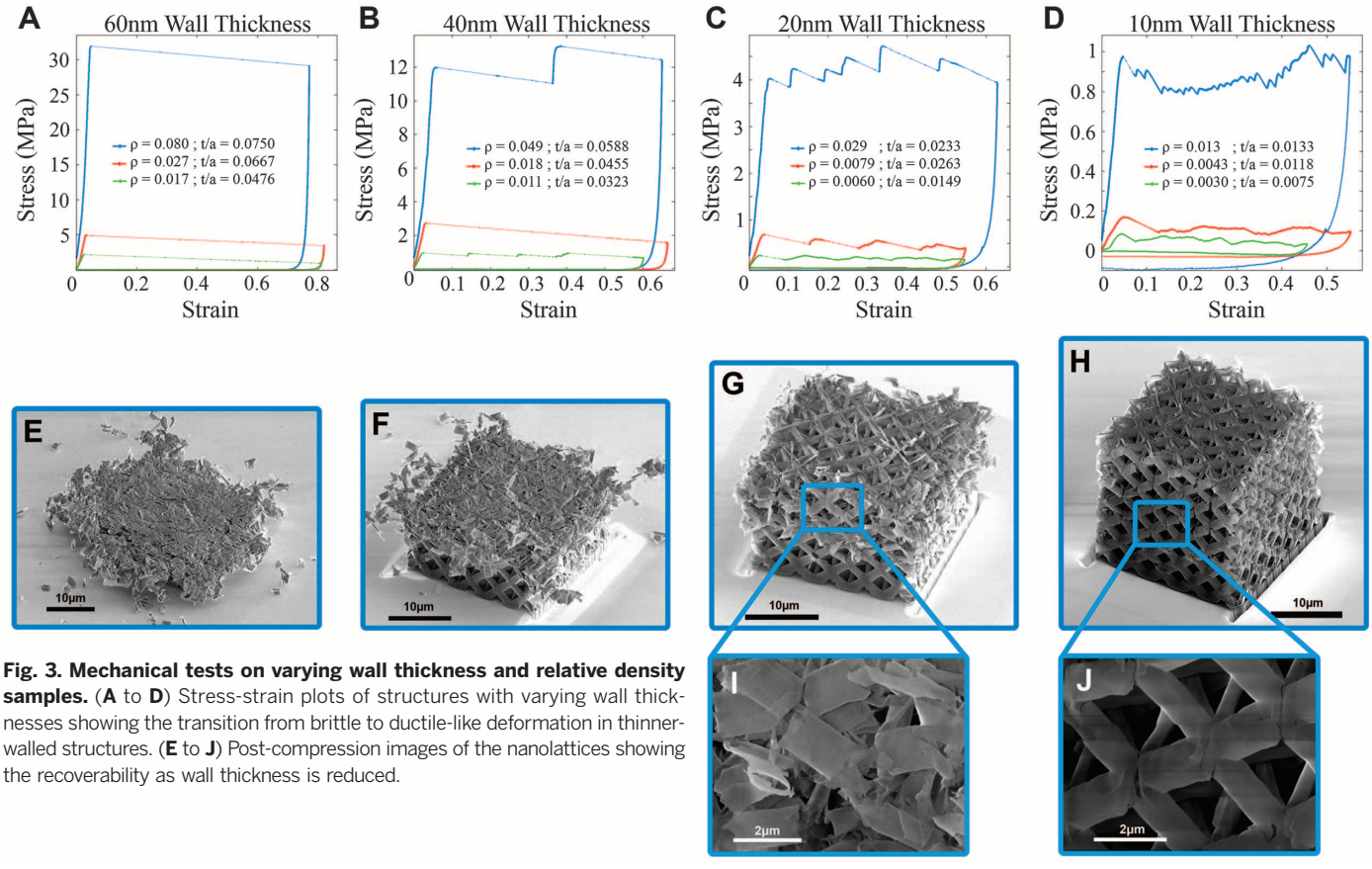

Fig. 3. Mechanical tests on varying wall thickness and relative density samples. (**A** to **D**) Stress-strain plots of structures with varying wall thicknesses showing the transition from brittle to ductile-like deformation in thinner-walled structures. (**E** to **J**) Post-compression images of the nanolattices showing the recoverability as wall thickness is reduced.

and warping of the tubes near the nodes (Figs. 2, D and E; and 3, H and J; and movie S1). The ductile-like deformation and recoverability observed in our experiments on the thin-walled nanolattices probably arise as a result of such shell buckling.

The proposed shell buckling model does not take into account the microstructural or material details, nor is it capable of predicting the deformation of structures in the transition regime of $0.020 \le t/a \le 0.030$. It is helpful in qualitatively explaining deformation in this regime, where nanolattices experience a complex stress state with compressive, tensile, and shear components. Fracture occurs primarily under tension, and shear and buckling occur only in compression, which means that the stress state within the beams can simultaneously satisfy fracture and buckling conditions. This is observed experimentally as a mixing of fracture and buckling failure modes, along with suppressed strain burst behavior and some recoverability (Fig. 3, G and I, and movie S2).

Elastic recovery has been studied previously in metallic and polymer lattices, and models have been proposed for their recoverability (*21*, *30*–*32*). None of these works account for the observed ductile-like behavior of the ceramic nanolattices, and elastically deformable structures composed of intrinsically brittle materials such as ceramics are virtually unexplored. We postulate that reducing the t/a ratio to below $(t/a)_{crit}$ derived in Eq. 1 enables failure via shell buckling, an elastic failure mode that causes minimal damage to the beams and nodes and allows the structure to recover. The transition to elastic failure is a necessary condition to prevent initial yielding or fracture of the constituent material but not a sufficient condition to ensure recovery of the structure. Figure 2D shows that during shell buckling, the global deformation is accompanied by localized wrinkling and warping of the tube walls. This results in confined regions of high stress that can subsequently lead to localized fracture (Figs. 2E and 3J). The propagation of these localized microcracks depends on the overall stress landscape and flaw distribution. If a crack extends into a region of high tensile stresses, or if numerous flaws reside near a crack tip, it is likely to propagate through the node and can potentially result in fracture of the tube. If an existing crack extends into a region of compressive stress, or if the stress field is insufficient to continue the crack extension, its propagation will be suppressed so that the tubes may never fully fracture. In this mechanism, a sufficient number of nodal connections remain intact to enable the structure to recover nearly fully to its original shape. The applied compressive load reduces the local tensile stresses within the tube walls that are generated by bending of the beams, which generates a compressive stress state at the nodes that can impede the propagation of a crack. As the t/a is reduced, shell buckling will commence at a lower applied load (Eq. 1), which lowers the probability of initiating and/or propagating an existing crack. The wall thicknesses of alumina are on the order of tens of nanometers, a length scale that has been shown to exhibit enhanced strengths and damage tolerance caused by a statistically lower probability of finding a weak defect (*1*). These are some of the phenomena that collectively give rise to recoverability of the alumina nanolattices (Figs. 2E and 3H and figs. S3 and S4).

We discovered that the strength and Young's modulus of all our octet-truss nanolattices follow a power law scaling with relative density as $\sigma_y \sim \bar{\rho}^{1.76}$ and $E \sim \bar{\rho}^{1.61}$ (Fig. 4, A and B). This scaling outperforms traditional lightweight and ultralight bending-dominated structural materials, whose properties scale as $E \sim \bar{\rho}^2$ or $E \sim \bar{\rho}^3$ (*21*), but does not follow the analytic prediction for an ideal stretching-dominated structure, $\sigma_{ys} \sim \bar{\rho}$ and $E \sim \bar{\rho}$ (*20*). Such a deviation from the analytic prediction can be explained, in part, by factors such as the ellipticity of the tubes, structural imperfections, and non-idealities of the experimental setup. We attribute this deviation primarily to the hollowness of the tubes, which affects the structural integrity of the nodes, where the highest stress concentrations will occur (*30*, *31*).

The strength and deformation of an ideal, monolithic, stretching-dominated cellular solid is governed by stretching of the beams, with the nodes acting as rigid pin-jointed elements that perfectly transfer load between truss members

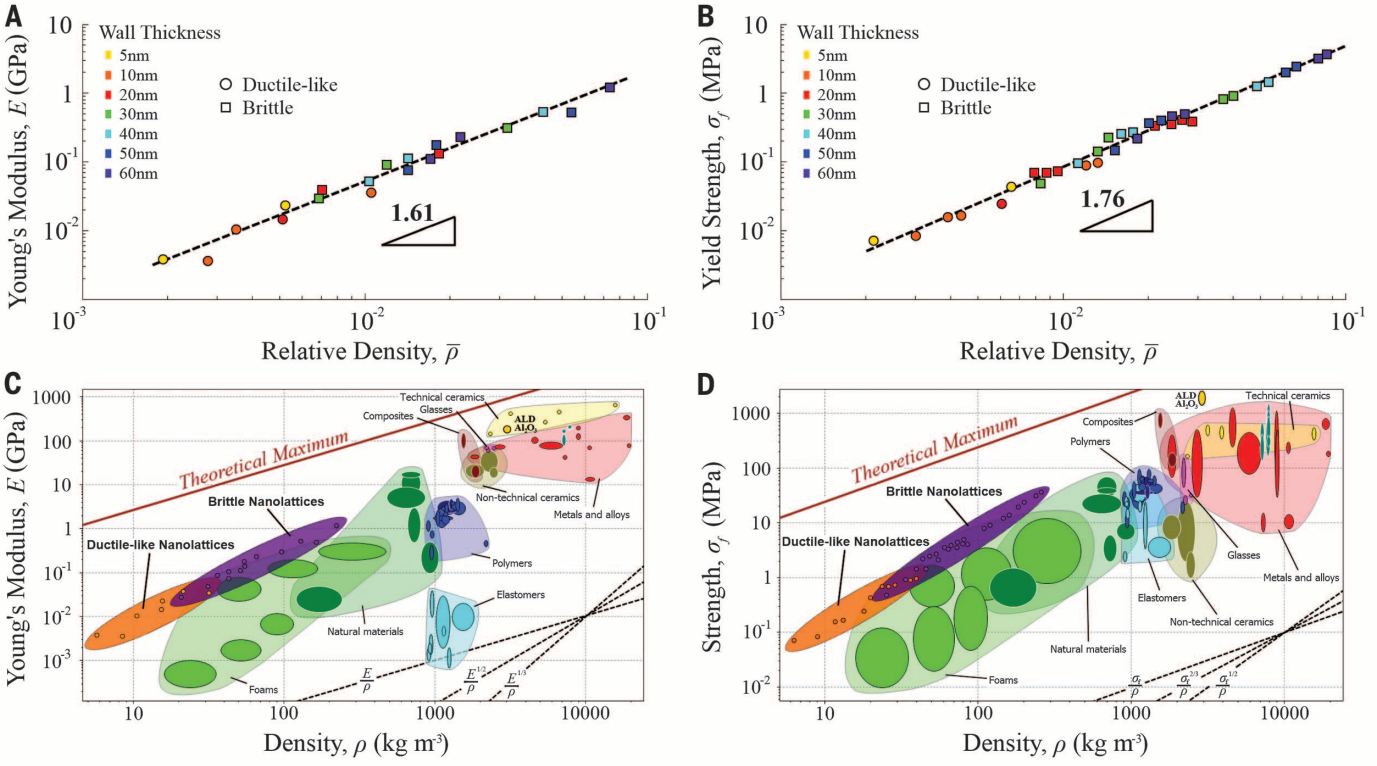

Fig. 4. Strength and stiffness versus density of alumina nanolattices. (**A** and **B**) Stiffness and strength plotted against relative density for all tested samples. Data clearly obey a power law, with little deviation across wall thicknesses and failure modes. (**C** and **D**) Material property plots (Materials Property CES Selector software by Granta Design) of the experimental stiffness and strength data against density for existing materials, showing that the materials created in this work reach a new niche in the high-strength and -stiffness lightweight material parameter space.

(*20*). In a hollow lattice, the nodes are constrained only by the shell walls, which has a detrimental effect on strength and stiffness because load transfer at the nodes occurs via shell wall bending. This, together with the sharp angles between the tubes, leads to an uneven distribution of stress and induces large stress concentrations in the vicinity of the nodes (Fig. 1, B and E). Bending of the tubes also causes large deflections and additional ovalization at the nodes, which further increases the compliance and stress concentrations. In situ experiments and postcompression analysis revealed that most of the deformation is localized to the nodes (Figs. 2, D and E, and 3J), which implies that improving nodal strength is a critical factor in enhancing the scaling of strength and stiffness with density.

We demonstrated the creation of ultralight hollow ceramic nanolattices that absorb energy, recover after significant compression, and reach an untapped strength and stiffness material property space. This is achieved using high-strength ALD alumina engineered into a thin-walled nanolattice that is capable of deforming elastically via shell buckling. The ultralight ceramic nanolattices represent the concept of materials by design, where it is possible to transform a strong and dense brittle ceramic into a strong, ultralight, energy-absorbing, and recoverable metamaterial. These results serve to emphasize the critical connection between material microstructure, hierarchical architecture, and mechanical properties at relevant length scales.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the Defense Advanced Research Projects Agency under the Materials with Controlled Microstructure and Architecture program managed by J. Goldwasser (contract no. W91CRB-10-0305) and to the Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office. The content of the information does not necessarily reflect the position or the policy of the government, and no official endorsement should be inferred. The authors are grateful to the Kavli Nanoscience Institute at Caltech for the availability of critical cleanroom facilities, and to R. Liontas and C. Garland for TEM assistance. Part of this work was carried out in the Lewis Group facilities at Caltech.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1322/suppl/DC1
Materials and Methods
Figs. S1 to S6
Table S1
Reference (*33*)
Movies S1 to S3

12 May 2014; accepted 11 August 2014
10.1126/science.1255908

WATER SPLITTING

Decoupled catalytic hydrogen evolution from a molecular metal oxide redox mediator in water splitting

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The electrolysis of water using renewable energy inputs is being actively pursued as a route to sustainable hydrogen production. Here we introduce a recyclable redox mediator (silicotungstic acid) that enables the coupling of low-pressure production of oxygen via water oxidation to a separate, catalytic hydrogen production step outside the electrolyzer that requires no post-electrolysis energy input. This approach sidesteps the production of high-pressure gases inside the electrolytic cell (a major cause of membrane degradation) and essentially eliminates the hazardous issue of product gas crossover at the low current densities that characterize renewables-driven water-splitting devices. We demonstrated that a platinum-catalyzed system can produce pure hydrogen over 30 times faster than state-of-the-art proton exchange membrane electrolyzers at equivalent platinum loading.

Hydrogen is vital for the production of commodity chemicals such as ammonia and has great potential as a clean-burning fuel (*1*, *2*). However, currently around 95% (~15 trillion mol year^{-1}) of the world's supply of H_2 is obtained by reforming fossil fuels (*3*), a process that is both unsustainable and leads to a net increase in atmospheric CO_2 levels. Of the alternative methods for H_2 production that are not linked to fossil resources, the electrolysis of water stands out as a mature, scalable technology for which the only required inputs are water and energy (in the form of electricity) (*4*). Hence, if the energy source is renewable, H_2 can be produced sustainably from water using electrolysis (*5*, *6*).

Renewable energy inputs tend to be sporadic and fluctuating, and thus the systems that are developed to harness this energy and convert it to H_2 [such as proton exchange membrane electrolyzers (PEMEs) (*7*), solar-to-fuels systems (*8*), and artificial leaves (*9*)] must be able to deal with varying energy inputs effectively and have rapid startup times. At the low power loads that are characteristic of renewable power sources, the rate at which H_2 and O_2 are produced may in fact be slower than the rate at which these gases permeate the membrane (*10*). At the very least, this will severely affect the amount of hydrogen that can be harvested from such devices (*11*), and in extreme cases could give rise to hazardous O_2/H_2 mixtures. The PEME is the most mature technology cited for renewables-to-hydrogen conversion, but prevention of such gas crossover at low current densities remains a challenge. PEMEs use nontrivial amounts of precious metal catalysts and so tend to operate at high current densities (1 A cm^{-2} or above) and high pressure, where the cost of their components can be offset to some extent (*7*). However, these optimal conditions may be hard to maintain in all cases of renewables-driven electrolysis (for example, in small-scale facilities), where less-expensive and lower-power devices would therefore be beneficial. Meanwhile, the high-pressure and high-current-density conditions under which PEMEs work most effectively are also not without drawbacks: These conditions can also lead to gas crossover through the membrane, and the coexistence of H_2, O_2 and catalyst particles produces reactive oxygen species

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(ROS) that degrade the membrane and shorten its lifetime (*12*, *13*). There is thus a need to develop new electrolyzer systems that can prevent product gases from mixing over a range of current densities and that make more effective use of the precious metal catalysts they employ, in order to make renewables-to-hydrogen conversion both practically and economically more attractive.

Previously, we introduced the concept of the electron-coupled proton buffer (ECPB), which can act to decouple electrolytic H_2 and O_2 production, producing these gases at separate times (*14*, *15*). Here we describe a redox mediator that can be reversibly reduced in an electrolytic cell (as water is oxidized at the anode) and then transferred to a separate chamber for spontaneous catalytic H_2 evolution, without the need for additional energy input after reduction of the mediator (Fig. 1). This approach leads to a device architecture for electrolyzers that has several important advantages. First, it allows the electrochemical step to be performed at atmospheric pressure, while potentially permitting H_2 to be evolved at elevated pressure in a distinct compartment. Second, virtually no H_2 is produced in the electrolytic cell itself, which (taken with the feature above) obviates the need to purge H_2 from the anode side of the cell and could significantly reduce ROS-mediated membrane degradation and the possibility of explosive gas mixtures forming at low current densities or upon membrane failure. Third, H_2 evolution from such a system is no longer directly coupled to the rate of water oxidation, and thus the decoupled H_2 production step can be performed a rate per milligram of catalyst that is over 30 times faster than that for state-of-the-art PEMEs (movie S1). Finally, the hydrogen produced has the potential to have an inherently low O_2 content, both on account of its production in a separate chamber from water oxidation and by virtue of the fact that the reduced mediator reacts rapidly with O_2 in solution. This final point could render the H_2 produced suitable for applications requiring high-purity H_2 such as fuel cells (*16*) or the Haber-Bosch process (*17*), without the need for post-electrolysis purification or built-in recombination catalysts.

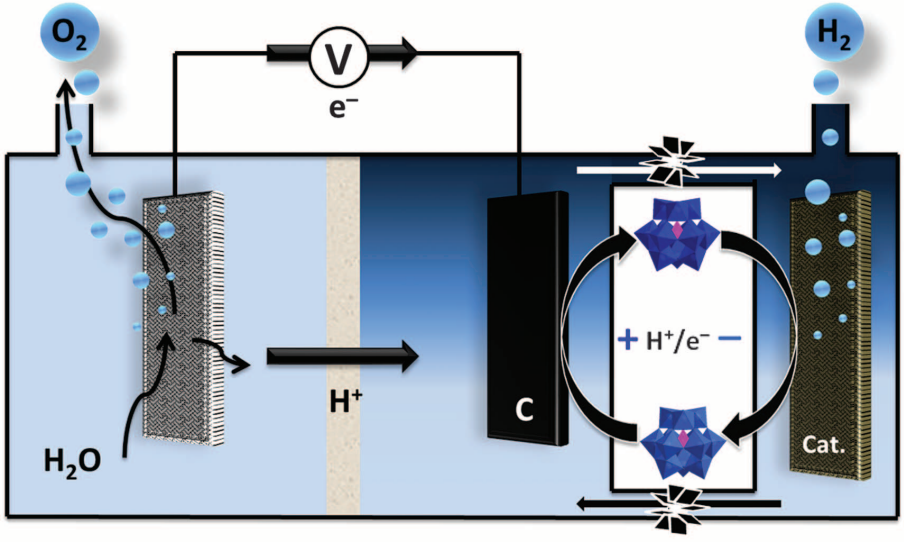

Fig. 1. A schematic of silicotungstic acid–mediated H_2 evolution from water. At the anode, H_2O is split into O_2, protons, and electrons, while the mediator is reversibly reduced and protonated at the cathode in preference to direct production of H_2. The reduced $H_6[SiW_{12}O_{40}]$ (dark blue) is then transferred to a separate chamber for H_2 evolution over a suitable catalyst and without additional energy input after two-electron reduction of the mediator to $H_6[SiW_{12}O_{40}]$.

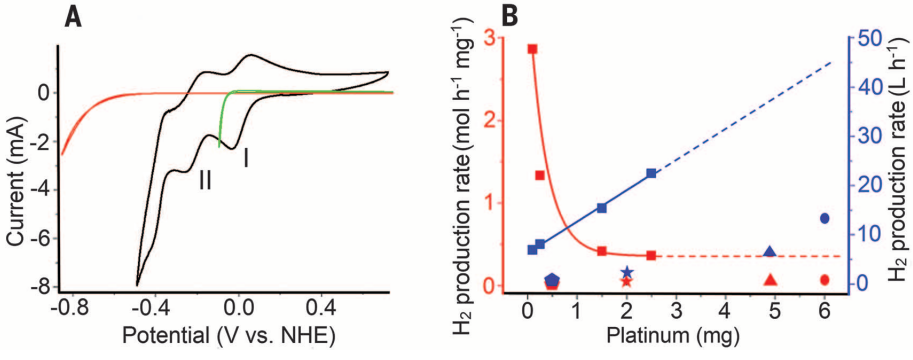

Fig. 2. Performance of silicotungstic acid as an electrochemical mediator for water splitting. (**A**) Reductive CVs under Ar and at room temperature: black, $H_4[SiW_{12}O_{40}]$ in water (0.5 M, pH 0.5), at a glassy carbon working electrode (area = 0.071 cm^2); red, 1 M H_3PO_4 (pH = 1.0) on a glassy carbon working electrode; green, 1 M H_3PO_4 (pH = 1.0) on a Pt disc working electrode (area = 0.031 cm^2). A Pt-mesh counterelectrode and Ag/AgCl reference electrode were used at a scan rate of 0.1 V s^{-1}. (**B**) Comparison of the rate of H_2 production possible using electrolysis mediated by silicotungstic acid (this work) and a selection of state-of-the-art electrolyzers from recent years. Square symbols indicate data obtained for a mediated system in this paper. Red data (left-hand *y* axis): the rate of H_2 production per milligram of Pt. Blue data (right-hand *y* axis): the absolute rate of H_2 production determined for H_2 production from $H_6[SiW_{12}O_{40}]$ (this work, squares) and the various literature electrolyzer systems. Dashed lines are provided solely as guides to the eye. Code for literature data is as follows: pentagons, reference (*30*); stars, reference (*26*); triangles, reference (*31*); dots, reference (*32*). This comparison does not include the time taken to reduce the mediator to $H_6[SiW_{12}O_{40}]$. For more details on how these metrics were obtained, see table S1.

The redox mediator investigated in this work was silicotungstic acid ($H_4[SiW_{12}O_{40}]$), the cyclic voltammogram (CV) of which on a glassy carbon electrode in aqueous solution is shown in Fig. 2A (black line). $H_4[SiW_{12}O_{40}]$ was chosen for investigation on account of its high solubility in water (up to 0.5 M), in which solvent it is a strong acid (*18*). $H_4[SiW_{12}O_{40}]$ has reversible one-electron redox waves centered at +0.01 V (wave I) and −0.22 V [wave II; all potentials are versus the normal hydrogen electrode (NHE)], the positions of which are critical to the following discussion (*18*). Also shown in Fig. 2A are reductive scans taken at a similar pH in the absence of $H_4[SiW_{12}O_{40}]$ on carbon and Pt electrodes (red and green lines, respectively). Given that the onset of H_2 evolution on Pt occurs at essentially the same potential as the first reduction of $H_4[SiW_{12}O_{40}]$, but that H_2 evolution on carbon is not appreciable above −0.6 V, we hypothesized that the reduction of $H_4[SiW_{12}O_{40}]$ at a carbon electrode at potentials slightly more positive than −0.6 V would give the two-electron reduced form ($H_6[SiW_{12}O_{40}]$) without any competing H_2 evolution. If $H_6[SiW_{12}O_{40}]$ were then exposed to Pt, it should spontaneously evolve H_2 until equilibrium between H_2 and the reduced mediator was reached, which Fig. 2A suggests will correspond to a mixture of $H_4[SiW_{12}O_{40}]$ and the one-electron reduced form, $H_5[SiW_{12}O_{40}]$.

To test this hypothesis, an airtight electrolysis cell was constructed with a Pt mesh or carbon felt anode (for water oxidation) and a carbon felt cathode (for $H_4[SiW_{12}O_{40}]$ reduction), as shown in fig. S1. Reduction of the mediator and concomitant water oxidation were performed, and the composition of the gases in the separated headspaces was monitored by gas chromatographic headspace analysis (GCHA). Full faradaic efficiency for O_2 evolution could be observed (using

Pt anodes), whereas complete two-electron reduction of the mediator could be achieved with only trace H_2 being evolved [see supplementary materials (SM) section SI-4 and figs. S2 and S3]. This two-electron reduced $H_6[SiW_{12}O_{40}]$ could then be stored without significant spontaneous H_2 evolution (<0.002% loss of H_2 per hour; fig. S3). Taken together, these data suggest that O_2 evolution and H_2 evolution can be effectively decoupled from each other using $H_4[SiW_{12}O_{40}]$, potentially allowing the O_2 produced during electrolysis to be vented to the atmosphere without the need for additional H_2 removal processes (*19*).

The two-electron reduced mediator could then be removed from the electrolysis cell and introduced into sealed reaction flasks under an atmosphere of Ar. The addition of various metal foils to this solution catalyzed H_2 evolution, with Pt exhibiting the best performance (SM section SI-5 and fig. S4A). Powdered samples of MoS_2 (*20*, *21*) and Ni_2P (*22*) were also found to be effective catalysts for H_2 evolution from $H_6[SiW_{12}O_{40}]$ (fig. S4B). However, by far the greatest rate of H_2 evolution was found when using precious metal catalysts supported on carbon. Figure 2B shows that per milligram of Pt used, the rate of H_2 production from $H_6[SiW_{12}O_{40}]$ exceeds the rate of H_2 evolution possible using a state-of-the-art PEME by a factor of 30 (red data). This more effective use of the precious metal H_2 evolution catalyst could be a result of the better dispersion of catalyst that is possible when it is not confined to an electrode.

The kinetics of H_2 evolution from solutions of $H_6[SiW_{12}O_{40}]$ as a function of time and catalyst are examined in Fig. 3A. Based on the volume of mediator solution used in these experiments, full conversion of two-electron reduced $H_6[SiW_{12}O_{40}]$ to one-electron reduced $H_5[SiW_{12}O_{40}]$ would be expected to liberate 122.4 ml of H_2, whereas complete reversion to $H_4[SiW_{12}O_{40}]$ would release 244.7 ml of H_2 (SM section SI-6). In practice, somewhat more than 122.4 ml of H_2 were liberated in under 30 min with all the catalysts examined in Fig. 3A, suggesting complete and rapid transformation of $H_6[SiW_{12}O_{40}]$ to $H_5[SiW_{12}O_{40}]$, followed by limited further conversion (10 to 36%) of $H_5[SiW_{12}O_{40}]$ to $H_4[SiW_{12}O_{40}]$ under these conditions.

Initial rates were then extrapolated to rates of H_2 produced per milligram of precious metal per hour (Table 1 and table S2), giving a maximum rate of 2861 mmol of H_2 mg^{-1} $hour^{-1}$ when using low loadings of Pt/C. The rate of H_2 evolution decays from the initial value in Fig. 3B on account of the process $H_6[SiW_{12}O_{40}] \rightarrow H_5[SiW_{12}O_{40}]$ being 80% complete within 30 s for all the Pt/C loadings shown. Hence, in a continuous flow system, it should be possible to achieve rates very similar to the initial rate measured here for as long as the flow of $H_6[SiW_{12}O_{40}]$ is maintained (the mediator could then be recycled to the cathode for recharging). Table 1 compares the rate of H_2 production by the mediator-based system with that achieved by a selection of state-of-the-art PEMEs from the recent literature (a more extensive comparison can be found in table S1). With PEMEs, the rate of H_2 production is necessarily coupled to the rate of water oxidation occurring at the anode. In a mediated electrolysis cell, the rates of water oxidation and mediator reduction are coupled, but the rate of H_2 production depends on the availability of the reduced mediator. This allows a mediated system to make more effective use of the H_2 evolution catalyst, as illustrated by Table 1. The time required to reduce the mediator is not included in the calculations for Table 1: Only the rate of H_2 production (and hence how long it would take to obtain all the H_2 from the mediator for compression and/or storage) is considered. Details on mediator reduction can be found in the

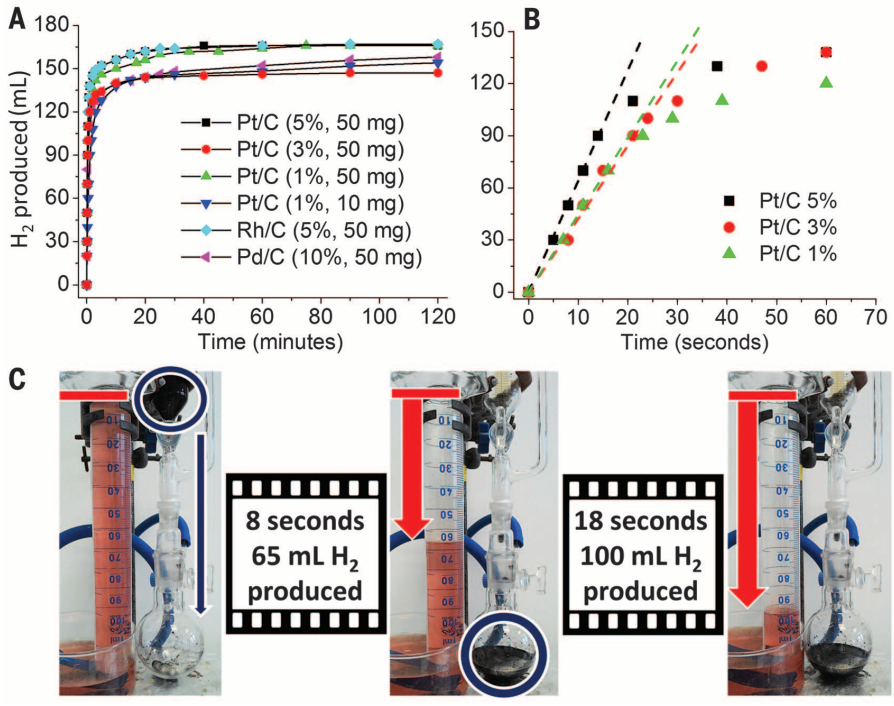

Fig. 3. Catalytic H_2 evolution from $H_6[SiW_{12}O_{40}]$ when in contact with various catalysts. (**A**) Rate of H_2 production from a 20-ml sample of 0.5 M $H_6[SiW_{12}O_{40}]$ under an Ar atmosphere. (**B**) Magnification of the first 2 min of the H_2 evolution process from $H_6[SiW_{12}O_{40}]$ in the presence of 50 mg Pt/C (5, 3, and 1 weight %). Dashed lines indicate the derived initial rates. (**C**) A typical apparatus configuration for determining volumes of H_2 evolved when $H_6[SiW_{12}O_{40}]$ was exposed to powdered catalysts. Reduced mediator in the upper flask (blue circle in left-hand photograph) is allowed to flow into the lower chamber containing catalyst (blue circle in middle photograph), and H_2 is rapidly evolved and collected (as indicated by the red arrows). A movie showing rapid H_2 evolution (movie S1) and full experimental details can be found in the supplementary materials (SM section SI-6).

Table 1. Comparison of the rate of H_2 production possible with silicotungstic acid–mediated electrolysis and a selection of leading PEMEs from the current literature. Literature values are based on the highest rate of H_2 production reported in those works. A table with more examples and a full description of how these metrics were calculated can be found in the supplementary materials.

H_2 evolution catalyst	Amount of powder/size of electrode	Total catalyst used (mg)	H_2 production rate (mmol $hour^{-1}$ mg^{-1})	Reference
Pt/C (0.5 mg of Pt cm^{-2})	9 × 100 cm^2	450	22	(*33*)
Pt/C (0.4 mg of Pt cm^{-2})	5 cm^2	2	47	(*26*)
Pt/C (0.7 mg of Pt cm^{-2})	7 cm^2	4.9	53	(*31*)
Pt/C (0.3 mg of Pt cm^{-2})	20 cm^2	6	93	(*32*)
Pd/C 10%	50 mg	5	143	This work
Rh/C 5%	50 mg	2.5	241	This work
Pt/C 5%	50 mg	2.5	368	This work
Pt/C 3%	50 mg	1.5	423	This work
Pt/C 1%	50 mg	0.5	1275	This work
Pt/C 1%	25 mg	0.25	1336	This work
Pt/C 1%	10 mg	0.1	2861	This work

supplementary materials (SM sections SI-3 and SI-7).

Next, we examined the purity of the H_2 that was produced by this silicotungstic acid–mediated method. GCHA indicated that the level of electrolysis-derived O_2 in this H_2 was below detectable limits (±0.08%; SM sections SI-4 and SI-6). Moreover, if 10% O_2 were deliberately introduced into the headspace of the vessel containing $H_6[SiW_{12}O_{40}]$, this extraneous O_2 was completely removed by reaction with $H_6[SiW_{12}O_{40}]$ (% O_2 in the headspace was only 0.04% after 30 min), ultimately producing water and reoxidized mediator (*23*) and further guaranteeing that the H_2 evolved is O_2-free (SM section SI-9 and fig. S9). This has obvious implications for electrolyzer safety, because gaseous mixtures of H_2 and O_2 on the cathode side are now precluded by the reduced mediator's rapid reaction with O_2. This reaction is spontaneous and does not require any precious metal–based recombination catalysts such as those often employed in PEMEs.

As noted earlier, a primary mode of degradation of the perfluorinated membranes used in PEMEs is attack by ROS (*12*). These ROS form in the presence of O_2, H_2, and precious metals (including the catalytic recombination layers that are designed to prevent mixtures of O_2 and H_2 forming in electrolysis product streams). Moreover, recombination of H_2 and O_2 is an exothermic process that causes local heating, damaging the membrane through mechanical means; this route is especially prevalent at Pt sites on the cathode (*24*, *25*). The use of a mediator can help to mitigate against membrane degradation in three ways. First, the amount of H_2 produced in the electrolyzer itself is vastly diminished, removing the need to purify the O_2 product stream and preventing ROS formation on the anode side of the cell. Second, on the cathode side, the reduced mediator reacts rapidly with any O_2 present to produce water, and any peroxy species that do form will do so in bulk solution far from the membrane and will themselves rapidly react with reduced mediator to form water (*23*). Finally, the catalyst is now isolated in a second chamber and is not in contact with the membrane, lessening local heating effects. Hence, using a mediator could potentially allow increased lifetimes for the membranes used in such electrolyzers relative to the life span of similar membranes in PEMEs.

The efficiency of the electrochemical process to produce O_2 from water and $H_6[SiW_{12}O_{40}]$ from $H_4[SiW_{12}O_{40}]$ was calculated and compared to equivalent systems that would produce H_2 and O_2 directly by electrolysis (SM section SI-7 and fig. S6). In comparison to a system that uses a carbon cathode to reduce protons and a Pt anode to oxidize water, the mediated system was 16% more efficient, with an overall energy efficiency of 63%. A standard electrolysis system for direct O_2 and H_2 production from water, in which both electrodes are Pt, was found to have an efficiency of 67% [which agrees well with the efficiency of room-temperature PEMEs reported in the literature (*26*)]. Hence, given the potential for lower loadings of precious metal and high initial purity of the product gases when using mediated electrolysis (and other possible technoeconomic advantages; SM section SI-12), we believe that such systems will be competitive with PEMEs in terms of cost-efficiency metrics.

The redox reactions of silicotungstic acid are summarized in fig. S10A. Starting from fully reduced $H_6[SiW_{12}O_{40}]$, H_2 evolution in the presence of a catalyst such as Pt/C is rapid, leading to the one-electron reduced species $H_5[SiW_{12}O_{40}]$. This process can be reversed by electro-reducing $H_5[SiW_{12}O_{40}]$ at a carbon cathode. Alternatively, starting from the fully oxidized species $H_4[SiW_{12}O_{40}]$, the one-electron reduced species can be accessed either by electrochemical reduction or by reaction with H_2 in the presence of a suitable catalyst such as Pt/C (SM section SI-8 and fig. S8). Likewise, if one-electron reduced $H_5[SiW_{12}O_{40}]$ is placed in a sealed reaction vessel under Ar in the presence of Pt/C, H_2 evolves slowly into the headspace, as gauged by GCHA (fig. S7). This behavior implies that there exists an equilibrium between H_2 and $H_4[SiW_{12}O_{40}]$ on one hand and the one-electron reduced mediator ($H_5[SiW_{12}O_{40}]$) on the other in the presence of catalysts such as Pt/C.

Overall faradaic efficiencies for the roundtrip process were gauged by fully reducing a sample of $H_4[SiW_{12}O_{40}]$ to $H_6[SiW_{12}O_{40}]$ with coulometry. Pt/C was then added to this $H_6[SiW_{12}O_{40}]$, and H_2 was evolved. At the cessation of spontaneous H_2 evolution, an amount of H_2 corresponding to 68% of the charge passed in reducing $H_4[SiW_{12}O_{40}]$ to $H_6[SiW_{12}O_{40}]$ was obtained. In a cyclic system, any one-electron reduced $H_5[SiW_{12}O_{40}]$ could simply be returned to the electrolyzer for re-reduction to $H_6[SiW_{12}O_{40}]$. However in this case, once H_2 evolution had ceased, the Pt/C catalyst was removed by filtration under Ar, and the resulting Pt-free mediator solution was titrated with an Fe(III) source in order to oxidize all remaining $H_5[SiW_{12}O_{40}]$ to colorless $H_4[SiW_{12}O_{40}]$ and thus ascertain the amount of $H_5[SiW_{12}O_{40}]$ still present at the cessation of H_2 evolution (SM section SI-10). This value, when combined with the electrons already accounted for by the amount of H_2 evolved, gave a faradaic yield in excess of 98% for the roundtrip $H_4[SiW_{12}O_{40}] \rightarrow H_6[SiW_{12}O_{40}] \rightarrow H_4[SiW_{12}O_{40}]$.

The stability of the mediator to several cycles of oxidation and reduction was probed both electrochemically (by comparing the charges passed in oxidizing the reducing the mediator over a series of cycles, SM section SI-13) and by comparing ultraviolet-visible spectra of fresh and cycled samples and of reduced samples that were reoxidized by exposure to air (SM section SI-14). Figure S11A shows that 98% of the charge passed in fully reducing the mediator by one electron could be retrieved by reoxidation over nine full one-electron reduction-oxidation cycles, with no apparent degradation of the mediator. Figure S11B shows the stability of the mediator to four consecutive cycles of reduction to 80% of the maximum for full two-electron reduction, followed by reoxidation to 20% of this maximum. This experiment was designed to mimic the conditions under which the mediator would have to operate in a continuous-flow system. The data in fig. S11B suggest that there is no decay in the amount of charge that can be stored in the mediator (which would signal irreversible decomposition) within these bounds over the number of cycles probed. Similarly, fig. S12, A and B, show that a sample of silicotungstic acid subjected to 20 consecutive two-electron reduction and reoxidation cycles has an ultraviolet-visible spectrum indistinguishable from that of a fresh sample of silicotungstic acid. Taken together, these data suggest that the mediator is stable to redox cycling under these conditions and that $H_4[SiW_{12}O_{40}]$ might thus be suitable as a mediator in a continuous-flow system.

Because of the high molecular weight of $H_4[SiW_{12}O_{40}]$, it does not constitute an especially effective static storage medium for H_2 (or H_2 equivalents). Clearly, lower–molecular-weight mediators, or systems capable of storing more electrons, would therefore be at a practical advantage (*15*), allowing greater buffering capacity to be built into the system and providing more flexibility with regard to the temporal separation of water oxidation and H_2 generation. We are currently pursuing the identification of such mediators, and we see great potential for optimized mediator systems to be combined with other recent breakthroughs in catalysis (*27*, *28*) and device design (*9*, *29*), facilitating the use of low-power inputs (or those subject to large fluctuations) in renewables-to-hydrogen conversion.

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ACKNOWLEDGMENTS

This work was supported by the Engineeering and Physical Sciences Research Council (UK). L.C. thanks the Royal Society/Wolfson Foundation for a Merit Award. M.D.S. thanks the University of Glasgow for a Lord Kelvin Adam Smith Research Fellowship. We are grateful to J. Liddell (University of Glasgow) for production of the H cells used in this work. Supplementary materials are available, which include full experimental details for electrochemical procedures, GCHA, and catalytic H_2 evolution, as well as a movie showing the H_2 evolution step (movie S1). The advances presented in the work form part of a patent filing.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1326/suppl/DC1
Supplementary Text Sections SI-1 to SI-14
Figs. S1 to S12
Tables S1 and S2
References (*34–42*)
Movie S1

16 June 2014; accepted 6 August 2014
10.1126/science.1257443

BLACK HOLE PHYSICS

Rapid growth of seed black holes in the early universe by supra-exponential accretion

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Mass accretion by black holes (BHs) is typically capped at the Eddington rate, when radiation's push balances gravity's pull. However, even exponential growth at the Eddington-limited *e*-folding time t_E ~ few × 0.01 billion years is too slow to grow stellar-mass BH seeds into the supermassive luminous quasars that are observed when the universe is 1 billion years old. We propose a dynamical mechanism that can trigger supra-exponential accretion in the early universe, when a BH seed is bound in a star cluster fed by the ubiquitous dense cold gas flows. The high gas opacity traps the accretion radiation, while the low-mass BH's random motions suppress the formation of a slowly draining accretion disk. Supra-exponential growth can thus explain the puzzling emergence of supermassive BHs that power luminous quasars so soon after the Big Bang.

Optically bright quasars powered by accretion onto black holes (BHs) are now detected at redshifts as high as $z \sim 7$, when the universe was 6% of its current age (<1 billion years) (*1*). Their luminosities imply supermassive BHs (SMBHs) with the mass of the BH ($M_\bullet$) $\gtrsim 10^9$ solar masses ($M_\odot$) (*2*). The main obstacles to assembling such SMBHs so rapidly are the low masses of the hypothesized initial seed BHs, born of first-generation (Pop III) stars, coupled with the maximal growth rate for radiatively efficient accretion, the Eddington limit (*3–5*). Proposed ways to circumvent these limitations invoke super-Eddington accretion for brief periods of time (*6*); the ab initio formation of more-massive BH seeds (*7–10*) from the direct collapse of self-gravitating pre-galactic gas disks at high redshifts (*11–14*); and the formation of a very massive star from runaway stellar mergers in a dense cluster (*15*, *16*). Discriminating between these scenarios is challenging, because seed formation redshifts ($z > 10$) are observationally inaccessible. Current data require finely tuned, continuous early BH growth and massive initial BH seeds (*17–20*). Recent results from high-resolution simulations of early star formation at $z \sim 15$ to 18 exacerbate the problem by indicating that efficient fragmentation and turbulence (*21–25*) lead to the efficient formation of stellar clusters embedded in the flow, which prevents the formation of massive seeds (>>10 $M_\odot$) by limiting the mass of their potential Pop III progenitor stars. On the other hand, theoretical and numerical results on larger scales suggest that ubiquitous dense cold gas flows (*26*) stream in along filaments and feed protogalactic cores (*27*, *28*). Adaptive mesh refinement simulations track the fate of these sites (collapsed 10^7 $M_\odot$ dark-matter halos) from 1 Mpc scale at $z \sim 21$ with resolutions as low as ~ 2×10^{-10} pc in the central regions. These simulations find isothermal density cusps that reach extreme central densities, with an average density of $\rho_\infty \gtrsim 10^{-16}$ g cm^{-3} ($\gtrsim 10^8$ cm^{-3} for pure H) on 0.1 pc scales (*29*). They also reveal a marginally unstable central gas reservoir of few × 10^5 $M_\odot$ in the inner few parsecs (*30*), where the dynamical time scale is ~10^6 years. Although these simulations are somewhat idealized, we adopt the properties of this high-density environment as the initial conditions for the model presented here.

We consider a scenario in which a low-mass Pop III remnant BH remains embedded in a nuclear star cluster fed by dense cold gas flows (*26*) (Fig. 1 and Table 1). The stars and gas are virialized in the cluster potential, and the BH is initially a test particle in equipartition with the stars. Gas within the accretion (capture) radius of the BH, $r_a = [2c^2/(c_\infty^2 + v_\bullet^2)]r_g$, is dynamically bound to it, where $r_g = G M_\bullet/c^2$ is the gravitational radius of the BH; c_∞ is the gas sound speed in the cold flow far from the BH, which is a measure of the depth of the cluster's gravitational potential; and $v_\bullet$ is the BH velocity relative to the gas. Gas bound to the BH inside r_a is not necessarily accreted by it. Prompt accretion requires gas to flow from r_a into the BH on a plunging trajectory with low specific angular momentum $j < j_{ISO} \simeq 4 r_g c$, through the innermost stable periapse distance r_{ISO}. It is this angular momentum barrier, rather than the Eddington limit, that is the main obstacle to supra-exponential growth.

The BH is more massive than a cluster star, so $v_\bullet^2 < c_\infty^2$, and the accretion flow is quasi-spherical. In the idealized case where the flow is radial and adiabatic, it is described by the Bondi solution (*31*), $\dot{M}_B = (\pi/\sqrt{2}) r_a^2 \rho_\infty c_\infty$ (adiabatic index $\Gamma = 4/3$ assumed), which can be written compactly in terms of $\mu = M_\bullet/M_i$, where M_i is the initial BH mass, as $\dot{\mu} = \mu^2/t_B$, with time scale $t_B = c_\infty^3/\, 2^{3/2}\pi G^2 M_i \rho_\infty$. The stronger-than-linear dependence of the accretion rate on the BH mass leads to a solution that diverges supra-exponentially in a finite time t_B as $\mu(t) = 1/(1 - t/t_B)$. Physical flows, where gravitational energy is released as radiation, are not strictly adiabatic. As the mass accretion rate grows, the local luminosity can far exceed the Eddington luminosity $L_E = 4\pi c G M_\bullet/\kappa = \dot{M}_E c^2$ (κ is the gas opacity), for which radiation flux pressure balances gravity. However, radiation produced inside the photon-trapping radius $r_\gamma \sim (\dot{M}/\dot{M}_E) r_g$ is carried with the flow into the BH, because the local optical depth $\tau(r) \sim \kappa\rho(r) r$ makes photon diffusion outward slower than accretion inward (*32*) [which is a manifestation of the $\mathcal{O}(v/c)$ effect of relativistic beaming (*33*)]. The luminosity L_∞ that escapes to infinity from $r \gtrsim r_\gamma$ translates to a lowered radiative efficiency $\eta_\gamma = L_\infty/\dot{M}c^2 \sim \min(r_g/r_\gamma, r_g/r_{ISO})$, so it does not exceed ~L_E, thereby allowing supra-exponential Bondi mass accretion rates (*34*). Detailed calculations show that $L_\infty \lesssim 0.6\, L_E$ (*35*).

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The associated radiation pressure enters the dynamics of the flow as an effective reduction of gravity by a factor of 0.4, and, consequently, as a reduction of the accretion rate by $0.4^2 \simeq 1/6$. The supra-exponential divergence of spherical accretion is therefore

$$M_\bullet(t) = \frac{M_i}{1 - t/t_\infty}, t_\infty \cong 6t_B = \frac{3}{2^{1/2}\pi}\frac{c_\infty^3}{G^2 M_i \rho_\infty} \quad (1)$$

The typical lifetime of cold flow streams seen in simulations is $\gtrsim 10^7$ years (*29*), which is long enough for the ~10^5 $M_\odot$ of gas in the marginally unstable reservoir on the few-parsec scale to accrete onto the growing BH, and yet short enough to be relevant for forming $z > 7$ quasars. As a demonstration of concept, we adopt the gas properties found in these simulations, and match a divergence time of t_∞ ~ few × 10^7 years to the mean physical parameters of the 4×10^4 $M_\odot$ of gas on the 0.25-pc scale. We further assume that half of that mass is in a star cluster with 1 $M_\odot$ stars in a nonsingular distribution (a Plummer law), containing a 10 $M_\odot$ stellar BH, and that the escaped radiation from $r > r_\gamma$ does not substantially affect the cold flow on larger scales. This cluster, while dense, is dynamically stable on time scales >> t_∞. Table 1 lists the input physical parameters of the model, the derived gas and cluster properties, and the predicted accretion properties.

Simulations (*29*) find that the cold flow is nearly pressure-supported and thus has little angular momentum. It is also plausible that there is little net rotation between the gas and the stars that were formed from it. However, unavoidable gravitational interactions of the low-mass BH with cluster stars accelerate it at orbital frequencies of $\Omega(r_\bullet) \sim \Omega_0$, which induce a velocity gradient across the capture radius. Gas captured by the BH then acquires specific angular momentum relative to it, j_a, due to the opposite velocity and density gradients and the velocity-skewed capture cross-section ($j_{a,v} \sim \Omega r_a^2$ and $j_{a,\rho} \sim (d\log\rho/d\log r)_{r_\bullet} \Omega r_a^2$) (*36*). Gas with $j_a > j_{ISO}$ cannot plunge directly into the BH but rather circularizes at a radius $r_c = j_a^2/GM_\bullet$, and accretion then proceeds on the slow viscous time scale rather than the fast, near–free fall time scale. Analytic and numeric results on the capture efficiency of angular momentum by inhomogeneous wind accretion are currently available only in the ballistic (supersonic) limit (*37*, *38*). We adapt these results to provide a rough estimate of the angular momentum in the accretion flow on the BH seed in the subsonic regime considered here (see details in the supplementary materials).

The formal divergence of $j_a/j_{ISO} \propto M_\bullet$ in the test particle limit, where $\Omega \sim \Omega_0$, is reversed by the accretion itself. The nonrotating gas accreted by the BH from the cluster exerts a drag on it, $\ddot{r}_* = -2\gamma_a \dot{r}_\bullet$, where $\gamma_a = \dot{M}/2M_\bullet = M_\bullet(t)/2M_i t_\infty$. This provides additional damping beyond dissipation by dynamical friction against the gas and stars, $\gamma_{df} \propto [M_\bullet(t)/M_c]\Omega_0$ (*39*), which is balanced by the two-body fluctuations. Accretion damping drives the BH to subequipartition

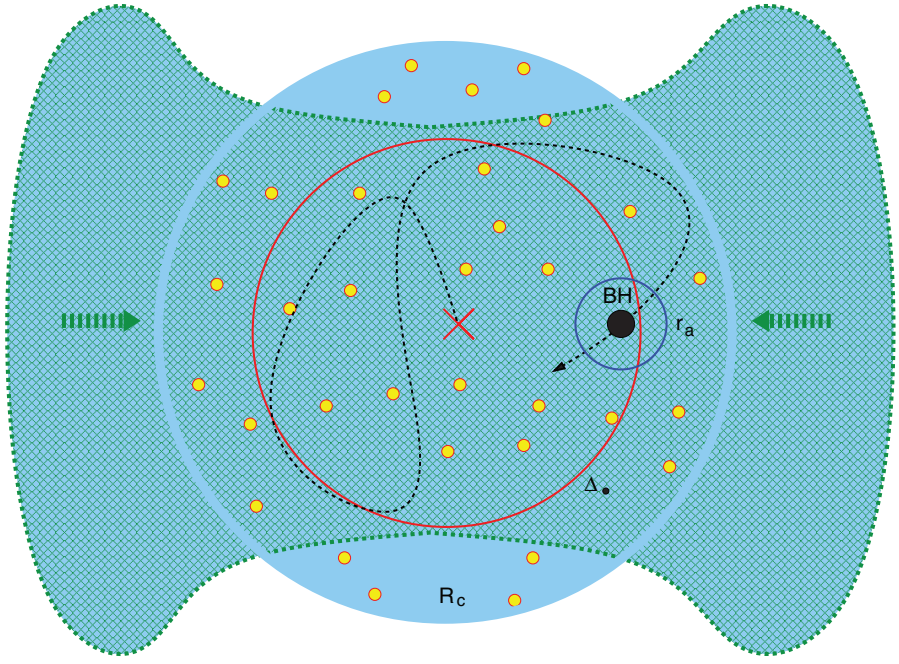

Fig. 1. A schematic depiction of accretion by a low-mass BH in a dense gas-rich cluster. Dense cold gas (green) flows to the center (red cross) of a stellar cluster (light blue region) of total mass $M_c = N_* M_* + M_g$ and radius R_c, which contains N_* stars (yellow circles) of mass M, each with velocity dispersion σ_*, and gas of mass M_g. The gas is nearly pressure-supported and close to the virial temperature. A stellar BH (black circle) of mass $M_* < M_\bullet << M_c$, which is accreting from its capture radius r_a (dark blue circle), is initially in fluctuation-dissipation equilibrium with the stars and is scattered by them (black dashed line) with velocity dispersion $s_\bullet \sim \sqrt{M_*/M_\bullet}\, s_*$ over a distance scale $D_\bullet \sim \sqrt{M_*/M_\bullet}\, R_c$ (red circle).

Table 1. Supra-exponential BH growth model.

Property*	Notation	Value
Model parameters		
Initial BH mass	$M_\bullet$	10 $M_\odot$
Star mass	M_*	1 $M_\odot$
Core radius	R_c	0.25 pc
Stellar core mass	M_s	2×10^4 $M_\odot$
Gas mass in core	M_g	2×10^4 $M_\odot$
Total core mass	M_c	4×10^4 $M_\odot$
Cold flow adiabatic index	Γ	5/3
Derived cluster properties		
Mean gas density	ρ_∞	2.1×10^{-17} g cm^{-3}
Jeans sound speed	c_s	14.5 km s^{-1}
Velocity dispersion	σ_*	18.0 km s^{-1}
Initial BH rms velocity	$v_\bullet$	11.4 km s^{-1}
Initial BH rms scattering distance	$\Delta_\bullet$	6.5×10^{-2} pc
Cluster orbital frequency	Ω_0	1/5545 year^{-1}
Central two-body relaxation time	t_{r0}	1.4×10^7 years
Vector resonant relaxation time	t_{vRR}	6.2×10^5 years
Evaporation time	t_{evap}	8.0×10^9 years
Collisional destruction time	t_{coll}	1.5×10^{11} years
Gas reservoir dynamical time	t_{res}	~10^6 years
Predicted accretion properties		
Initial accretion radius	r_a^0	2.5×10^{-4} pc
Mass divergence time	t_∞	3.5×10^7 years
Initial specific accretion ang. mom.	j_a/j_{ISO}	1.6
Ang. mom. suppression by resonant relaxation	$\sqrt{t_\infty}/t_{vRR}$	7.6

*See also definitions and discussion in the supplementary materials.

energy and angular momentum. Two-body interactions with the cluster stars can reestablish equipartition on the central relaxation time scale t_{r0} ~2×10^7 years only as long as the BH growth rate $2\gamma_a$ is slower than the relaxation rate, up to time $t_{eq}/t_\infty = 1 - t_{r0}/t_\infty \sim 0.6$ (Eq. 1), when the BH has grown by a factor of only $t_\infty/t_{r0} \sim 2.5$ to $M_{eq} \simeq 25\,M_\odot$. At later times, j_a is expected to fall below the extrapolated test particle limit, because both the BH wandering radius $\Delta_\bullet$ and the orbital frequency Ω are increasingly damped by dynamical friction and by the accretion drag, which are both $\propto M_\bullet(t)$.

Figure 2 shows the evolution of j_a/j_{ISO} with $M_\bullet$ for the cold flow cluster model of Table 1. A key property of acceleration-induced angular momentum accretion is the existence of a BH mass scale M_0 where $j_a(M_0) \rightarrow 0$ on typical orbits, because the density and velocity gradients cancel each other. For a cluster in dynamic equipartition and pressure-supported gas, M_0 depends weakly only on the shape of the density/potential cluster model near the origin: For the Plummer model, $M_0 \simeq 20\,M_\bullet$. This low mass scale is significant because $M_i < M_0 < M_{eq}$, and therefore j_a/j_{ISO} remains low during the critical stage of early growth, before damping can become effective.

In addition, vector resonant relaxation (*40*, *41*), a rapid process of angular momentum relaxation that operates in nearly spherical potentials, further suppresses the growth of j_a by randomizing the BH's orbital orientation on a time scale (*42*) $t_{vRR} \sim 6 \times 10^5$ years. This decreases j_a by a factor of $\sqrt{t_\infty/t_{vRR}} \sim 8$ over the divergence time. Randomization by resonant relaxation can be effective until time $t_{rnd}/t_\infty = 1 - t_{vRR}/t_\infty \sim 0.98$. By that time, the BH has grown by a factor of $t_\infty/t_{vRR} \sim 60$ to $M_{rnd} \simeq 600\,M_\odot$ and >0.95 of its mass has been accreted from low–angular-momentum gas in the absence of efficient equipartition. The effect of resonant relaxation can be estimated analytically in the early growth stages, up to $M_\bullet \sim M_{eq}$, when both the growth rate and j_a can be approximated as near-constant: Dynamical randomization quickly pushes j_a/j_{ISO} below 1 (independently of the decrease in j_a/j_{ISO} near $M_\bullet = M_0$) (Fig. 2). The damped and randomized motions of the BH suppress the accumulation of angular momentum in the accretion flow and allow Bondi accretion to proceed supra-exponentially.

The BH mass up to t_{rnd}, $M_\bullet \le M_{rnd} \sim 0.02\,M_c$ is still small enough to justify both treating the BH as a test particle in the fixed potential of the gas and star cluster and representing cluster dynamics by a simple model. It is much more difficult to self-consistently predict the subsequent joint evolution of the BH and cluster. However, the physical arguments for the gradual deceleration of the BH and the decline of j_a beyond $M_{eq} \sim 25\,M_\odot$, suggest that a substantial fraction of the available $10^5\,M_\odot$ gas reservoir can be accreted in $t_\infty \sim$ few $\times 10^7$ years at $z > 15$. Even if the supra-exponential growth phase terminates with a modest BH seed of only $M_{rnd} = 600\,M_\odot$ at $z = 16$ ($t \simeq 0.25$ billion years for $H_0 = 0.7, \Omega = 1, \Omega_M = 0.28$), this would allow the subsequent Eddington-limited growth (with radiative efficiency $\eta_\gamma = 0.1$ and electron-scattering opacity $\kappa = 0.35\ cm^2\ g^{-1}$) of a $3.4 \times 10^8\,M_\odot$ SMBH by $z = 7$ ($t \simeq 0.78$ billion years) and a $2.5 \times 10^{10}\,M_\odot$ one by $z = 6$ ($t \simeq 0.95$ billion years). Even if the process operates efficiently only in 1 to 5% of the dark-matter halos where the first stars form, it can adequately account for the SMBHs seen to be powering the detected luminous quasars at $z > 6$.

We conclude that low-mass stellar BHs in very dense, low–angular-momentum cold flows at redshifts $z > 15$ can be launched by stellar dynamical processes into a phase of supply-limited supra-exponential accretion and can grow rapidly in ~ few $\times 10^7$ years into $\gtrsim 10^4\,M_\odot$ BH seeds. Subsequent slower Eddington-limited growth by disk accretion suffices to produce the supermassive BHs that power the brightest early quasars.

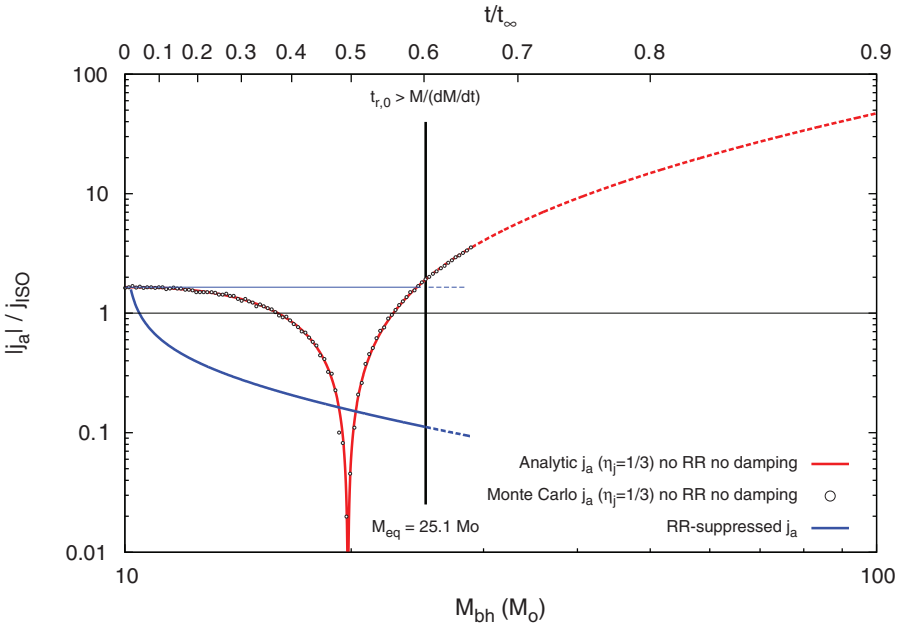

Fig. 2. The specific angular momentum ratio, j_a/j_{ISO}, in gas captured by the BH, as function of the BH mass $M_\bullet$ (and the corresponding t/t_∞ for Bondi accretion). The evolution of j_a/j_{ISO} during the initial stages of the BH growth is calculated in the ballistic wind accretion limit by a first-order analytic estimate with an angular momentum capture efficiency of $\eta_j = 1/3$ (red line), which is validated against results from a Monte Carlo integration over the exact capture cross section (circles). j_a falls to zero at $M_0 \simeq 20\,M_\odot$, where the density and velocity gradients cancel each other. The vertical line at $M_{eq} \simeq 25\,M_\odot$ marks the transition to a dynamical regime where two-body relaxation can no longer establish equipartition between the growing BH and the stars, and the acceleration frequency Ω is damped. The early dynamical suppression of the angular momentum down to $j_a < j_{ISO}$ by resonant relaxation of the BH orbit (thick blue line) is approximated by conservatively assuming a constant $j_a(t) = j_a(0)$ (thin blue line). At $M_\bullet > M_{eq}$, where the BH dynamics are sub-equipartition, the actual value of j_a/j_{ISO} is expected to progressively drop below the extrapolated one (dashed lines) (see the detailed discussion in the supplementary materials).

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ACKNOWLEDGMENTS

We thank P. Armitage, B. Bar-Or, M. Begelman, F. Bournaud, M. Colpi, A. Dekel. J.-P. Lasota, C. Reynolds, and N. Sapir for helpful discussions and comments. T.A. acknowledges support by European Research Council Starting Grant no. 202996, Deutsch-Israelische Projektkooperation–Bundesministerium für Bildung und Forschung grant no. 71-0460-0101, and the I-CORE Program of the Planning and Budgeting Committee and Israel Science Foundation (center no. 1829/12). P.N. acknowledges support from a NASA-NSF Theoretical and Computational Astrophysics Networks award, number 1332858. The authors thank the Kavli Institute for Theoretical Physics, University of California, Santa Barbara, where this work was initiated and supported in part by NSF grant PHY11-2591. T.A. is grateful for the warm hospitality of A. Millán and L. Arkwright of La Posada San Marcos, Alájar, Spain, who hosted the Alájar Workshop where this work was continued.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1330/suppl/DC1
Supplementary Text
Fig. S1
References (*44*–*54*)

20 January 2014; accepted 21 July 2014
10.1126/science.1251053

QUANTUM INFORMATION

Ultrafast optical control of orbital and spin dynamics in a solid-state defect

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Atom-scale defects in semiconductors are promising building blocks for quantum devices, but our understanding of their material-dependent electronic structure, optical interactions, and dissipation mechanisms is lacking. Using picosecond resonant pulses of light, we study the coherent orbital and spin dynamics of a single nitrogen-vacancy center in diamond over time scales spanning six orders of magnitude. We develop a time-domain quantum tomography technique to precisely map the defect's excited-state Hamiltonian and exploit the excited-state dynamics to control its ground-state spin with optical pulses alone. These techniques generalize to other optically addressable nanoscale spin systems and serve as powerful tools to characterize and control spin qubits for future applications in quantum technology.

Many proof-of-principle quantum information protocols have been implemented in recent years using a handful of semiconductor spin systems (*1*). One such system is the nitrogen-vacancy (NV) center in diamond, which has shown promise as a computational (*2*), memory (*3*), and communication (*4*, *5*) element for quantum information processing and for nanoscale sensing (*6*). Other related systems include donor atoms in silicon (*7*), whose electron spins can be controllably "switched off" to provide exceptionally long-lived nuclear spin memories (*8*), and defect spins in silicon carbide (*9*), which offer NV-center–like properties in a more technologically mature substrate material with luminescence closer to telecommunications wavelengths. There is a growing need for general methods to characterize and rapidly control such potential qubit systems.

Using the negatively charged diamond NV center as a model, we introduce a technique to probe the intrinsic structure of optically active spin systems and control their dynamics using ultrafast optical pulses. Our scheme complements previous demonstrations of all-optical NV-center spin control using off-resonant laser fields (*10*, *11*), but it enables faster control by using picosecond optical pulses of light to resonantly excite the NV center. By combining time-domain control of these fast excitations with quantum tomography of the ground-state (GS) spin, we demonstrate arbitrary, all-optical, coherent spin rotations and develop a technique to precisely map the excited-state (ES) Hamiltonian, H_{ES}, through the coherent evolution it produces.

The spin-triplet/orbital-singlet GS of a negatively charged diamond NV center is connected to a spin-triplet/orbital-doublet ES by dipole-allowed optical transitions that can be excited either resonantly (637 nm) or nonresonantly (≈500 to 630 nm). Optical spin polarization and photoluminescence (PL) readout is facilitated by a spin-dependent intersystem crossing (ISC) through a set of singlet states. At temperatures below ≈20 K, fine structure appears in the resonant optical transition due to the interplay of spin-spin/spin-orbit interactions with strain, electric, and magnetic fields (*12*, *13*). Crystal strain breaks the ES orbital degeneracy, yielding two separated orbital branches ($|L\rangle$ and $|U\rangle$) that are connected to the GS ($|G\rangle$) through orthogonal linear-polarization optical selection rules (Fig. 1A). Over multiple cool-downs of the same device, we measured strain splittings between $|L\rangle$ and $|U\rangle$ in the range ≈10 to 20 GHz. While phenomenological models successfully describe the NV center's overall electronic structure (*12*, *13*), previous estimates of the parameters in H_{ES} based on spectroscopic measurements (*14*, *15*) have limited precision (Table 1), and dynamical effects related to phonons, hyperfine coupling, and the ISC still lack a complete description.

We use a home-built confocal microscope (*16*) to isolate single NV centers in an electronic-grade, synthetic diamond (Element Six Ltd., UK) that was irradiated with 2-MeV electrons (10^{14} cm^{-2}) and annealed (*16*). A solid immersion lens milled using a focused ion beam (*17*) enhances the optical collection and excitation efficiencies, and nearby metal gates radiate microwave magnetic fields (Fig. 1C). A frequency-doubled optical parametric oscillator pumped by a mode-locked Ti:sapphire laser generates subpicosecond optical pulses, a tunable diode laser (~637 nm) enables resonant spin readout (*18*) and frequency-domain spectroscopy, and a 532-nm diode laser provides PL imaging and stabilizes the NV-center charge state (*19*).

We use ultrafast optical pulses to manipulate the NV center's orbital state in much the same way that microwave pulses rotate the GS spin. They affect only the orbital state directly, being effectively instantaneous from the perspective of the spin while preserving the spin coherence (*20*). By tuning the polarization of the optical field, we can couple $|G\rangle$ to either ES orbital eigenstate, $|L\rangle$ or $|U\rangle$, or to a coherent superposition of the two (*16*). We collect the PL in the red-shifted phonon side band (650 to 750 nm), which is proportional to the probability that the NV center was optically excited by a given operation.

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Figure 1D shows the measured excitation probability for individual pulses, P_{EX}, as a function of the pulse energy, corrected for ionization effects (conversion of NV^{-1} to NV^{0}) that occur at higher pulse powers (*16*). The excitation from $|G\rangle$ to the ES exhibits a coherent adiabatic passage behavior, manifested by saturation at $P_{EX} = 1$ at high powers, due to frequency chirp introduced by dispersive elements in the optical path. Numerical simulations of the orbital dynamics using the optical pulse waveform reconstructed through a nonlinear interferometric technique (*16*, *21*) confirm the observed power dependence.

In analogy with microwave spin resonance techniques, we probe orbital coherence using a two-pulse (Ramsey) scheme (Fig. 2A) in which each pulse is tuned to excite the NV center with $P_{EX} \approx 0.5$. A Mach-Zehnder interferometer splits each laser pulse into two identical copies, with a variable time delay set by an optical delay line in one leg (*16*). The combination of a motorized stage for coarse positioning with a fine-control piezoelectric-mounted mirror provides variable delays between 0 and 4 ns with subfemtosecond precision, enabling the study of orbital coherence with time scales spanning six orders of magnitude. Figure 2B shows sweeps of the femtosecond-scale relative delay ($\Delta\tau$) at two settings of absolute delay (τ) marked in Fig. 2C. The final excitation probability oscillates with a period of $\lambda/c = 2.1$ fs (where the wavelength $\lambda = 637$ nm and c is the speed of light), reflecting the quantum coherence between the GS and ES orbitals. With the polarization tuned to couple $|G\rangle$ to a superposition of $|L\rangle$ and $|U\rangle$, we create a superposition state composed of all three orbital levels and observe collapses and revivals in the Ramsey amplitude on picosecond scales indicative of coherent evolution within the ES orbital doublet manifold (Fig. 2C). These measurements are similar to quantum-beat spectroscopy techniques (*22*) used to probe ES molecular structure in gases, liquids, and solids.

Over nanosecond time scales (Fig. 2D), this approach enables orbital-selective, time-domain measurements of orbital dephasing and decoherence effects such as spectral diffusion and the dynamic Jahn-Teller effect (*23*). For example, we measure different orbital dephasing times $T_{orbital} = 7.1 \pm 0.3$ ns and 5.8 ± 0.4 ns for a two-component state, $\alpha|G\rangle + \beta|L\rangle$, and a three-component state, $\alpha|G\rangle + \beta(|L\rangle + |U\rangle)$, respectively (*16*), where α and β are complex amplitudes. This difference is not fully understood and needs to be explored further. The demonstration of time-domain orbital control also suggests extensions to multipulse dynamical decoupling sequences, potentially to improve the coherence of emitted photons to link distant NV centers (*5*).

The ability to coherently manipulate the NV center's orbital state on picosecond time scales provides new opportunities to control its electronic spin. Due to interactions (e.g., spin-orbit, spin-spin, strain) that depend on the electron's orbital wave function, the spin eigenstates of H_{ES} differ from those in the GS. Because the eigenstates set the basis for free evolution, ultrafast pulses can serve as gates to control the spin dynamics by coherently driving the system into different orbitals and abruptly changing the spin Hamiltonian. By fixing the spin evolution time within the ES manifold, we can produce a controlled spin rotation without microwaves.

To demonstrate this effect, we first consider a qubit constructed from the GS subspace $\{|G,0\rangle, |G,+1\rangle\}$ and optically excited to the ES subspace $\{|L,0\rangle, |L,+1\rangle\}$ for a short duration via optical pulses with polarization $\hat{H}$. Here, $|\varepsilon, m_s\rangle$ denotes the $m_s \in \{0,+1,-1\}$ spin sublevel in the $\varepsilon \in \{G,L,U\}$ orbital branch. Due to an ES level-avoided crossing (LAC) between $|L,0\rangle$ and $|L,+1\rangle$ mediated by the spin-spin interaction (Fig. 1B), the ES spin eigenstates vary dramatically as a function of axial magnetic field, changing from GS-like S_z eigenstates $\{|0\rangle, |+1\rangle\}$ far from the LAC to equal superposition states of $|0\rangle$ and $|+1\rangle$ at the center of the crossing. In a Bloch sphere representation of the qubit, the ES basis makes an angle θ_{ES} relative to the GS basis (z axis), defining the rotation axis for coherent evolution in the ES. We probe the ES dynamics by performing time-dependent quantum tomography (TDQT) on the GS qubit as a function of the delay between two ultrafast pulses that excite and subsequently de-excite the NV center. Using microwave spin resonance pulses that maintain a constant phase relation with the timing of the

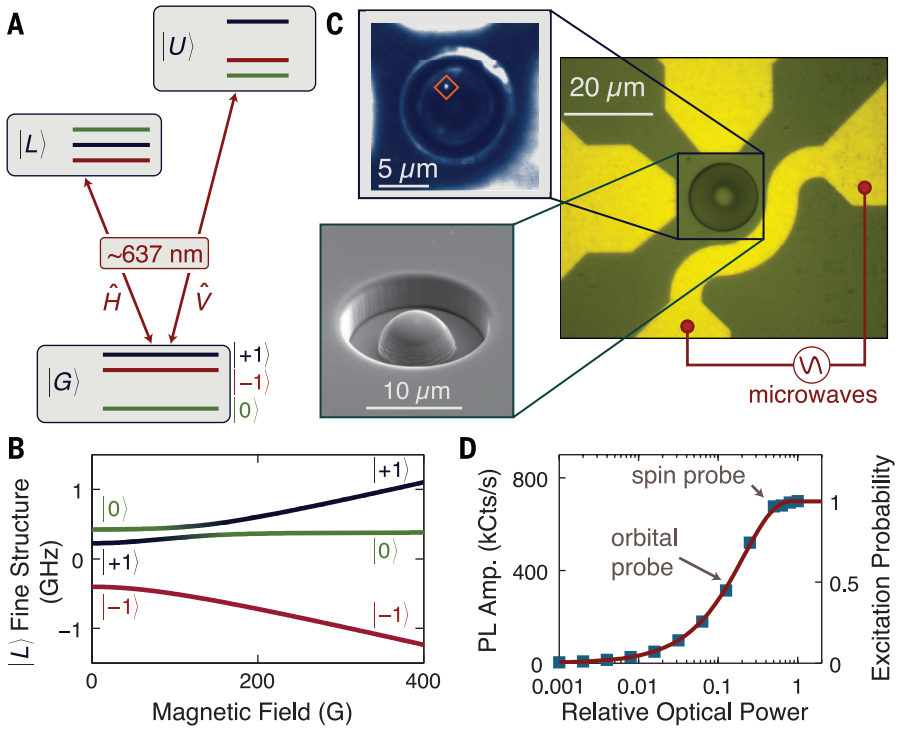

Fig. 1. Single NV-center optical transitions. (**A**) Schematic of the NV center's orbital and spin fine structure ($B < 100$ G). $\hat{H}$ and $\hat{V}$ define the orthogonal linear polarization basis vectors. (**B**) Spin sublevels in $|L\rangle$ calculated as a function of magnetic field applied along the NV center's symmetry axis. (**C**) Optical micrograph (right) of our device, consisting of metal surface gates used to generate microwave magnetic fields surrounding a solid immersion lens (SIL) milled from the diamond using a focused ion beam. (Lower left) Scanning electron micrograph of the SIL before gate fabrication. (Upper left) Scanning confocal PL micrograph. The single NV center studied in this work is marked by a diamond. (**D**) Spontaneous PL decay amplitude and corresponding optical excitation probability of the NV center as a function of the power of a single ultrafast pulse (squares). Numerical simulations (*16*) using the measured pulse characteristics (red curve) are also shown. Approximate settings used to probe orbital and spin coherence are marked by arrows. kCts/s, thousand counts per second.

Table 1. Hamiltonian parameters. Intrinsic parameters of H_{ES} inferred from fitting our numerical model to TDQT measurements and comparisons with values from previous frequency-domain measurements. See the supplementary materials (*16*) for parameter definitions.

Parameter	Best-fit value	Literature value	Reference
Axial spin-orbit (λ)	5.33 ± 0.03 GHz	5.3 GHz	(*14*)
Axial "ZZ" spin-spin (D_{ES})	1.44 ± 0.02 GHz	1.42 GHz	(*14*)
"XY" spin-spin (Δ_1)	1.541 ± 0.005 GHz	1.55 GHz	(*14*)
"XZ" spin-spin (Δ_2)	154 ± 13 MHz	200 MHz	(*15*)
Axial Landé g-factor ($g_{ES}^{\parallel}$)	2.15 ± 0.04	None available	N/A

ultrafast pulses (*16*), we successively initialize the GS qubit, drive it to the ES for variable time τ, and then project it in one of three orthogonal axes on the Bloch sphere.

Figure 3A contains a few examples of TDQT measurements recorded at magnetic field B = 110 G near the center of the LAC, where we expect $\theta_{ES} \approx \frac{\pi}{2}$. The projection $\langle Z \rangle$ oscillates at a frequency ≈260 MHz, in agreement with the minimum frequency separation observed in optical frequency-domain spectroscopy (Fig. 3C). The fast oscillations observed in the $\langle X \rangle$ and $\langle Y \rangle$ projections result from phase accumulation relative to the rotating frame of the microwave carrier resonant with the GS frequency, f_{GS} = 3.18 GHz. These data are quantitatively described by an analytic model based on a master-equation approach (*16*) that includes coherent ES spin evolution at frequency f_{ES} about a tilted axis with polar (azimuthal) angle θ_{ES} (ϕ_{ES}) together with decoherence effects due to incomplete optical inversion, spontaneous photon emission, the ISC, and both longitudinal (T_1-type) and transverse (T_2-type) relaxation processes in the ES, with rates $\Gamma_i = 1/T_i$. Best-fit curves are plotted with the corresponding data in Fig. 3A.

The best-fit parameters (f_{ES}, θ_{ES}) and (Γ_1, Γ_2) from a global fit to 11 sets of TDQT data at different magnetic field values are shown in Fig. 3, D and E, respectively. Within each set, ϕ_{ES} depends on the unknown relative phase between the microwave carrier and the timing of the optical pulses. To clarify the dynamics, we "unwind" the phase accumulation from the GS rotating frame using the best-fit simulated density matrix, recovering the lab-frame trajectories of the qubit state as a function of evolution time in the ES. Several lab-frame trajectories are shown in Fig. 3B, along with the rotation axes corresponding to the inferred ES eigenstates.

In principle, universal qubit control is possible with this scheme as long as $\theta_{ES} \neq 0$, in which case noncolinear rotation axes can be selected by varying ϕ_{ES} via the timing of optical pulse pairs. At the center of the LAC, the available rotation axes span the Bloch-sphere equator, enabling arbitrary single-qubit operations using a single pair of pulses. The LAC coupling strength of 260 MHz generates an equatorial π rotation in only 1.9 ns, approaching the fastest operation times demonstrated for NV centers with microwave spin resonance techniques (*24*). The process fidelity (*16*, *25*) of this equatorial π rotation is 0.59, while the fidelity of a π rotation about a nearly polar axis at B = 400 G (requiring only 160 ps in the GS rotating frame) is 0.77. The fidelities are limited by dephasing in the ES, the intersystem crossing, spontaneous photon emission, and incomplete optical inversion ($P_{EX} \approx 0.9$ in our TDQT experiments). Given complete inversion, we calculate improved π-rotation fidelities of 0.69 (equatorial) and 0.92 (polar), and further improvements should be possible by tuning f_{ES} and θ_{ES} through external magnetic, electric (*26*), or strain (*14*) fields. In principle, a tradeoff exists between direct excitation of the ES, which enables faster control, and off-resonant techniques (*10*, *11*), which reduce sensitivity to ES dissipation mechanisms, but in practice the reported fidelities are markedly similar in both cases.

Besides demonstrating all-optical spin control, the technique presented in Fig. 3 provides a powerful approach to characterize the optically excited states of systems like the diamond NV center: namely, the ability to directly map H_{ES}, in this case specified by (f_{ES}, θ_{ES}, ϕ_{ES}), through TDQT measurements. In addition to H_{ES}, the time-domain approach also provides detailed information about dissipative dynamics typically obscured in frequency-domain spectroscopy, such as the decoherence rates Γ_1 and Γ_2, which point to interactions with additional degrees of freedom. For example, the apparent increase in Γ_1 at low B in Fig. 3E actually results from coherent mixing with the third triplet state, $|L, -1\rangle$, outside the scope of our qubit model.

We can extend the TDQT technique to the full spin-triplet Hilbert space to map H_{ES} completely. Using phase-locked multicolor microwaves to address both the $|G, 0\rangle \leftrightarrow |G, -1\rangle$ and $|G, 0\rangle \leftrightarrow |G, +1\rangle$ transitions, we perform state tomography on the final spin triplet for a selection of prepared inputs states (*16*). Figure 4 shows a few example TDQT traces, detailing the evolution of the GS spin populations in $\{|0\rangle, |+1\rangle, |-1\rangle\}$ for an initial state $|G, +1\rangle$ that is excited to $|L\rangle$ or $|U\rangle$, respectively, at B = 110 G.

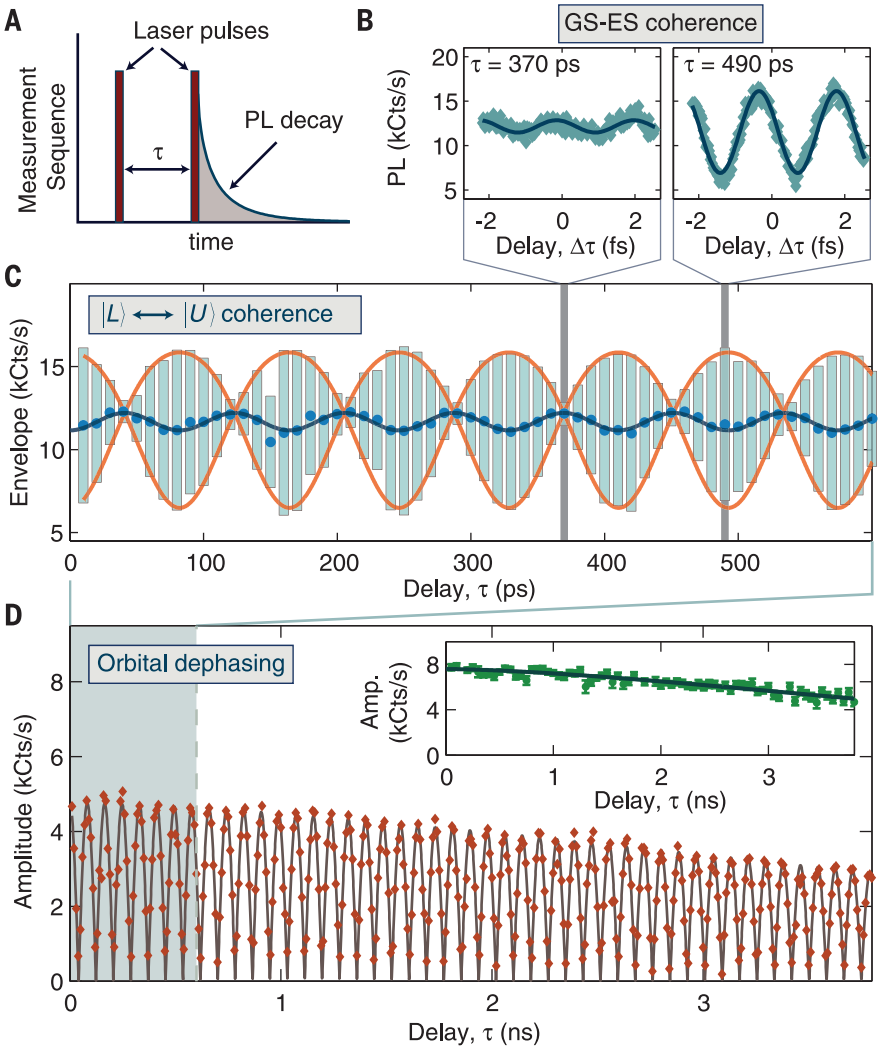

Fig. 2. Orbital coherence measurements. (**A**) Schematic of optical pulse sequence used to probe orbital coherence. (**B**) Optical excitation probability versus femtosecond-scale relative delay (Δτ) at the two points of absolute delay (τ) marked in (C), with sinusoidal fits of the form $y_0 + A \sin(\omega\Delta\tau + \phi)$, where $\omega/2\pi$ = 470 THz is the optical frequency at 637 nm. (**C**) Coherence envelopes from sinusoidal fits to individual Δτ scans as in (B), when the excitation polarization couples equally to $|L\rangle$ and $|U\rangle$. Rectangles show the sinusoid extrema ($y_0 \pm A$), and blue points denote the offset y_0. Oscillations reflect coherent evolution within the ES orbital doublet. (**D**) Best-fit coherence amplitude, A, versus τ, extending the data in (C) to nanosecond time scales (orange points), together with a corresponding measurement (inset) with the excitation polarization coupling only to $|L\rangle$ (green points). In both (C) and (D), solid curves show fits to functions described in the supplementary materials (*16*).

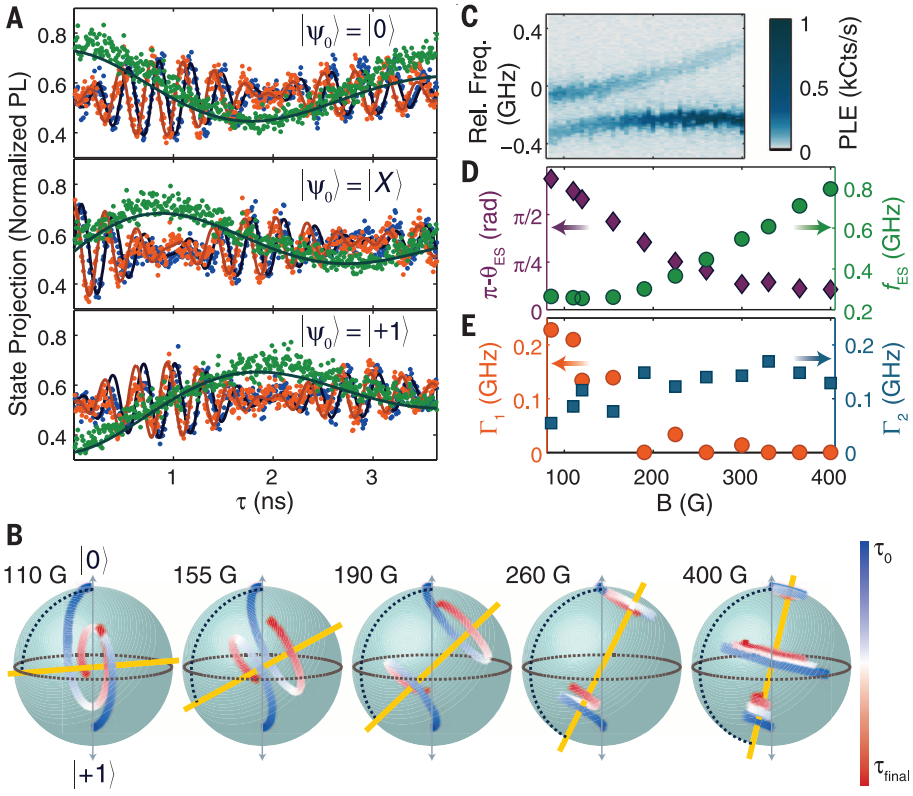

Fig. 3. All-optical qubit control. (**A**) TDQT of the $|m_s\rangle = \{|0\rangle, |+1\rangle\}$ GS qubit subspace as a function of the delay between two optical pulses coupling $|G\rangle$ to $|L\rangle$ near the LAC at B = 100 G. Each panel shows the final $\langle X\rangle$, $\langle Y\rangle$, and $\langle Z\rangle$ projections (orange, blue, and green points, respectively) for a different initial state $|\psi_0\rangle$, along with fits to our analytical qubit model as corresponding curves. (**B**) Best-fit trajectories in the lab-frame qubit Bloch sphere corresponding to TDQT measurements at different magnetic field values. The yellow axis in each plot shows the orientation of the ES eigenvectors relative to the GS, and θ_{ES} is indicated by dotted black lines. (**C**) Frequency-domain PL excitation (PLE) spectroscopy versus B [same axis as in (E)], showing the LAC between $|L, 0\rangle$ and $|L, +1\rangle$ around 110 G. (**D**) Best-fit ES free evolution precession frequency (f_{ES}) and polar angle (θ_{ES}) from measurements at varied B. (**E**) Best-fit decoherence rates (Γ_1, Γ_2) corresponding to population (T_1-type, orange circles) and coherence (T_2-type, blue squares) relaxation in the ES, respectively. Uncertainties in (D) and (E) are similar to the symbol sizes.

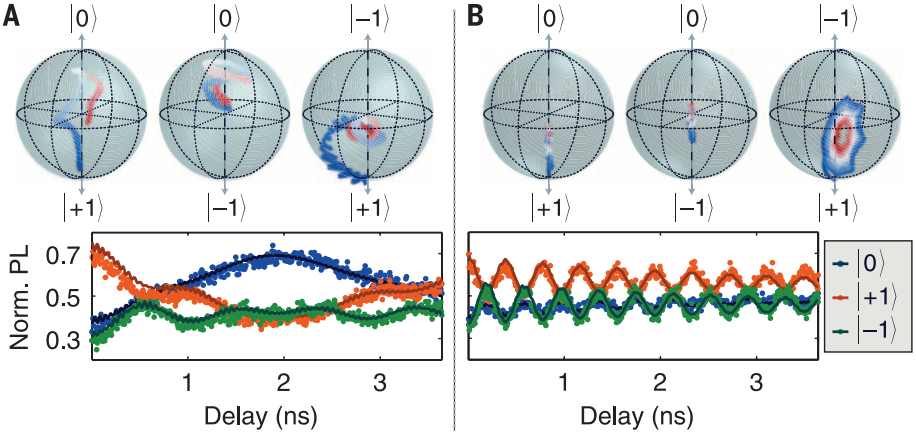

Fig. 4. Time-domain quantum tomography. (**A**) Evolution of the GS spin initialized in $|G, +1\rangle$ and coupled to the $|L\rangle$ orbital branch by a pair of ultrafast pulses. (Lower panel) Measurements of the GS populations in $\{|0\rangle, |+1\rangle, |-1\rangle\}$ (blue, orange, and green points, respectively) as a function of the delay of the second pulse. Curves of corresponding colors result from a global fit to 54 different initialization-projection pairs, of which these three are a subset. (Upper panel) Bloch spheres showing the best-fit lab-frame trajectories in three qubit subspaces. (**B**) Same as (A) but with the optical pulses coupling $|G\rangle$ to $|U\rangle$. All measurements were taken at B = 110 G, near the LAC between the product states $|L, 0\rangle$ and $|L, +1\rangle$. Norm., normalized (*16*).

By fitting these data to a numerical model, we extract the symmetry-allowed reduced matrix elements of H_{ES}, including intrinsic spin-spin/spin-orbit terms and perturbations due to external electric, strain, and magnetic fields (*12*, *13*). The model (*16*) also includes spectral diffusion and phonon relaxation within the ES orbitals, ES spin dephasing, and a detailed description of the ISC, with each process parameterized by corresponding rates. The global fit to 54 TDQT time traces contains 31 free parameters, all of which are well constrained by the data (*16*).

Table 1 provides best-fit values for several intrinsic parameters in H_{ES} together with previous estimates based on frequency-domain spectroscopy (*14*, *15*). We find general agreement and improved precision, particularly for the spin-spin parameter Δ_2, whose value is confounded in frequency-domain measurements by strain perturbations (*16*). In contrast to frequency-domain techniques, TDQT distinguishes individual contributions from both intrinsic (spin-orbit/spin-spin) and external perturbations by their phases and amplitudes throughout H_{ES}. Essentially, we directly probe the ES eigenstates as well as the eigenvalues and can therefore fully map H_{ES} without varying any external fields. Furthermore, TDQT yields quantitative information about dissipative effects; for example, we resolve the ISC decay rates of individual ES basis states from other dephasing and decoherence processes (*16*).

To visualize the spin evolution in triplet space, we use a set of Bloch spheres that span three qubit subspaces, as shown in Fig. 4. As before, the best-fit trajectories are "unwound" from their corresponding rotating frames to reveal the ES dynamics in the lab frame. Due to spin-orbit effects, the spin evolution in $|L\rangle$ and $|U\rangle$ proceeds in different bases. The LAC in $|L\rangle$ drives oscillations between $|L, 0\rangle$ and $|L, +1\rangle$, whereas the states $|U, +1\rangle$ and $|U, -1\rangle$ are strongly mixed by spin-spin interactions. By direct extension of the qubit-control scheme developed above, this enables all-optical coherent control spanning the full GS triplet, through the control of both the timings and polarizations of optical pulses.

Our all-optical approach could be used to address spins individually in spin arrays (*27*) or large-scale photonic networks (*28*). By engineering appropriate orbital-dependent coupling between defects through, for example, optical, electrical, or mechanical interactions, the scheme could be extended to multiqubit entangling gates, similar to ultrafast gate proposals for trapped ions (*29*, *30*) and superconducting qubits (*31*). Likewise, the TDQT technique can be applied to a broad range of atomic, molecular, and solid-state quantum systems, offering a powerful tool to characterize and control both new and established systems for future quantum technologies.

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ACKNOWLEDGMENTS

We thank K. Ohno for help with sample characterization and A. Falk for thoughtful comments. We gratefully acknowledge support from the Air Force Office of Scientific Research, the NSF DMR-1306300, and the German Research Foundation (SFB 767). All data described in this Report are presented in the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1333/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S19
Tables S1 to S4
References (*32*–*39*)

2 May 2014; accepted 23 July 2014
Published online 14 August 2014;
10.1126/science.1255541

MAGNETISM

All-optical control of ferromagnetic thin films and nanostructures

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The interplay of light and magnetism allowed light to be used as a probe of magnetic materials. Now the focus has shifted to use polarized light to alter or manipulate magnetism. Here, we demonstrate optical control of ferromagnetic materials ranging from magnetic thin films to multilayers and even granular films being explored for ultra-high-density magnetic recording. Our finding shows that optical control of magnetic materials is a much more general phenomenon than previously assumed and may have a major impact on data memory and storage industries through the integration of optical control of ferromagnetic bits.

The dynamic response of magnetic order to ultrafast external excitation is a fascinating issue of modern magnetism (*1*, *2*). Optical probing at the femtosecond time scale allows the investigation of ultrafast magnetization dynamics, including fundamental interactions between spins, electrons, and lattice degrees of freedom far from equilibrium (*1*, *3*–*5*). It further has the potential for new technologies such as heat-assisted magnetic recording (HAMR) (*6*, *7*). An important outcome from studies of ultrafast dynamics of magnetic systems is the demonstration that circularly polarized light can directly switch magnetic domains in ferrimagnetic GdFeCo alloy films without an applied magnetic field (*8*). This deterministic magnetization switching using circularly polarized laser pulses is referred to as all-optical helicity-dependent switching (AO-HDS).

Until now, ferrimagnets have been the only materials to show AO-HDS. Whereas initial studies focused on rare-earth transition-metal (RE-TM) GdFeCo alloys, there have been recent examples of AO-HDS in other RE-TM materials (*9*, *10*), as well as synthetic ferrimagnets (*11*). In all cases, AO-HDS was observed for ferrimagnetic systems with two distinct sublattices that are antiferromagnetically exchange-coupled. Models for AO-HDS were based on the existence of an effective field created by the circular polarized light via the inverse Faraday effect (*12*, *13*) or by the transfer of angular momentum from the light to the magnetic system (*14*). More recent models have been focused on the formation of a transient ferromagnetic state (*15*) due to different demagnetization times for RE and TM sublattices where the light's helicity plays a secondary role (*16*–*18*). There is also emerging evidence that laser-induced superdiffusive spin currents can flow in heterogeneous systems (*18*–*22*), potentially contributing to the AO-HDS process (*18*). An open question is whether AO-HDS is specific to a subset of ferrimagnetic materials or can be applied to ferromagnetic materials. Furthermore, can optical pulses also control technologically important high-anisotropy granular or patterned materials that are anticipated for future high-density magnetic recording (*23*)?

The optical response of ferromagnetic samples are studied by a combination of optical- and heat-assisted magnetic switching experiments where the samples are excited by a ~100-femtosecond pulsed laser source and subsequently imaged in a Faraday microscope (fig. S1). Figure 1 shows images of $[Co(0.4\ nm)/Pt(0.7)]_N$ multilayers, where N is the number of bilayer repeats in the multilayer structure (N = 8, 5, and 3 repeats), which have perpendicular magnetic anisotropy, so the magnetization easy axis is normal to the film and the image contrast results from the two possible directions of the magnetization. The laser helicity is either of two circular polarizations (σ^+ or σ^-) or linear polarized (L). For Fig. 1, A to C, the laser is scanned across a region of the films that has both magnetization directions, allowing the effect of the initial state of the magnetization on the magneto-optical response to be studied.

For N = 8 repeats (Fig. 1A), domain formation is observed in the region scanned by the laser that is filled with disordered stripe subdomains that minimize the dipole energy (*24*). This process is independent of the light polarization and represents laser-induced thermal demagnetization (TD). A rim is observed at the edge of the scanned area where the magnetic orientation is opposite the background stabilized by dipolar fields from the surrounding film. For N = 5 repeats (Fig. 1B), the formation of subdomains in the scanned region is again observed. The average domain size is larger for decreasing number of layers, since in the thin-film limit the equilibrium domain size increases with decreasing film thickness (*24*). More important, the resulting domain structure depends on the light polarization. For σ^+ light, white isolated bubble-like domains are observed in a dark background, whereas for σ^- light, dark domains are seen in a white background and the magnetization near the edges of the line scan favors the direction opposite the surrounding film.

In contrast, for N = 3 repeats (Fig. 1C), fully deterministic magnetization reversal is observed

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for both circular polarizations that is independent of the initial magnetization direction, a clear demonstration of AO-HDS in a ferromagnetic material. The random domains created for linear polarization are much larger for $N = 3$ repeats, in accord with the small dipolar energy gain for ultrathin films. Figure 1D shows images for the $N = 3$ repeats sample for various laser powers where the film is saturated in one direction and the laser spot is fixed and not scanned on the surface. For low power (362 nW), a reversed domain is written for σ^+, there is no change to the film for σ^-, and a region of random domains is observed for linear polarization. With increasing laser power, regions of demagnetized random domains develop in the center of the laser spot for all three polarizations, indicating that the power is such that the temperature exceeds a critical temperature for which domains are formed (e.g., the Curie temperature T_C). For σ^+, there is a rim at the edge of the demagnetized area that shows deterministic switching that is not present for σ^- or linearly polarized light.

We explored the effective driving energy for AO-HDS and domain formation (TD) by adding external magnetic fields to the experiments shown in Fig. 1, A to C (figs. S2 to S4). Although linear polarization leads to domain formation in zero applied field, the application of a magnetic field can stabilize a uniform magnetization state. This field increases from 3 to 4 Oe for $N = 3$ repeats, to ~12 Oe for $N = 5$ repeats and ~40 Oe for $N = 8$ repeats, demonstrating the increased dipolar energy with thickness. When the applied field is combined with circular polarization, the applied field can either support or oppose the circular polarization. For $N = 3$ repeats, a field of 7 Oe opposes the circular polarized light and yields a demagnetized film, whereas a field of ~12 Oe will saturate the film in the opposite direction as that expected for the helicity of the light (fig. S4). For $N = 5$ repeats, the field needed to yield a demagnetized film is ~12 Oe, whereas a field of ~25 Oe is needed to saturate the film opposite to the light helicity (fig. S3). However, when comparing the effects of circular polarization of the light to the applied magnetic field, one has to remember that the magnetic field is applied during the entire thermal process, whereas the role of the helicity of the pulse is believed to persist only for a few picoseconds after the laser excitation (*25*).

To determine how general AO-HDS is, we studied a range of ferromagnetic film materials, including $[Co(t_{Co})/Pt(t_{Pt})]_N$, $[Co(t_{Co})/Pd(t_{Pt})]_N$, $[Co_xNi_{1-x}(0.6\text{ nm})/Pt(0.7\text{ nm})]_N$, and $[Co/Ni]_N$ multilayer structures, where we have varied several material parameters (t_{Co}, t_{Pt}, N, and Ni concentration) that change materials' magnetic properties, such as magnetization, T_C, anisotropy, and exchange. AO-HDS is observed in all these ferromagnetic materials classes, including single Co layers sandwiched between Pt layers. Figure 2 summarizes selected results for the threshold laser power needed to achieve either AO-HDS (solid symbols) or TD (open symbols). For $[Co(t_{Co})/Pt(0.7\text{ nm})]_N$ multilayers, the results (Fig. 2A) show AO-HDS for $N = 2$ or 3 repeats and $t_{Co} \leq 0.6$ nm (i.e., the thinnest samples) and TD for thicker samples (consistent with Fig. 1). The threshold laser power increases linearly with both N and t_{Co}, and these trends are independent of AO-HDS or TD final states. For $[Co(0.4\text{ nm})/Pt(t_{Pt})]_2$ samples (Fig. 2B), the threshold power decreases slightly with increasing Pt thickness. For $N = 1$ trilayer structures—i.e., $Pt/Co(t_{Co})/Pt$ (Fig. 2C)—we observed AO-HDS for $0.6\text{ nm} \leq t_{Co} \leq 1.5$ nm. The upper limit is set by the thickness at which the sample maintains perpendicular magnetic anisotropy and the lower limit by the sensitivity of the optical detection. The threshold power increases linearly with t_{Co}, and the values for single Co layers are consistent with the extrapolation of the data in Fig. 2A to $N = 1$ repeats. For $[Co_{1-x}Ni_x(0.6\text{ nm})/Pt(0.7\text{ nm})]_N$ multilayers as a function of both N and Ni concentration (Fig. 2D), the threshold power increases with N as seen in Fig. 2A and decreases with Ni concentration. Finally, AO-HDS is seen for a Cu(10 nm)/$[Ni(0.5\text{ nm})/Co(0.1\text{ nm})]_2$/Ni(0.5 nm)/Cu(5 nm) multilayer.

These results show that AO-HDS is a rather general phenomenon for ferromagnetic films but is limited according to the thin-film limit, which is useful in a number of spintronic applications (e.g., magnetic random access memory). Applications such as high-density magnetic recording require small magnetic grains or patterned bits with high magnetic anisotropy (K_U) to remain thermally stable at the nanoscale (*26*). The current challenge is that the applied magnetic fields required to write high-anisotropy grains is above what can be achieved by electromagnetic write heads. HAMR is the leading technology to address the challenge when a laser spot heats the magnetic material close to T_C, allowing the magnetization to be written with a modest applied magnetic field (*6*, *7*). We studied the role of AO-HDS in combination with applied magnetic fields on high-anisotropy FePt-based HAMR media (*27*). The media are FePtAgC and FePtC granular films that form high-anisotropy FePt grains separated by C grain boundaries. The average grain size is ~9.7 and ~7.7 nm for the FePtAgC and FePtC, respectively (fig. S5). The room-temperature magnetic anisotropy and coercive fields are 7 T and 3.5 T, respectively (fig. S6). Figure 3 are optical results on the FePtAgC granular film, which is initially in a random magnetic state with equal up- or down-oriented magnetic grains. Because the grain size is well below the resolution of the Faraday

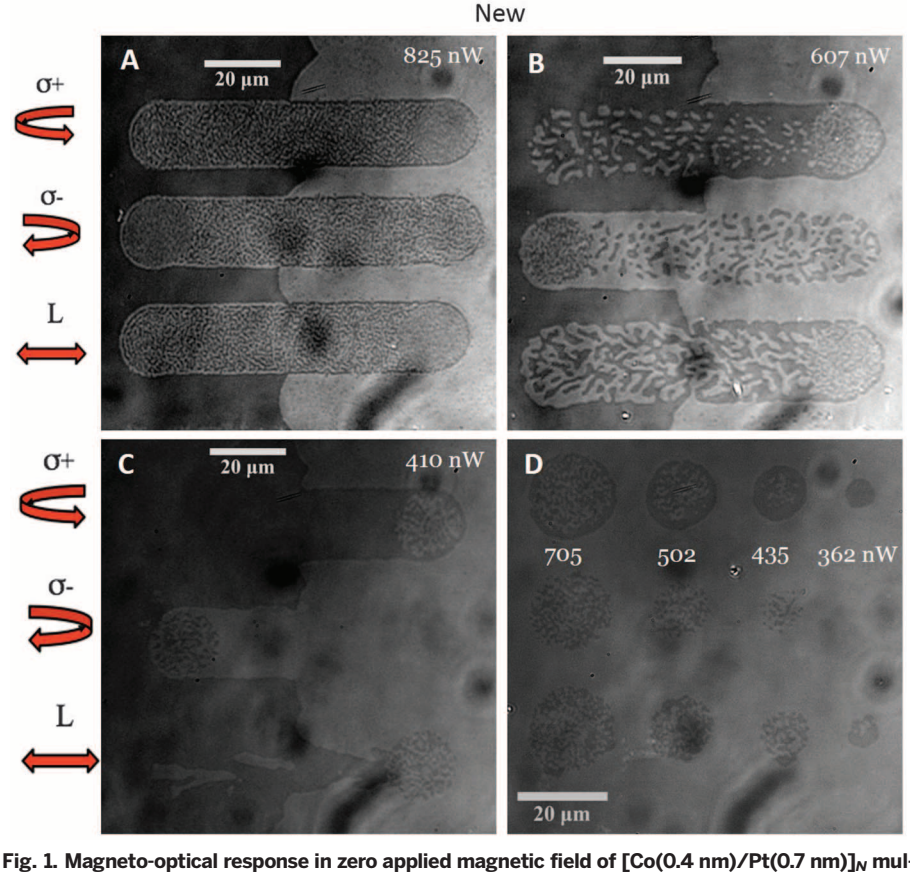

Fig. 1. Magneto-optical response in zero applied magnetic field of $[Co(0.4\text{ nm})/Pt(0.7\text{ nm})]_N$ multilayer samples to various laser polarizations. (**A**) $N = 8$ repeats. (**B**) $N = 5$ repeats. (**C** and **D**) $N = 3$ repeats. For each image, the laser is circularly polarized (σ^+ or σ^-) or linear polarized (L). For (A) to (C), the laser beam was swept over a region of the sample with two perpendicularly oriented magnetic domains showing black/white contrast in the images, with a domain wall that runs vertically in the middle of each image. In (D), the laser was fixed at individual spots over a region of the sample with uniform magnetization (white contrast). The average laser intensity at different spots is indicated in the image.

microscope, the randomly magnetized sample appears gray. Figure 3A shows that there is a net magnetization achieved that depends on the helicity of the circularly polarized light, whereas no change is observed with linear polarization. This clearly demonstrates that the film magnetization is controlled by the polarization of the light. Figure 3B shows images recorded without scanning the laser beam as in Fig. 1D. With increasing power above the threshold power (~420 nW,) there is a region of reversed grains. Above ~600 nW, a ring forms where AO-HDS occurs at a particular radius, consistent with a relatively narrow range of powers to achieve AO-HDS. The center of the ring, where the laser intensity is the highest, is demagnetized (TD-behavior), presumably exceeding T_C.

While AO-HDS is observed, the degree of magnetization is less than 100%. By comparing the contrast to the saturated film, the estimate of the induced magnetization is ~10 to 20% of saturation. The lack of saturation can arise from at least two effects. The first is that AO-HDS is only affecting a subset of the grains. The second, and more likely, is that the magnetic grains are highly thermally activated. Near T_C, there is a strong drop in K_U, the magnetic energy stored in the grain $K_U V$ (where V is the volume of the grain) becomes comparable to thermal energy $k_B T$, and the particles are superparamagnetic. During the sample cooling after the optical pulse, the grain assembly can partially demagnetize due to thermal switching of individual grains [see discussion in (*28*)]. This effect is further driven by the dipolar fields from the neighboring grains that support a demagnetized ground state.

To quantify the role of thermal activation on AO-HDS, an external magnetic field was applied while the sample was illuminated by the polarized light. Figure 4 shows the results of line scans with both circular polarizations for different external magnetic field. An applied field of ~700 Oe is sufficient to suppress the effects of the helicity of the light where no contrast is observed for σ^-. For σ^+, the contrast increases with increasing field. Similarly, we can excite the films with linear polarized light, and an applied field of ~600 Oe yields a similar optical contrast as the AO-HDS results (fig. S7). The fact that these modest fields (relative to the room-temperature coercive field) are sufficient to magnetize the sample and/or counter the polarization of the light indicates that the sample is heated by the laser near T_C. Moreover, the grains are highly thermally activated during the optical excitation, because an external field up to 1100 Oe is not sufficient to fully saturate the film.

Our results show that AO-HDS is not exclusive to ferrimagnetic materials, and therefore antiferromagnetic exchange coupling between two magnetic sublattices is not required. Given that we observe AO-HDS switching on single Co films as well as Co/Pt and Co/Ni multilayers, it is unlikely that super-diffusive currents that couple different magnetic regions in a heterogeneous sample are required. The results do suggest that heating near the Curie point is important for the AO-HDS in ferromagnetic materials, because the threshold intensities for both AO-HDS and TD (Fig. 2) generally track with the expected trends for T_C and do not scale with parameters such as interlayer exchange or anisotropy. Near T_C, the inverse Faraday effect or transfer of angular momentum from the light to the magnetic system is expected to be most effective. Recent modeling within the Landau-Lifshitz-Bloch formalism finds that achieving a nearly quenched magnetization is crucial for linear reversal under an ultrashort optomagnetic field pulse arising from the inverse Faraday effect (*29*). Well below T_C, the magnetization reversal is precessional and governed by the Landau-Lifshitz-Gilbert equation. In the current geometry, the optomagnetic field is parallel to the magnetization, the least efficient for precessional switching (*30*). Similarly, when approaching T_C, the transfer of the relatively modest angular moment in the light can lead to a symmetry breaking such that magnetization is deterministically switched.

The final ingredient for AO-HDS is that the magnetization state, once switched near T_C, be maintained while the sample cools. If demagnetization and thermal energies are too large, then

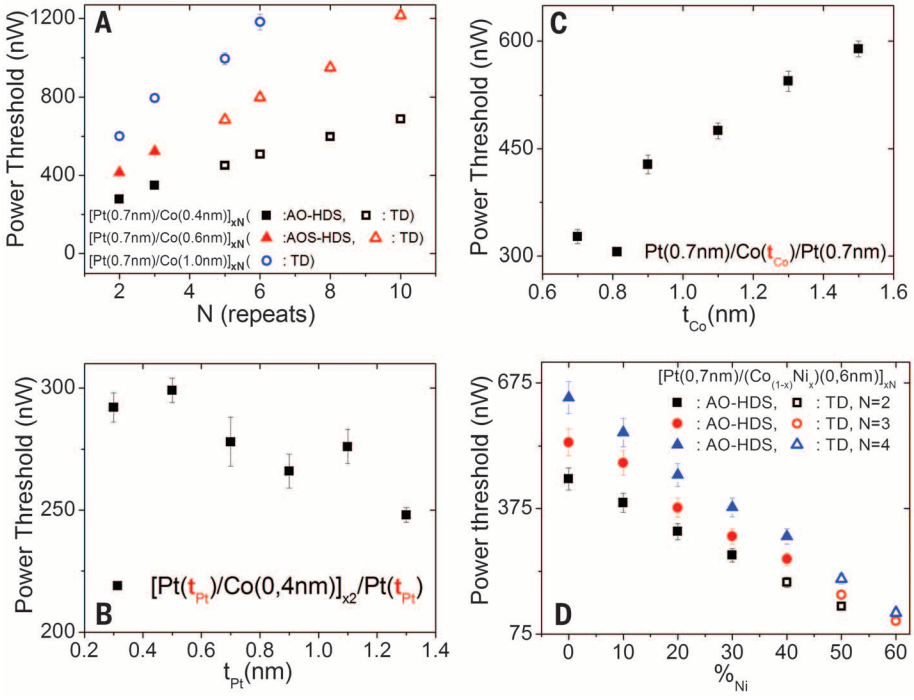

Fig. 2. Magneto-optical response of multilayer thin-film samples to circular polarization light and varying powers. Plotted is the evolution of the threshold power to achieve either TD or AO-HDS of various samples. For each sample, the threshold power is given either as a filled symbol for AO-HDS or open symbols for TD. (**A**) Threshold power versus N for $[Co(t_{Co})/Pt(0.7\ nm)]_N$ samples with t_{Co} = 0.4, 0.6 and 1.0 nm. (**B**) Threshold power versus t_{Pt} for $[Co(0.4\ nm)/Pt(t_{Pt})]_2$ multilayer samples. (**C**) Threshold power versus t_{Co} for $Pt/Co(t_{Co})/Pt$ trilayer samples. (**D**) Threshold power versus Ni concentration for $[Co_{1-x}Ni_x(0.6\ nm)/Pd(0.7\ nm)]_N$, with N = 2, 3 or 4 repeats.

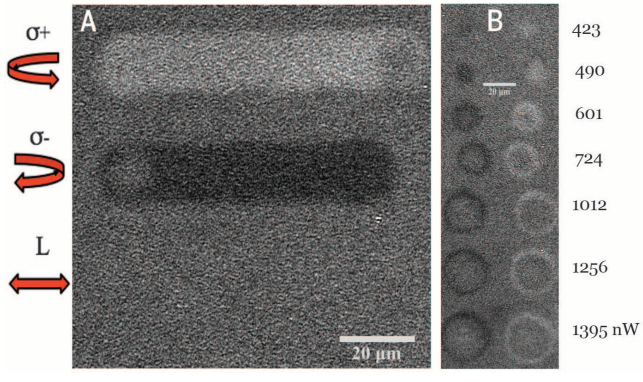

Fig. 3. Magneto-optical response in zero applied magnetic field of a 15-nm FePtAgC granular film sample starting with an initially demagnetized sample. (**A**) Line scans for σ^+, σ^-, and linear polarized light (L). The laser beam was swept over the sample, and the magnetization pattern was subsequently imaged. (**B**) Images of magnetic domains written by keeping the laser spot at a fixed position on the sample. The laser was either σ^+ polarized (left column) or σ^- polarized (right column). The laser power is given next to the image.

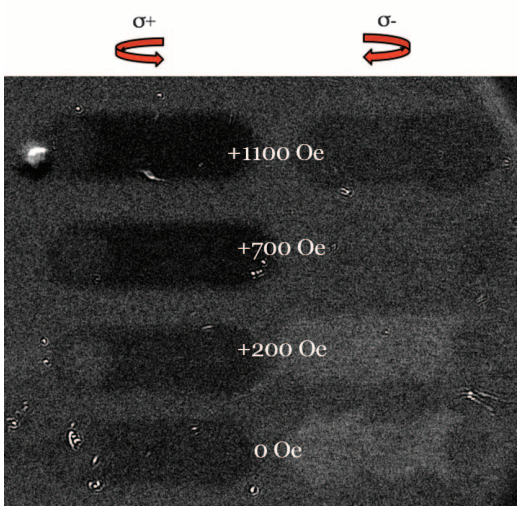

Fig. 4. Magneto-optical response in various applied magnetic field of a 15-nm FePtAgC granular film sample starting with an initially demagnetized sample. Shown are line scans for σ^+ circularly polarized light in the left column and σ^- circularly polarized light in the right column. The laser power was 677 nW. The magnitude of the external magnetic field is given in the figures, and the orientation of the field supports σ^+ polarization and opposes the σ^- polarization.

the sample will demagnetize during cooling. For perpendicular magnetized films, there are strong dipolar fields within the film that support domain formation. The dipolar energy gain for domain formation is strongly suppressed in the ultrathin film limit and explains the observation of AO-HDS only in the thin-film limit (Figs. 1 and 2). Domain formation is also suppressed for low magnetization materials, consistent with AO-HDS measurements of ferrimagnetic materials (*11*).

The present results on ferromagnetic materials demonstrate a new and technologically important class of materials showing AO-HDS and opens new directions in integrated magnetic-optical memory, data storage, and processing applications. This study further offers progress toward a better understanding of the interaction between pulsed polarized light and magnetic materials.

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ACKNOWLEDGMENTS

We thank R. Tolley and M. Gottwald for help on sample fabrication and M. Menarini and V. Lomakin for helpful discussions. This work was supported by the Agence Nationale de la Recherche (ANR), ANR-10-BLANC-1005 "Friends," and work at University of California San Diego was supported by the Office of Naval Research (ONR) Multidisciplinary University Initiative (MURI) program and a grant from the Advanced Storage Technology Consortium. It was also supported by the Partner University Fund "Novel Magnetic Materials for Spin Torque Physics" as well as the European Project (OP2M FP7-IOF-2011-298060) and the Region Lorraine. Work at the National Institute for Materials Science was supported by Advanced Storage Technology Consortium. Author contributions include the following: S.M., M.A., M.C., Y.S., and E.E.F. designed and coordinated the project; C.-H.L. grew, characterized, and optimized the thin films samples, while B.S.D.Ch.S.V., Y.K.T., and K.H. developed and grew the FePt-based granular media. C.-H.L., S.M., and Y.K.T. operated the Kerr microscope and the pump laser experiment. S.M. and E.E.F. coordinated work on the paper with contributions from C.-H.L, M.H., M.C., and G.M., with regular discussions with all authors.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1337/suppl/DC1
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Supplementary Text
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References

17 March 2014; accepted 4 August 2014
Published online 21 August 2014;
10.1126/science.1253493

SOCIAL PSYCHOLOGY

Morality in everyday life

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The science of morality has drawn heavily on well-controlled but artificial laboratory settings. To study everyday morality, we repeatedly assessed moral or immoral acts and experiences in a large (N = 1252) sample using ecological momentary assessment. Moral experiences were surprisingly frequent and manifold. Liberals and conservatives emphasized somewhat different moral dimensions. Religious and nonreligious participants did not differ in the likelihood or quality of committed moral and immoral acts. Being the target of moral or immoral deeds had the strongest impact on happiness, whereas committing moral or immoral deeds had the strongest impact on sense of purpose. Analyses of daily dynamics revealed evidence for both moral contagion and moral licensing. In sum, morality science may benefit from a closer look at the antecedents, dynamics, and consequences of everyday moral experience.

How people distinguish between actions that are "right" and "wrong" affects many important aspects of life. Morality science—informed by philosophy, biology, anthropology, and psychology—seeks to understand how the moral sense develops (*1*, *2*), how moral judgments are made (*3*, *4*), how moral experiences differ among individuals, groups, and cultures (*5*–*8*), and what the psychological implications of the morally "good" or "bad" life are (*9*).

Insights from contemporary morality research have mostly been gained through the analysis of moral vignettes, questionnaire data, and thought experiments such as trolley problems (*10*). As important as these approaches are, they are all limited to some extent by the artificial nature of the stimuli used and the non-natural settings in which they are embedded. Despite considerable scientific and practical interest in issues of morality, virtually no research has taken morality science out of these artificial settings and directly asked people about whether and how they think about morality and immorality in the course of their everyday lived experience. Here we present an attempt to capture moral events, experiences, and dynamics as they unfold in people's natural environments.

Using ecological momentary assessment (*11*), we addressed a number of fundamental key issues in scientific and public debates about morality:

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(i) How often do people commit moral and immoral acts in their daily lives? How often are they the targets of moral and immoral acts? How often do they witness moral and immoral acts in their environment, or learn about them through indirect channels such as social media? (ii) What are these moral experiences about? In particular, we examined how well an influential taxonomy of moral dimensions, moral foundations theory [MFT (*12*–*14*)], can account for descriptive content, and whether everyday moral experiences highlight understudied dimensions of morality. (iii) Given the ongoing debate about whether religion is a necessary foundation for morality (*15*, *16*), is there evidence that religious people actually commit more moral or fewer immoral deeds than nonreligious people? And can we replicate evidence for a political morality divide between liberals and conservatives (*6*, *17*)? (iv) What is the empirical connection between morality, momentary happiness, and meaning in life (i.e., sense of purpose)? For instance, does committing moral deeds likewise boost momentary happiness and sense of purpose? (v) Finally, our approach affords the possibility to study the temporal dynamics of morality. For instance, are people more likely to do something good if they have become the targets of a moral deed themselves (moral contagion)? And can we replicate moral self-licensing effects demonstrated in the lab (*18*) in the context of everyday social interaction, whereby committing a prior moral act leads people to relax their moral standards with regard to subsequent behavior?

We recruited a large, demographically and geographically diverse sample (1252 adults aged 18 to 68 years) from the United States and Canada. Each participant was randomly signaled five times daily on his or her smartphone for 3 days between 9 a.m. and 9 p.m. At each assessment, participants indicated whether they committed, were the target of, witnessed, or learned about a moral or immoral act within the past hour (they could also respond "none of the above"). For each moral or immoral event, participants described via text entry what the event was about. They also provided contextual information on the moral event (e.g., location) and completed state measures of nine distinct moral emotions such as guilt and disgust [on a scale from 0 (not at all) to 5 (very much)], momentary happiness ["How happy do you feel at the moment?" from –3 (very unhappy) to +3 (very happy)], and sense of purpose ["Do you feel that your life has a clear sense of purpose at the moment?" from 0 (not at all) to 4 (very much)]. Religiosity and political

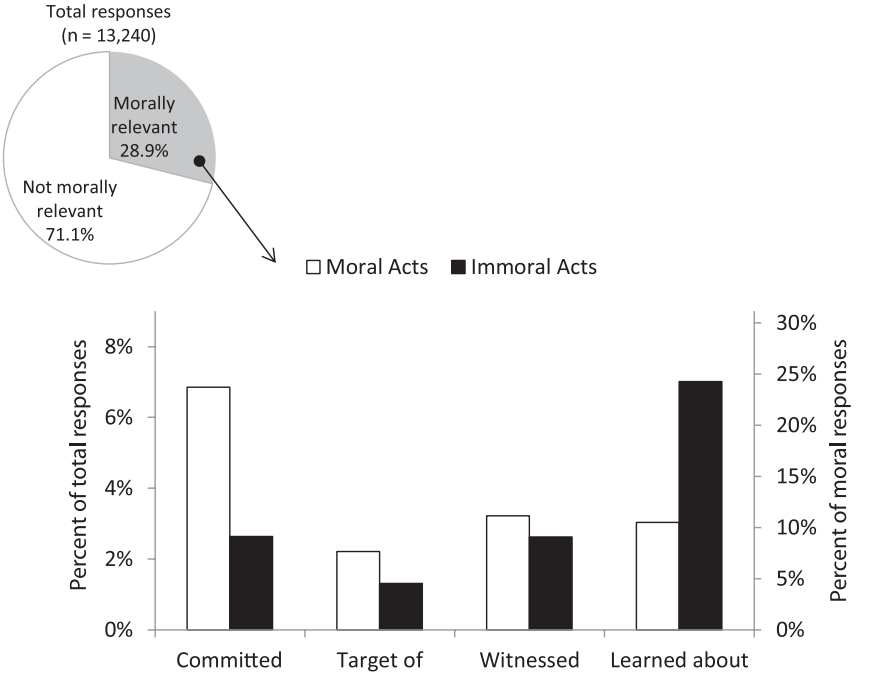

Fig. 1. Overall distribution of responses (pie chart) and of the eight moral event categories (bar graph) in relation to total responses (left axis) and morally relevant responses (right axis). Type of event and moral valence (moral versus immoral) were statistically associated; see main text.

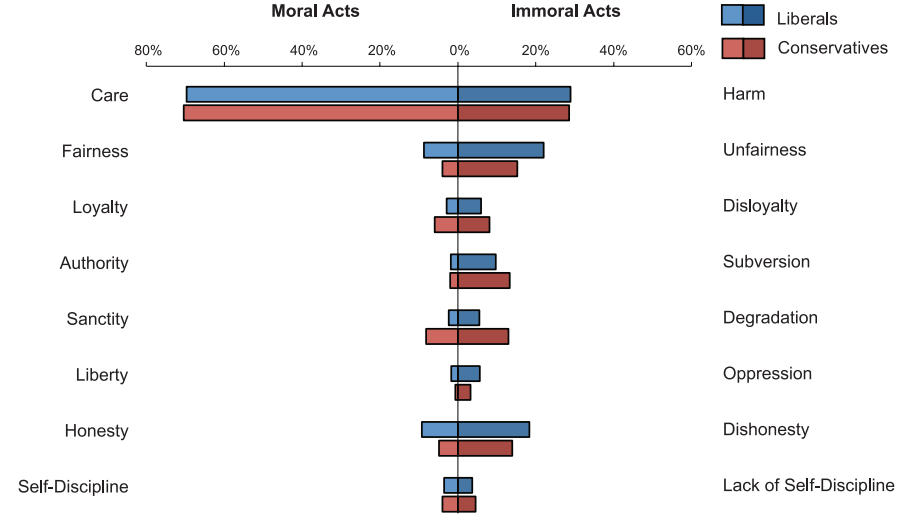

Fig. 2. Moral content and liberal versus conservative political ideology. Percentage distributions of moral and immoral event codings by political ideology (liberals versus conservatives) are shown on moral dimensions. Moral as well as immoral events within each political ideology add up to 100%. Moral content and political ideology were statistically associated; see main text.

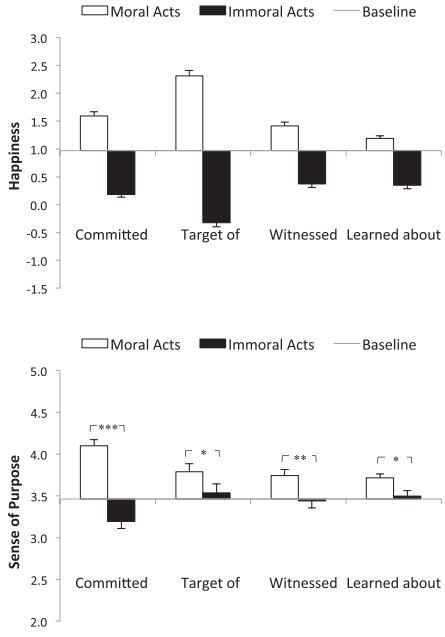

Fig. 3. Morality, happiness, and purpose. Moral acts were associated with relative gains in happiness relative to baseline, immoral acts with relative losses (upper panel). The happiness effect was strongest when people reported being the target of moral/immoral acts. Sense of purpose was most strongly affected by the commission of moral as compared to immoral acts (lower panel). *P < 0.05, **P < 0.01, ***P < 0.001.

ideology were assessed during an intake survey upon study registration.

Participants furnished a total of 13,240 reports (at a median response rate of 80%). On 28.9% of responses (n = 3828), participants reported a moral or immoral event. Moral events (15.3%; n = 2029) and immoral events (13.6%; n = 1799) had similar overall frequencies. As shown in Fig. 1, type of event and moral valence were associated such that people were more likely to report committing or being the target of a moral versus an immoral act, and were more likely to learn about an immoral rather than a moral act [$\chi^2(3) = 483.6$, $P < 0.001$, Cramer's $V = 0.36$]. The finding that people were more than twice as likely to find out about immoral rather than moral acts through personal communication (e.g., gossiping) and other channels (see fig. S1 for more information on this category) fits well with social-psychological theories of the function of gossip and evolutionary theories of reputation management (*19*, *20*). Excluding learned-about acts (for which location data are often unknown), most moral or immoral acts happened in public settings (64.3%), followed by participants' homes (23.4%), the homes of close others such as family and friends (6.5%), and other settings (5.7%). There was no statistical association between location and whether an act was moral or immoral [$\chi^2(3) = 3.3$, $P = 0.343$, Cramer's $V = 0.04$].

Building on MFT (*14*), we reliably classified moral events into five originally proposed core dimensions (Care/Harm, Fairness/Unfairness, Loyalty/Disloyalty, Authority/Subversion, Sanctity/Degradation) as well as a newly proposed Liberty/Oppression dimension (*17*) and two additional categories derived from our data (Honesty/Dishonesty, Self-Discipline/Lack of Self-Discipline) (supplementary materials and table S1). The first five core dimensions accounted for 80.1% of moral events mentioned in daily life (86.1% of moral acts, 73.5% of immoral acts). Care/Harm was by far the most frequently mentioned dimension (50.6%, in particular accounting for a large share of morally good acts; table S2), followed by Fairness/Unfairness (13.9%), Honesty/Dishonesty (12.8%), Authority/Subversion (5.6%), Sanctity/Degradation (5.2%), Loyalty/Disloyalty (4.8%), Self-Discipline/Lack of Self-Discipline (3.8%), and Liberty/Oppression (3.3%). These results confirm MFT's original core dimensions in spontaneously generated participant responses from everyday life, but also suggest additional categories associated with honesty and self-discipline.

Political ideology was reliably associated with moral content [$\chi^2(7) = 81.9$, $P < 0.001$, Cramer's $V = 0.18$]. Descriptive percentage distributions (Fig. 2) show that liberals mentioned events related to Fairness/Unfairness, Liberty/Oppression, and Honesty/Dishonesty more frequently than did conservatives, whereas conservatives mentioned events related to Loyalty/Disloyalty, Authority/Subversion, and Sanctity/Degradation more frequently than did liberals. More focused tests established that these differences were most reliable for Fairness/Unfairness, Loyalty/Disloyalty, Sanctity/Degradation, Liberty/Oppression, and Honesty/Dishonesty, and remained largely stable when controlling for religiosity, which accounted for significant portions of variance on four of the eight dimensions (see table S3). Thus, our everyday-life approach largely corroborates the idea that political ideology relates to different moral emphases, even though real-world effects appear to be more a matter of nuance rather than stark contrast.

Comparing religious and nonreligious participants, there was no discernible difference in the frequency of positive moral experience (both overall and by event; table S4). Thus, we did not find evidence for religious people committing moral acts more frequently than nonreligious people. Religious people reported fewer immoral experiences overall, but this difference was mostly attributable to religious people reporting having learned about immoral acts less often—a possible result of selective exposure—rather than having committed immoral deeds less often than nonreligious people (table S4). Moreover, a second sample of independent judges (N = 249) who were presented with participants' open text descriptions rated moral and immoral deeds committed by religious people as equally right and wrong, respectively (table S5). However, assessing the average emotional "footprints" of these acts revealed that, relative to nonreligious people, religious people experienced more intense self-conscious emotions such as guilt, embarrassment, and disgust in response to the immoral deeds they had committed, and more pride and gratefulness in response to moral deeds (fig. S3). Viewed in concert, these findings suggest that religious and nonreligious people commit comparable moral and immoral deeds and with comparable frequency. However, religious people respond more strongly in psychological terms to the immoral and moral deeds they commit.

Does morality have implications for happiness and sense of purpose? Figure 3 (upper panel) displays momentary happiness levels for the four types of events relative to the baseline level of happiness observed for nonmoral events. Moral acts were associated with higher levels of momentary happiness than immoral acts, as indicated by a large main effect ($F_{1,3441} = 758.4$, $P < 0.001$). This happiness effect was moderated by type of event ($F_{3,3382} = 22.7$, $P < 0.001$) such that the strongest gain and loss in happiness was observed when participants were the targets of moral and immoral acts, respectively (Cohen's $d = 1.34$). Happiness effect sizes were comparatively smaller when committing moral or immoral acts ($d = 0.85$), witnessing them ($d = 0.73$), or learning about them ($d = 0.57$). In contrast, sense of purpose (Fig. 3, lower panel) was most strongly affected by the commission of moral as compared to immoral acts (interaction effect $F_{3,2172} = 5.1$, $P = 0.002$). Hence, whereas benefiting from others' good deeds grants the highest observed levels of momentary happiness among types of events, doing good lends the most purpose to people's lives.

Finally, we investigated whether the likelihood of committing a moral or immoral deed can be predicted by moral events that happened earlier to a person on a given day (table S6). In support for moral contagion, becoming the target of a moral act was associated with an above-average likelihood

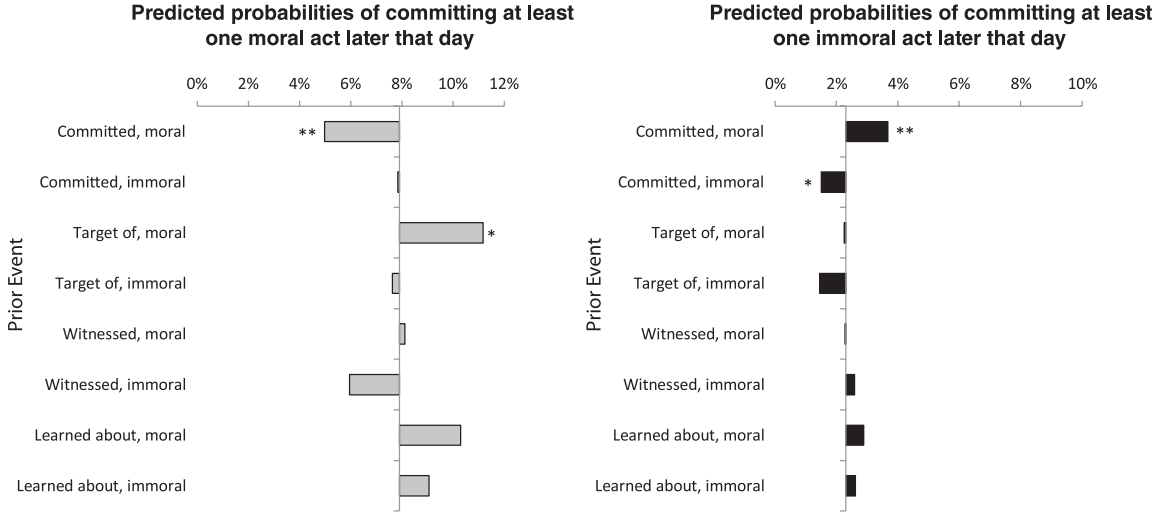

Fig. 4. Moral dynamics. Predicted probabilities with which a prior event is followed by at least one committed moral act (left panel) or immoral act (right panel) on the same day, relative to the average sample probability (vertical axis), are shown. Having been the target of an earlier moral act was associated with an above-average likelihood of subsequent moral behavior. Having committed an earlier moral act was associated with an above-average likelihood of subsequent immoral behavior. *$P < 0.05$, **$P < 0.01$.

of committing a moral act later (Fig. 4). In addition, a moral self-licensing pattern emerged (*18*), such that committing a moral act earlier in the day was associated with an above-average likelihood of a subsequent immoral act and a decreased likelihood of a subsequent moral act (Fig. 4). Together, the analysis of everyday moral dynamics revealed evidence both for moral contagion through other people's good deeds and moral self-licensing through one's own good deeds outside of the laboratory. Given these different mechanisms, it seems important to find out more about how the principles of moral contagion can be used in public policy interventions, and how moral slacking may be prevented.

By tracking people's everyday moral experiences, we corroborated well-controlled but artificial laboratory research, refined prior predictions, and made illuminating discoveries about how people experience and structure morality, as well as about how morality affects people's happiness and sense of purpose. A closer, ecologically valid look at how morality unfolds in people's natural environments may inspire new models and theories about what it means to lead the "good" or "bad" life.

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ACKNOWLEDGMENTS

Supported by a SAGE Award from the Foundation for Personality and Social Psychology and a research grant from the University of Chicago Booth School of Business (W.H.), where portions of this work were completed. The study was approved by the institutional review board of the University of Chicago. W.H. is a co-founder of SurveySignal, a University spin-off of the Web-based experience-sampling software used for this project, and has been advising the development of SurveySignal on an unpaid basis. The replication data are available at the Harvard Dataverse Network (doi:10.7910/DVN/26910).

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S3
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References (*21*–*27*)

30 January 2014; accepted 5 August 2014
10.1126/science.1251560

BIODIVERSITY LOSS

Loss of avian phylogenetic diversity in neotropical agricultural systems

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Habitat conversion is the primary driver of biodiversity loss, yet little is known about how it is restructuring the tree of life by favoring some lineages over others. We combined a complete avian phylogeny with 12 years of Costa Rican bird surveys (118,127 detections across 487 species) sampled in three land uses: forest reserves, diversified agricultural systems, and intensive monocultures. Diversified agricultural systems supported 600 million more years of evolutionary history than intensive monocultures but 300 million fewer years than forests. Compared with species with many extant relatives, evolutionarily distinct species were extirpated at higher rates in both diversified and intensive agricultural systems. Forests are therefore essential for maintaining diversity across the tree of life, but diversified agricultural systems may help buffer against extreme loss of phylogenetic diversity.

As human-converted habitats expand over Earth's surface, the fate of global biodiversity will depend increasingly on the quality and characteristics of farming landscapes (*1*, *2*). Agricultural systems vary widely in their ability to support biodiversity, with many species extirpated from some but sustained in others (*1*, *3*). Additionally, characteristics of the species themselves, evolved over millions of years, may predispose some lineages to benefit (or suffer) from human environmental impacts (*4*–*6*).

Phylogenetic diversity, the total evolutionary history or phylogenetic branch lengths of all species in a community (*7*), is recognized as having intrinsic conservation value (*8*, *9*). Also, ecological experiments in small plots indicate that communities with more phylogenetic diversity are more stable (*10*), possess higher productivity (*11*), and support more species at other trophic levels (*12*). Despite the known impact of agriculture on species loss, how habitat conversion affects phylogenetic diversity remains unknown. Studies of plants and invertebrates have established that local environmental disturbances (e.g., lake acidification and species invasion) favor subsets of closely related clades and often result in phylogenetic diversity loss (*13*–*15*). Further, some studies that examine the global extinction risk of birds and mammals suggest that particular branches of the tree of life are at greater risk than others (*5*, *6*, *16*), although whether evolutionarily distinct species are more at risk than species with many living relatives remains contested (*6*, *16*, *17*).

We quantified changes in phylogenetic diversity across multiple landscapes in Costa Rica, combining a recent complete avian phylogeny (*18*) with temporally and spatially extensive tropical bird censuses to assess how habitat conversion is restructuring the avian phylogeny (*19*). The data set comprised 44 transects, surveyed in wet and dry seasons over 12 years (2001 to 2012) across four regions in two biomes (fig. S1). Transects were located in three land-use types: forest reserves, diversified agricultural systems, and intensive monocultures. Compared with intensive monocultures, diversified agricultural systems had more crop types, complex configurations of vegetation, and substantial surrounding tree cover (*1*) (table S1). Our analysis focused on three unresolved questions. First, do certain bird clades thrive in agriculture, or is this capacity broadly distributed across the tree of life? Second, how much phylogenetic diversity is lost when native forest is replaced with agriculture? Last, are evolutionarily distinct species capable of persisting in agriculture?

We found that clades from across the bird phylogeny thrived in agriculture (Fig. 1). Affinity for different habitats showed phylogenetic signal, meaning that closely related species were more likely to share habitat preferences than species that were distantly related (table S2) (*20*). The phylogenetic signal was best described by using Pagel's lambda transformation of the phylogeny (*21*), which reduces the degree of correlation of traits between species below the Brownian motion expectation (across habitat types and seasons, $\lambda = 0.25$ to 0.48; table S3). Although most taxonomic families had species associated with all habitat types, some families tended to affiliate with particular habitats. For example, pigeons, seedeaters, swallows, and blackbirds were

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agriculture-affiliated, whereas trogons, antbirds, ovenbirds, and manakins were forest-affiliated (Fig. 1 and table S4).

Despite the variety of lineages that were found in agriculture, average within transect phylogenetic diversity was 40% lower in intensive monocultures than in forest reserves and 15% lower in diversified agricultural systems than in forest reserves (Fig. 2A and table S5). Across all transects and years, forest reserves, diversified agricultural systems, and intensive monocultures housed 4.10, 3.85, and 3.26 billion years of evolutionary history. Two processes were responsible for changes in phylogenetic diversity: species loss and increasing species relatedness. We found roughly the same number of bird species in diversified agricultural systems (N = 59 species) as in forest reserves [N = 62 species; likelihood ratio test (LRT) P = 0.75] but half as many species in intensive monocultures (N = 29 species; LRT P < 0.001; Fig. 2B and table S5).

However, species loss alone did not account for declining phylogenetic diversity in agriculture (fig. S2). Species in forest reserves were less related to one another than expected by chance, whereas species in diversified agricultural systems and intensive monocultures were more closely related (Fig. 2C, fig. S3, and table S5). These patterns indicate that phylogenetic diversity loss in agriculture occurs in two steps. First, habitat conversion from forest to diversified agricultural systems causes a shift in community composition while maintaining species richness: Agricultural species are not nested subsets of forest species (fig. S4). Because species in diversified agricultural communities are closely related, this shift results in a moderate decline in phylogenetic diversity within a transect. Then, as agricultural practices intensify, species loss from this agricultural bird community causes another more-substantial decline in phylogenetic diversity.

Whether phylogenetic diversity loss will substantially reshape the global tree of life depends on the capability of species from evolutionarily distinct lineages to persist in agricultural lands. We quantified each species' evolutionary distinctness (fair proportion metric) as its contribution to the phylogenetic diversity of the world's 9993 bird species (*17*). Species in forest reserves had slightly greater average evolutionary distinctness than those in diversified agricultural systems (LRT P < 0.001) or intensive monocultures (LRT P < 0.001; Fig. 2D, fig. S5, and table S5). This pattern did not result from a small number of highly distinct, forest affiliated species—repeating the analysis after removing the top 10% most distinct species did not alter results (table S5). Conversely, communities in intensive monocultures hosted younger species with more-rapid diversification rates (DRs) (*18*) (LRT P < 0.001; fig. S6 and table S5). At the species level after accounting for phylogenetic covariance, DR was negatively correlated with forest affiliation and positively correlated with affinity for diversified agricultural systems (table S6).

To explore how habitat conversion affects the temporal population dynamics and local extirpation risks of evolutionarily distinct species, we developed a temporal, multispecies, hierarchical occupancy model that accounted for detection bias (fig. S7). The model provided a dynamic assessment of which species were extirpated from and/or recolonized sites at the greatest rates from year to year (*22*). Extirpation was estimated as the probability that a species did not persist from one year to the next, whereas colonization was the probability that a species was absent one year but present the next. We modeled occupancy dynamics

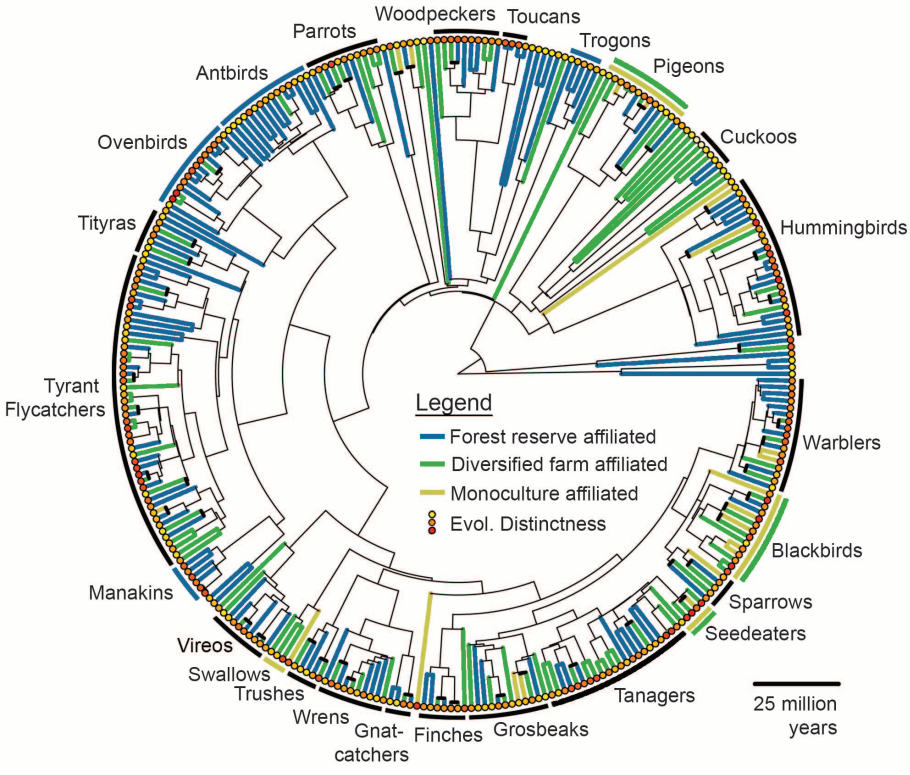

Fig. 1. Agricultural affiliation is distributed across the avian phylogeny. Tips represent the 308 species detected more than 25 times. Terminal branches are colored by primary habitat affiliation. Families with more than four species present are labeled, grouped by arcs, and colored if more affiliated with a particular habitat type than expected by chance (randomization test P < 0.05). A species' evolutionary distinctness is indicated by a colored dot at the branch tip (yellow, high evolutionary distinctness; red, low evolutionary distinctness).

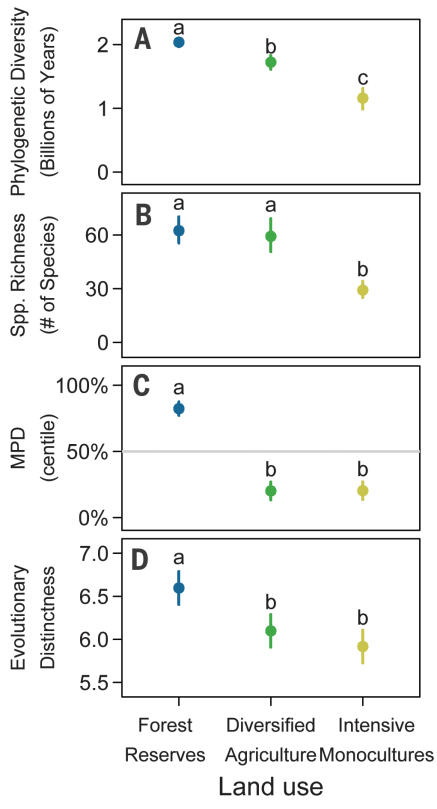

Fig. 2. Habitat conversion causes phylogenetic diversity declines. (**A**) Diversified agriculture maintains intermediate phylogenetic diversity, measured as the total evolutionary history present at a site. Loss of phylogenetic diversity in agriculture was caused by two processes: (**B**) loss of species richness and (**C**) increasing relatedness among species in agricultural communities. In (C), larger values indicate greater mean phylogenetic distance (MPD; i.e., overdispersion and low relatedness), whereas smaller values indicate communities contain more close relatives (i.e., phylogenetic clustering). (**D**) The median evolutionary distinctness of the community was higher in forests than in agricultural habitats. Points depict estimated mean ± SEM from generalized linear mixed models (table S5). Different letters denote significant differences between groups (LRT P < 0.05). N_{total} = 528 ($N_{transects}$ = 44, N_{years} = 12).

over 12 years, validating our model through examining dry and wet season surveys separately.

We found that extirpation probability was highest in intensive monocultures and lowest in forest reserves (Fig. 3). More evolutionarily distinct species experienced higher extirpation rates than less-distinct species in both diversified agricultural systems and intensive monocultures. Evolutionarily distinct species fared worst in intensive monocultures, where the top 10% most-distinct species experienced extirpation rates ~two times greater than in diversified agricultural systems. Between-year colonization probabilities were low in all land-use types, but evolutionarily distinct species colonized both diversified agricultural systems and intensive monocultures less frequently than less-distinct species (fig. S8). Repeating analyses at the genus level confirmed that the results were not driven by a few clades. These findings suggest that, over time, evolutionarily distinct species will face challenges in maintaining populations in agricultural systems, especially in intensive monocultures.

We offer two possible explanations for why evolutionarily distinct species and phylogenetic diversity should decline in agriculture. First, species that today inhabit tropical agriculture may have evolved primarily in open habitats, such as grasslands. During geologically recent periods of glaciation when open grassland habitats in the Neotropics proliferated (*23*), several clades may have undergone increased speciation (or experienced less extinction), leading to the enrichment of younger species in agriculture (*24*, *25*). Indeed, we found that species that use natural open habitats (e.g., shrub-brush, savannas, or along waterways) were more likely to thrive in agriculture (table S6). However, whereas species associated with savannas had slightly higher diversification rates than other species (N_{spp} = 308, LRT $P < 0.05$), there were no consistent differences in diversification rates between species that use natural open habitats and those that do not (table S6).

Another explanation is that birds in agriculture represent a novel community. In comparison to forest, agricultural habitats are less stable (*26*) and more regularly disturbed (e.g., pruned, harvested, and replanted). These attributes could preclude sustained competition (*27*) and favor related species with traits that allow them to persist in agriculture's novel and variable conditions (Fig. 2C) (*14*, *15*). Previous work with the same data set demonstrated that bird functional diversity is lower in intensive monocultures than in forest reserves or diversified agricultural systems, suggesting that agriculture can act as an ecological filter (*1*). We further explored this idea by analyzing whether land use affects the distribution of several functional traits thought to regulate bird responses to environmental disturbances (*28*). Indeed, granivores and birds with wider diet breadths and larger clutch sizes had higher colonization rates in agriculture (figs. S9 and S10). Additionally, compared with smaller species, larger birds experienced higher extirpation rates in intensive monocultures but lower extirpation rates in diversified agricultural systems (fig. S9).

Our results suggest that both agricultural expansion and intensification threaten evolutionarily distinct species, aligning with earlier findings of heightened global endangerment among birds from basal lineages (*6*). In contrast, species from recently diversifying clades appear best able to exploit agricultural habitats and may thus benefit from ongoing agricultural expansion. The persistence of some species from younger lineages cannot prevent the species losses, and concomitant declines in phylogenetic diversity, that accompany agricultural intensification.

Ultimately, protected areas are essential for preserving evolutionary history. Yet in the absence of a much-expanded global reserve system, prioritizing diversified agricultural systems over intensive monocultures, especially surrounding reserves, provides a strategy for enhancing the conservation value of human-modified landscapes. About the same number of species persisted in diversified agricultural systems as in forest reserves, and, as a result, diversified agricultural systems maintained 1.5 times the phylogenetic diversity of intensive monocultures. Shepherding biodiversity through the human pressures of the 21st century will require a shared vision for conservation and agriculture, one that simultaneously preserves species and ecosystem functions while also enhancing food production and human well-being.

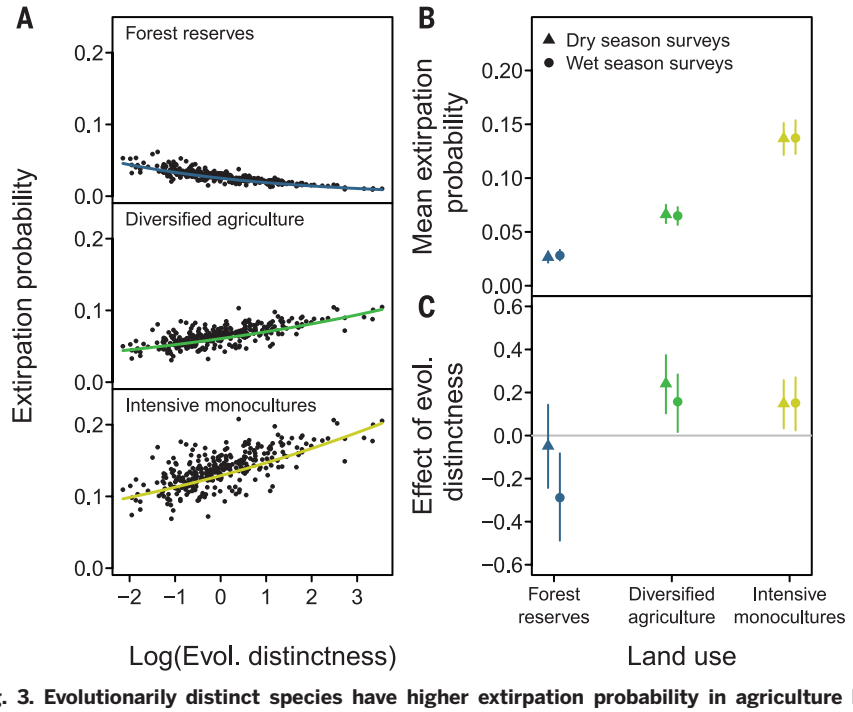

Fig. 3. Evolutionarily distinct species have higher extirpation probability in agriculture but not in forest. (**A**) Species-specific extirpation probabilities as a function of evolutionary distinctness (standardized and on a log scale) from wet season surveys in each of the three land uses. (**B**) Average extirpation probabilities across species (means from the left panels) for both wet and dry seasons. (**C**) How evolutionary distinctness modifies the effect of habitat type on the extirpation probability of a species [slopes from (A)]. For (B) and (C), points indicate mean ± 95% Bayesian credible interval. N_{spp} = 308, $N_{transects}$ = 44, N_{years} = 12, $N_{visits/season}$ = 3.

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ACKNOWLEDGMENTS

We thank the Organization for Tropical Studies and Los Cusingos Bird Sanctuary for facilitating collection of field data and P. Ehrlich for comments on this manuscript. This work was supported by the Ward Wilson Woods Jr. Environmental Studies Fund and an NSF Doctoral Dissertation Improvement Grant to L.O.F., a NatureNet fellowship from the Nature Conservancy to D.S.K., an Natural Sciences and Engineering Research Council postdoctoral fellowship to L.K.M., and a Bing Ecology and Conservation Graduate Fellowship to C.D.M. Data collection was made possible by donors to the Center for Conservation Biology and grants to G.C.D. from the Heinz, Moore Family, and Winslow foundations and the LuEsther T. Mertz and the Pew Charitable trusts. Original data are available at Dryad (10.5061/dryad.fv921).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1343/suppl/DC1
Materials and Methods
Tables S1 to S6
Figs. S1 to S10
References (*29*–*31*)

9 April 2014; accepted 29 July 2014
10.1126/science.1254610

MICROBIAL ECOLOGY

Biogeographic patterns in ocean microbes emerge in a neutral agent-based model

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A key question in ecology and evolution is the relative role of natural selection and neutral evolution in producing biogeographic patterns. We quantify the role of neutral processes by simulating division, mutation, and death of 100,000 individual marine bacteria cells with full 1 million–base-pair genomes in a global surface ocean circulation model. The model is run for up to 100,000 years and output is analyzed using BLAST (Basic Local Alignment Search Tool) alignment and metagenomics fragment recruitment. Simulations show the production and maintenance of biogeographic patterns, characterized by distinct provinces subject to mixing and periodic takeovers by neighbors (coalescence), after which neutral evolution reestablishes the province and the patterns reorganize. The emergent patterns are substantial (e.g., down to 99.5% DNA identity between North and Central Pacific provinces) and suggest that microbes evolve faster than ocean currents can disperse them. This approach can also be used to explore environmental selection.

An important ongoing endeavor in ecology and evolution is to understand the mechanisms underlying the geographic distribution patterns of organisms. One mechanism that can create such patterns is natural selection by contemporary environmental factors acting on adaptive mutations or a persistent seed bank of species. Neutral evolution (selectively neutral mutations and genetic drift) coupled with dispersal limitation or isolation is another mechanism (*1*–*6*). These processes are not mutually exclusive, and for microbes in the global surface ocean, molecular observations [e.g., shotgun sequencing (*7*)] provide support for the role of both mechanisms (*8*–*11*).

How does neutral evolution influence the biogeographic distribution of surface ocean microbes? To what extent does dispersion allow for different operational taxonomic units (OTUs) to develop and persist? Are there emerging spatial patterns [e.g., provinces (*12*)], and how do these change in time?

Several approaches are available to quantify the contribution of the various processes in generating and maintaining biogeographic patterns among ocean microbes (*2*). A common empirical approach involves correlating observations (e.g., microbial composition) with environmental variables, subtracting out this environment effect, and then correlating with geographic distance. In the ocean, hydrodynamic models coupled with tracers (either Eulerian concentration or Lagrangian particles) can be used as a measure

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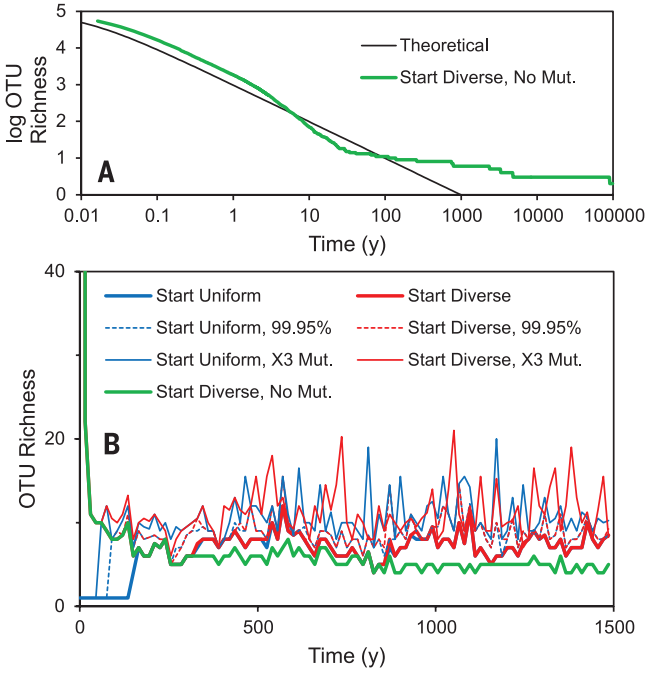

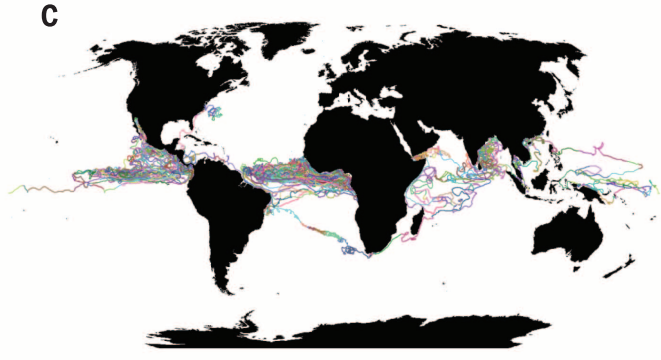

Fig. 1. Diversity (OTU richness) in global surface ocean microbes predicted by a neutral agent-based model. Results from several simulations are presented: "Start Diverse" indicates that all initial cells have an individual, completely random genome, whereas "Start Uniform" denotes that all initial cells have the same, completely random genome. "No Mut." and "X3 Mut." represent zero and three times higher mutation rates, respectively. "99.95%" is representative of a 99.95% cutoff (versus 99.9% used in other analyses). "Theoretical" denotes a model based on neutral theory, not considering dispersal limitation (*31*). (**A** and **B**) OTU richness over time. Note the *x*- and *y*-axis scales. Start diverse (red) and start uniform (blue) lines overlap after 200 years. (**C**) Life history of an individual cell isolated near Bermuda at 1000 years. Color changes demarcate mutation events. This cell was initialized with the *P. ubique* HTCC1062 genome (*34*).

of connectivity to supplement empirical relations (*10*, *13*). A problem with this approach is that one can never be sure that the distance effect is not actually caused by an unmeasured environmental variable. Mechanistic models constitute an alternative approach. Eulerian models with coupled phytoplankton ecology and biogeochemistry can simulate biogeographic patterns produced by environmental selection (*14*). However, the Eulerian approach generally assumes all species are present everywhere and does not consider dispersal limitation.

An alternative approach is to use a neutral model, where environmental selection is excluded. Such a model can function as a null hypothesis, where deviation from observed patterns can help quantify the effect of the omitted factors (e.g., selection) (*6*, *15*, *16*). A neutral model can also be used to directly predict biogeographic patterns resulting from neutral processes. Of course, this is only meaningful if the model parameters are sufficiently constrained. For bacteria in the open ocean, hydrodynamic transport is relatively well understood, and rates of growth and base pair mutation are available (*17*–*19*), allowing us to make meaningful predictions without prior calibration [e.g., dispersal parameter (*15*)].

We model individual bacteria using an agent-based (i.e., Lagrangian- or individual-based) approach (*20*–*24*). The large number of ocean microbes—one estimate is 3.6×10^{28} prokaryotes in the surface ocean (*25*)—makes simulating all individuals impossible, so we model a smaller number of representative cells, referred to as "super-individuals" (*21*, *24*). This prohibits us from explicitly simulating absolute diversity or species richness. However, biogeographic distribution, quantified as nucleotide divergence between cells at different locations (total nucleotide difference of cells from different locations minus local nucleotide diversity) (*26*), is independent of population size. This is supported by theory (*27*) and an application of our full model to a simple two-box system (*28*), and it can be understood as follows: Consider two locations (provinces) with equal-sized populations and limited mixing. Cells continuously migrate between these provinces, and periodically, one succeeds to take over the recipient province (coalescence). At that time, the nucleotide divergence between the subpopulations is eliminated and then it increases in a linear, clockwise manner that is independent of the population size until the next coalescence event. Thus, the frequency of coalescence events determines the average divergence. The rate at which cells migrate between provinces is proportional to the population size, but the probability of a migrant cell taking over the population is inversely proportional to the population size. As a result, the frequency of coalescence (migration and takeover) is independent of the population size. Despite the large scaling in population size (model versus reality), the super-individual approach should produce meaningful estimates of nucleotide divergence.

Our model simulates a population of individual cells. We use a logistic approach in which cells die at a constant rate and divide at a rate that depends on the local cell concentration. Death and division are stochastic. As the local cell density approaches a specified carrying capacity, the growth rate slows down. The parameters were adjusted to produce reasonable growth rates [0.14 day^{-1} (*29*)] and a population of ~100,000 cells distributed at a relatively constant density. Cells have a 1 million–base-pair (Mbp) genome (i.e., an array of A, T, C, and G letters) subject to mutation, where the mutation rate was increased from the point mutation rate [i.e., mutation rate $m = 5.4 \times 10^{-10}$ base pairs per division for *Escherichia coli* (*17*)] to account for base pair changes by recombination [$r/m = 63.1$ (where r is the recombination rate) for *Pelagibacter ubique* (*18*, *19*), $m + r = 3.5 \times 10^{-8}$ base pairs per division]. Cells are transported in a Lagrangian manner horizontally within the surface layer using a 1/10° eddy-resolving circulation model, spanning the oceans from 75°S to 75°N (*30*), with cycling of a 31-year period to allow for simulations up to 100,000 years (*28*).

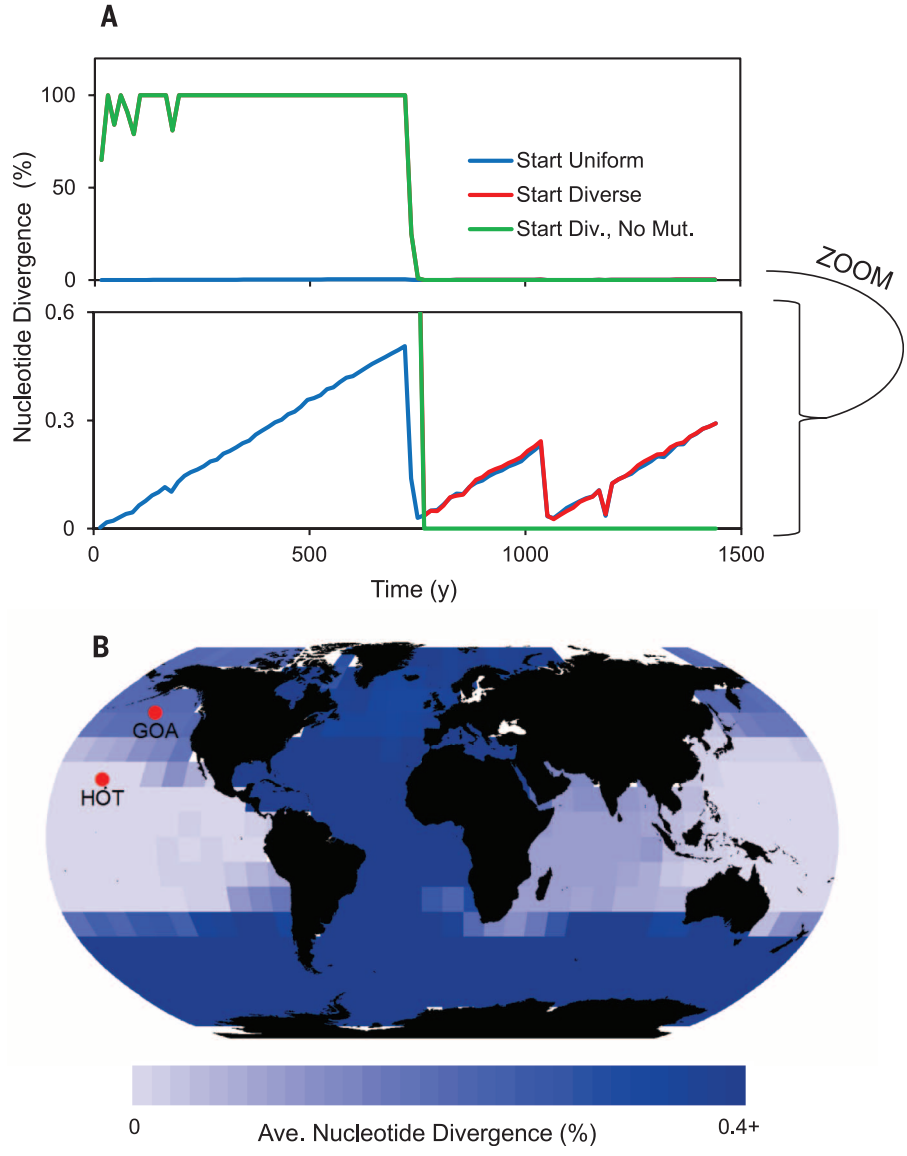

Fig. 2. Biogeographic pattern (nucleotide divergence) in global surface ocean microbes predicted by a neutral agent-based model and quantified by genome alignment. (**A**) Cells from Hawaii (HOT) compared with cells from the Gulf of Alaska (GOA) over time. See (B) for locations. See the Fig. 1 legend for a description of the simulations. SCGs collected at the Gulf of Alaska (n = 10) were aligned against SCGs collected at Hawaii (n = 10). 1-Mbp genome size. Start diverse with no mutation (green) and start diverse (red) lines overlap up to 700 years. Start diverse (red) and start uniform (blue) lines overlap after 700 years. (**B**) Cells from Hawaii compared with cells from all locations. SCGs collected on a 10°-by-10° grid (n = 5 at each box) were aligned to SCGs from Hawaii (n = 5). Genome size: 100,000 base pairs. "Start Uniform" simulation, where all initial cells have the same, completely random genome, is shown. Average of nucleotide divergence over 1500 years is shown.

We first consider the diversity in the model, which is not a realistic estimate of the actual diversity in the surface ocean microbe population because we simulate super-individuals, but it illustrates the behavior of model. When the model starts with a diverse population (that is, each cell has a different genome) and no mutation, diversity decreases monotonically as OTUs are lost by extinction and not gained by mutation (Fig. 1A). After ~100 years, the population consists of ~10 OTUs, resident in relatively distinct spatial regions, and the rate of OTU loss becomes limited by dispersal between these provinces (*28*). At that time, the model starts to predict higher OTU richness than a neutral theory model that does not consider dispersal limitation (*31*). The rate of OTU loss becomes low, but the populations continue to mix (*28*) and the probability of extinction remains greater than zero. At 100,000 years, the model includes two OTUs in the Southern Ocean and everywhere else. The model should eventually reduce to one OTU, although this is not realized in the 100,000-year simulation.

When the model is initialized with a diverse population and includes mutation, it also exhibits an initial rapid loss in diversity but then levels off at an OTU richness slightly higher than the simulation without mutation (Fig. 1B). For these simulations, we determined the diversity from a sample of the population (100 cells) by performing pairwise whole-genome BLAST (Basic Local Alignment Search Tool) alignment, identifying OTUs using 99.9% whole-genome identity cutoff and then up-scaling to the true richness (in the model) using Chao1, a nonparametric species estimator that extrapolates from the sample data to "true" richness (see supplementary materials and methods). The OTU richness is variable over time because of stochastic transport and sampling (see fig. S4), but that is identical for all simulations. Therefore, the difference in OTU richness between the simulations with and without mutation can be attributed solely to mutation. The difference is relatively small but increases with higher taxonomic resolution (99.95% cutoff) or mutation rate (×3). For a simulation starting with a uniform population (all cells have the same genome) and including mutation, the OTU richness starts at one and then increases once sufficient mutations accumulate to exceed the OTU threshold. After ~200 years, the simulations starting diverse and uniform converge. At that time, model has reached a dynamic steady state where the rate of OTU loss by extinction is balanced by the rate of OTU gain by mutation. From a practical perspective, this shows that specifying different initial conditions or running the model any longer would not change the diversity.

The model is then used to explore the role of neutral evolution in producing biogeographic patterns. As an example, we compared the genomes of cells from Hawaii and the Gulf of Alaska (Fig. 2B). For the simulation starting diverse without mutation, the difference (nucleotide divergence) is 100% until ~700 years when it abruptly decreases to 0% (Fig. 2A). This is caused by a takeover of the Central Pacific province by a cell from the North Pacific province or a coalescence of these two subpopulations (*27*) (see also movie S1 around 700 years). The simulation starting diverse with mutation also starts at 100% and decreases at the coalescence event, but then increases again as the two subpopulations diverge. The simulation starting uniform initially has 0% difference but immediately starts to increase and then converges with the simulation starting diverse and including mutation. Coalescence events are stochastic (see also fig. S4B), and we observed two such events over the 1500-year simulation period. There are also occasional abrupt drops in nucleotide divergence, which are due to vagrant cells that enter a province but do not establish. The magnitude of nucleotide divergence is a function of the growth and mutation rates (figs. S5 and S6).

The time between coalescence events puts a limit to how much two provinces can diverge, and in this case, the model predicts up to 0.5% difference (99.5% identity). These results can be related to observations. If two cells are sampled from these two locations and their genomes are sequenced and compared, 0 to 0.5% of the observed difference can be attributed to neutral processes. This level of divergence is substantial but considerably lower than what is commonly considered a species (>95% identity). We map out the biogeographic pattern produced by neutral evolution for Hawaii compared with all locations across the globe (Fig. 2B). The model predicts that nucleotide divergence generally increases with distance from Hawaii. However, the divergence is larger for the North Pacific than the Indian Ocean, so distance and/or the presence of landmasses are not necessarily good proxies of dispersal barriers. We also compile this information for all locations into an atlas of neutral biogeography (table S1).

We mapped out the biogeographic pattern produced by neutral evolution using fragment recruitment (*7*), which is akin to in silico DNA hybridization. Specifically, we took the single-cell genomes (SCGs) of the OTUs that were maintained in the simulation starting uniform at 1400 years (see Fig. 1B) and recruited fragments collected on a 10°-by-10° grid. We assigned each grid box to the highest-recruiting SCG (i.e., the dominant OTU) and colored them accordingly, illustrating the provinces produced by neutral evolution and maintained by dispersal limitation

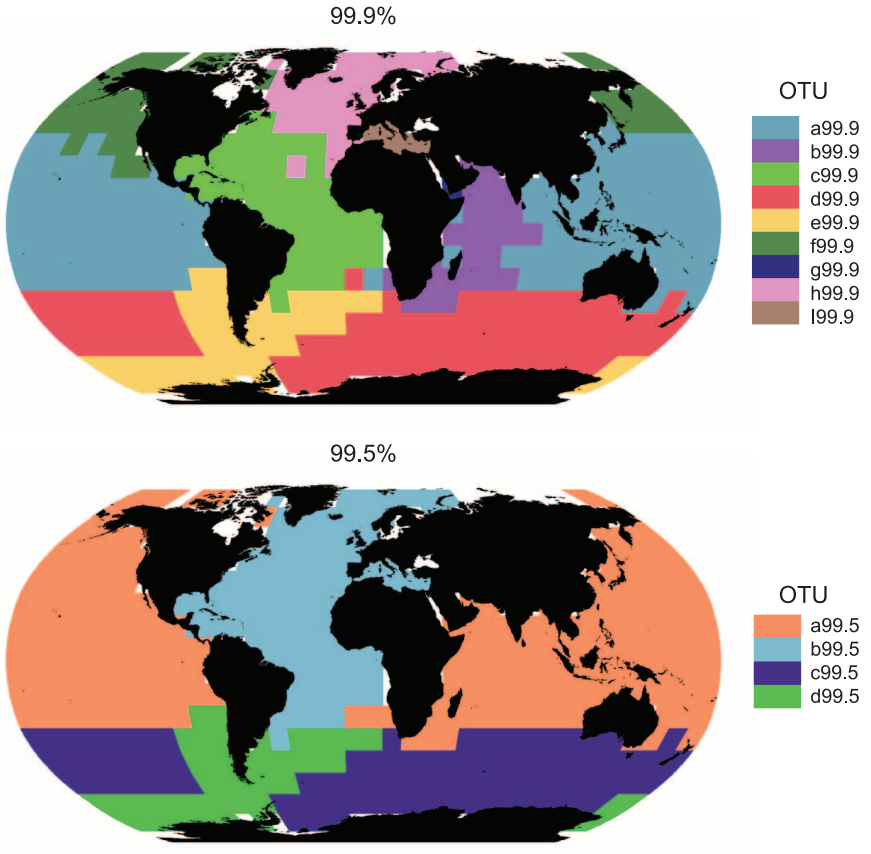

Fig. 3. Biogeographic patterns (OTU provinces) in global surface ocean microbes predicted by a neutral agent-based model and quantified by metagenomics fragment recruitment. Alignment of fragments collected on a 10°-by-10° grid (number of samples n = 10,000 at each box, fragment length l = 1000 base pairs) with SCGs from OTUs remaining at 1400 years (see Fig. 1B). (**Top**) 99.9% and (**bottom**) 99.5% BLAST identity. "Start Uniform" simulation, where all initial cells have the same, completely random genome, is shown. The colors demarcate areas with common dominant OTUs.

(Fig. 3). The resulting map is a function of the taxonomic cutoff used. At less than 99%, the model does not show any diversity or biogeographic patterns. This is expected because over 1400 years the genomes evolve by only 0.5%, corresponding to a 99% identity. However, at this time there are still provinces that have not yet experienced a takeover or coalescence event (Fig. 1A), and those are expected to continue to diverge beyond the 99% threshold for longer times. This distribution can change temporarily as a result of takeover events. For example, at times the Central Pacific and North Pacific provinces were distinguishable at 99.5% (0.5% difference, see Fig. 2A).

We conclude that neutral evolution (neutral mutations and genetic drift) coupled with dispersal limitation can produce substantial biogeographic patterns in the global surface ocean microbe population. Microbes evolve faster than the ocean circulation can disperse them, a feature that can also be seen in molecular observations (*10*). The patterns are dynamic. Provinces gradually emerge as subpopulations diverge by neutral evolution and periodically collapse due to coalescence. Neutral processes, along with environmental selection, must be considered in future research on microbial biogeography, and our results provide a quantitative benchmark for their potential role. Our results conflict with the notion that "everything is everywhere" (*11*, *32*) and may have important implications for how the oceans will respond to global change. Our model provides insights into the role of neutral evolution in shaping biogeographic patterns. The biology in the model is relatively simple, and future work may build on this by considering more spatial and temporal patterns (e.g., carrying capacity, as well as division and death rates based on ocean productivity) and more explicit representation of processes (e.g., recombination). Our modeling approach can theoretically be used to explore environmental selection as well. This will require relating genes to function, which is difficult but can be done for select genes or at the genome level (*23*, *33*).

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ACKNOWLEDGMENTS

We thank R. Stepanauskas for interesting discussion that inspired this research. Three anonymous reviewers provided constructive criticism. F.L.H. and N.D.F. are supported by grants from the NSF and National Oceanic and Atmospheric Administration Massachusetts Institute of Technology SeaGrant. E.v.S. is supported by ARC via grant DE130101336. The source code is available at www.coe.neu.edu/~ferdi/Code/.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1346/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S9
Table S1
References (*35*–*43*)
Movie S1

4 April 2014; accepted 1 August 2014
10.1126/science.1254421

ERROR MEMORY

A memory of errors in sensorimotor learning

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The current view of motor learning suggests that when we revisit a task, the brain recalls the motor commands it previously learned. In this view, motor memory is a memory of motor commands, acquired through trial-and-error and reinforcement. Here we show that the brain controls how much it is willing to learn from the current error through a principled mechanism that depends on the history of past errors. This suggests that the brain stores a previously unknown form of memory, a memory of errors. A mathematical formulation of this idea provides insights into a host of puzzling experimental data, including savings and meta-learning, demonstrating that when we are better at a motor task, it is partly because the brain recognizes the errors it experienced before.

How does the brain alter behavior after experiencing an error? Classic theories assumed that the brain learns some fraction of the error regardless of its history or magnitude (*1*, *2*). However, recent experiments (*3*–*6*) demonstrate that the brain learns relatively more from small errors than large errors, and can modulate its error sensitivity (*7*–*9*).

Understanding error sensitivity is important because it may provide insight into the phenomena of "savings" and "meta-learning." Savings refers to the observation that when a person adapts to perturbation (A), and then the perturbation is removed (i.e., washout), they exhibit faster readaptation to (A) (*10*). Remarkably, savings of (A) is present even when washout is followed by adaptation to (-A), a perturbation in the opposite direction (*11*, *12*). Current error-dependent models of learning cannot account for these observations (*13*, *14*), nor explain meta-learning, where prior exposure to a random perturbation produces savings (*15*, *16*).

We begin with a standard model of motor learning (*17*–*20*) in which on trial n, a perturbation x is imposed on action u so that the sensory consequences are $y^{(n)} = u^{(n)} + x^{(n)}$. Based on their belief about the environment $\hat{x}^{(n)}$, the learner predicts the sensory consequences $\hat{y}^{(n)} = u^{(n)} + \hat{x}^{(n)}$, and then updates his or her belief from the prediction error $e^{(n)} = y^{(n)} - \hat{y}^{(n)}$. Such learning typically depends on a decay factor a, and error sensitivity η

$$\hat{x}^{(n+1)} = a\hat{x}^{(n)} + \eta^{(n)}e^{(n)} \quad (1)$$

Consider an environment in which the perturbations persist from trial to trial, and another

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environment in which the perturbations switch (Fig. 1A). In a slowly switching environment, the brain should learn from error because the perturbations are likely to persist (learning from error in one trial will improve performance on the subsequent trial). However, in a rapidly switching environment, the brain should suppress learning from error because any learning will be detrimental to performance on subsequent trials.

Three groups of volunteers (n = 9 per group) made reaching movements while experiencing force perturbations from either a slow, medium, or rapidly switching environment (Fig. 1A). The mean of the perturbations was zero for all blocks (consisting of 30 trials). We measured error in a given trial and then computed the amount that was learned from that error (probe trials, purple bars, Fig. 1A). To quantify learning from error on trial n, we measured the change in force from the trial before to the trial after the perturbation, $f^{(n+1)}(t) - f^{(n-1)}(t)$ (Fig. 1C). In block 1, learning from error was similar in the three groups ($P > 0.99$), and in all probe trials the perturbation produced similar errors [Fig. 1B; repeated measure–analysis of variance (RM-ANOVA), effect of group $P > 0.8$, interaction, $P > 0.7$]. However, individuals who experienced the slowly switching environment increased their learning from error (Fig. 1C), whereas those who experienced the rapidly switching environment suppressed this learning.

We measured the force produced on a given trial and computed a coefficient representing percent ideal (Fig. 1D). RM-ANOVA indicated a significant block by group interaction ($P < 0.05$), suggesting that the history of perturbations altered the amount of learning from error. Posthoc tests showed that in the slowly switching environment, participants learned more from error than in the rapidly switching environment ($P < 0.03$). This change in error sensitivity developed gradually with training (Fig. 1D). The slowly switching environment induced an increase in error sensitivity (Fig. 1E; changes in sensitivity from the first half to second half of the experiment, ANOVA, $P < 0.05$).

Is control of error sensitivity local to the experienced errors? In experiment 2, participants performed rapid out-and-back movements for which no visual feedback was available during the outward part of the reach, with the aim of hitting a target at the turn-around point of their movement. An occasional perturbation altered the feedback regarding hand position at the turn-around point (Fig. 2A). We measured the relation between error $e^{(n)}$ and learning from error (change in reach extent).

Group 1 (n = 10) experienced a perturbation schedule that transitioned from slow, medium, to rapid switching (Fig. 2B), whereas group 2 (n = 10) experienced the reverse. In group 1, error sensitivity decreased, whereas in group 2, error sensitivity increased (Fig. 2C). We measured the mean error sensitivity in each environment, resulting in three measurements for each subject across the experiment. RM-ANOVA showed a significant main effect of group ($P < 0.005$) and block ($P < 0.001$) and group-by-block interaction

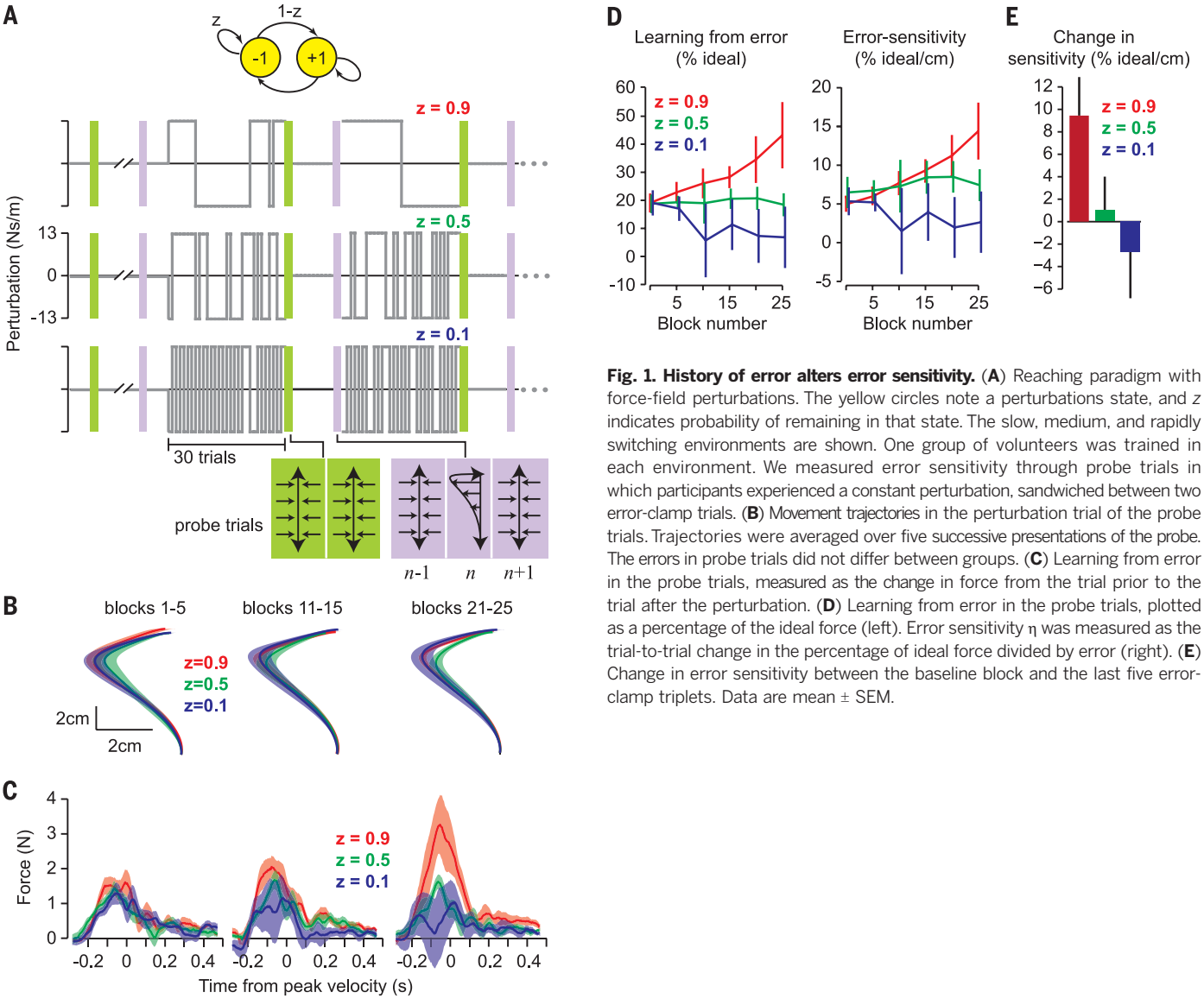

Fig. 1. History of error alters error sensitivity. (**A**) Reaching paradigm with force-field perturbations. The yellow circles note a perturbations state, and z indicates probability of remaining in that state. The slow, medium, and rapidly switching environments are shown. One group of volunteers was trained in each environment. We measured error sensitivity through probe trials in which participants experienced a constant perturbation, sandwiched between two error-clamp trials. (**B**) Movement trajectories in the perturbation trial of the probe trials. Trajectories were averaged over five successive presentations of the probe. The errors in probe trials did not differ between groups. (**C**) Learning from error in the probe trials, measured as the change in force from the trial prior to the trial after the perturbation. (**D**) Learning from error in the probe trials, plotted as a percentage of the ideal force (left). Error sensitivity η was measured as the trial-to-trial change in the percentage of ideal force divided by error (right). (**E**) Change in error sensitivity between the baseline block and the last five error-clamp triplets. Data are mean ± SEM.

($P < 0.001$). As the statistics of the perturbation changed, so did the error sensitivity.

We measured learning from error as a function of error in each environment (Fig. 2D). A given error produced greater learning when that error was experienced in a slowly switching environment (Fig. 2D, red line) (RM-ANOVA main effects of error size $P < 10^{-4}$, and environment $P < 0.001$, posthoc between slow versus medium or fast, $P < 0.001$). We quantified error sensitivity at each error size (Fig. 2E) and found that error sensitivity had not changed globally, but had changed predominantly for smaller error sizes. RM-ANOVA of the absolute sensitivities between 0.25 and 2 cm showed a significant main effect of environment ($P < 10^{-4}$), as well as a significant environment by error size interaction ($P < 0.05$). We found a significant difference in error sensitivity across environments for an error size of 0.25 cm ($P < 0.05$), but no significant difference for an error size of 2 cm ($P > 0.1$). Interestingly, the small error sizes for which the participants had shown the largest change in error sensitivity were also the most frequent errors (Fig. 2F). This hinted that control of error sensitivity was a function of error.

Current models of sensorimotor learning assume that error sensitivity $\eta^{(n)}$ is independent of error $e^{(n)}$. This is true for state-space models of learning (*18*, *21*–*24*), as well as Kalman filter models of learning (*5*, *25*–*27*). However, suppose that sensory prediction errors are encoded in the nervous system with a set of basis elements, where each basis element g_i has a preferred error $\breve{e}_i$. Further, suppose that error sensitivity is determined by a population coding

$$\eta(e^{(n)}) = \sum_i w_i g_i(e^{(n)})$$
$$g_i(e^{(n)}) = \exp\frac{-(e^{(n)} - \breve{e}_i)^2}{2\sigma^2} \quad (2)$$

On trial $n - 1$, the motor command $u^{(n-1)}$ produces an error $e^{(n-1)}$, as illustrated in the top part of Fig. 3A. The nervous system learns from this error and produces motor command $u^{(n)}$ on the subsequent trial, resulting in $e^{(n)}$. In a slowly switching environment (top part of Fig. 3A), $e^{(n)}$ has the same sign as $e^{(n-1)}$. In this case, error sensitivity should increase around $e^{(n-1)}$ (Fig. 3B, red line). By contrast, in a rapidly switching environment (Fig. 3A, bottom), $e^{(n)}$ has a different sign than $e^{(n-1)}$. In this case, error sensitivity should decrease

$$\mathbf{w}^{(n+1)} = \mathbf{w}^{(n)} + \beta \text{sign}(e^{(n-1)}e^{(n)}) \frac{\mathbf{g}(e^{(n-1)})}{\mathbf{g}^T(e^{(n-1)})\mathbf{g}(e^{(n-1)})} \quad (3)$$

In Eq. (3), $\mathbf{w} = [w_1 \quad w_2 \quad \cdots \quad w_N]^T$, $\mathbf{g} = [g_1 \quad g_2 \quad \cdots \quad g_N]^T$, and superscript T is the transpose operator. This rule is similar to the RPROP algorithm, a heuristic for adjusting the learning rate of machines (*28*), but has the unique feature of assuming that error sensitivity is via population coding of the error space.

Equations 1 to 3 represent a learner that stores two kinds of memory: a memory of the state of environment ($\hat{x}$, Eq. 1) and a memory of errors ($\mathbf{w}$, Eq. 3). We simulated the model (Fig. 3C, gray line) and found that in the slowly switching environment, error sensitivity increased in the neighborhood of the experienced errors, whereas in the rapidly changing environment, error sensitivity decreased (Fig. 3D).

Our model made a critical prediction: If the brain controlled error sensitivity via memory of errors, then it should be possible to simultaneously increase sensitivity for one error, while decreasing it for another. In experiment 3, we considered an isometric task in which participants ($n = 16$) produced a force to match a target (16 N) in the face of a perturbation. The perturbations were designed so that, according to our model, individuals would increase their sensitivity to –4 N errors, while simultaneously decreasing their sensitivity to +8 N errors.

In the baseline block, we probed sensitivity to +8 N and –4 N perturbations (probe 1, Fig. 3E). The resulting learning from error is plotted in Fig. 3F (probe 1). At baseline, participants responded to the +8 N and –4 N perturbations by learning a fraction of each error (Fig. 3F). We next produced 20 repetitions of a rapidly switching environment in which the perturbations were ±8 N (Fig. 3E, inset). After a period of washout, we then produced 15 repetitions of a slowly switching environment in which the perturbations were 0 N or +4 N. The critical aspect of our design was that the participants were never exposed to a –4 N perturbation. They nevertheless experienced –4 N errors (because removal of a learned +4 N perturbation results in a –4 N error).

The 8 N environment induced a decrease in sensitivity to a +8 N error, and subsequent exposure to the +4 N environment resulted in an increase in sensitivity to a –4 N error [Fig. 3G; RM-ANOVA showed a significant main effect of perturbation ($P < 0.03$) as well as a perturbation by block

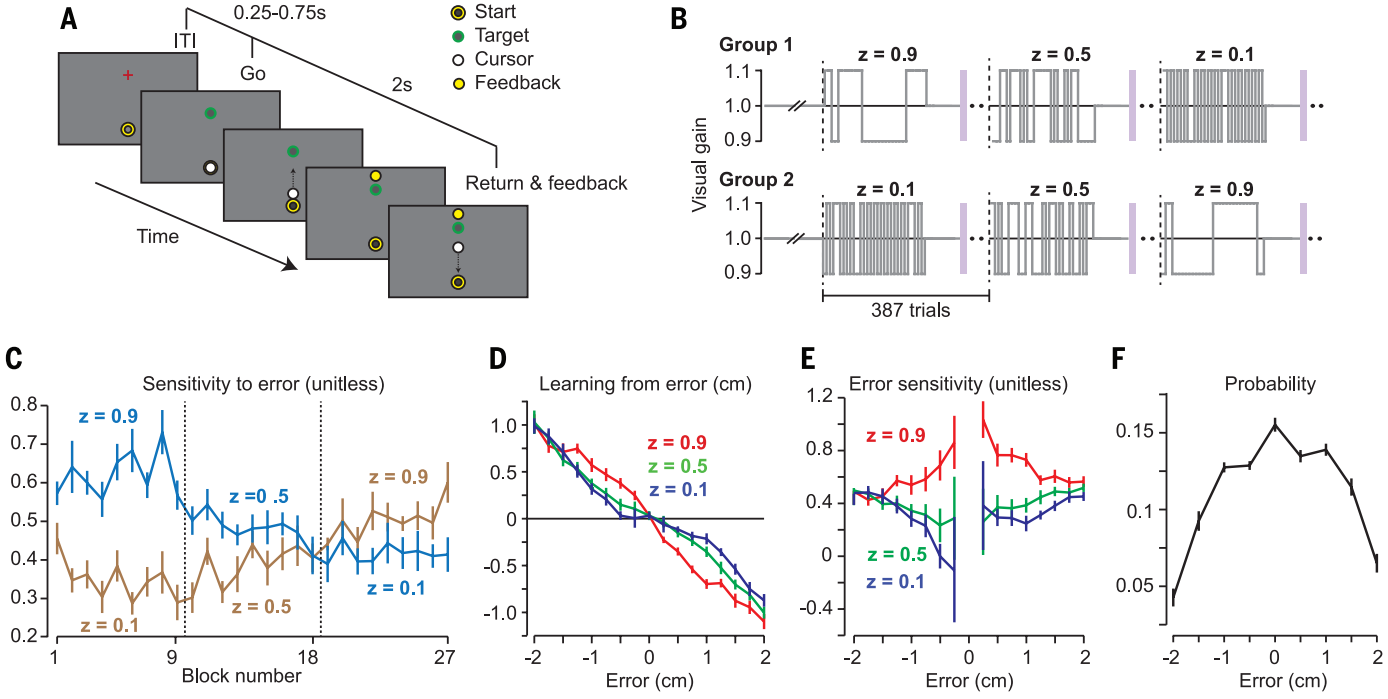

Fig. 2. Error sensitivity is a local function of experienced errors. (**A**) Paradigm with visuomotor gain perturbations. (**B**) Perturbation schedule. Dashed lines indicate changes in the statistics of the environment. (**C**) Error sensitivity averaged over all error sizes measured over each environment block. (**D**) Learning from error measured at various error sizes. (**E**) Error sensitivity as a function of error magnitude. (**F**) Probability of error.

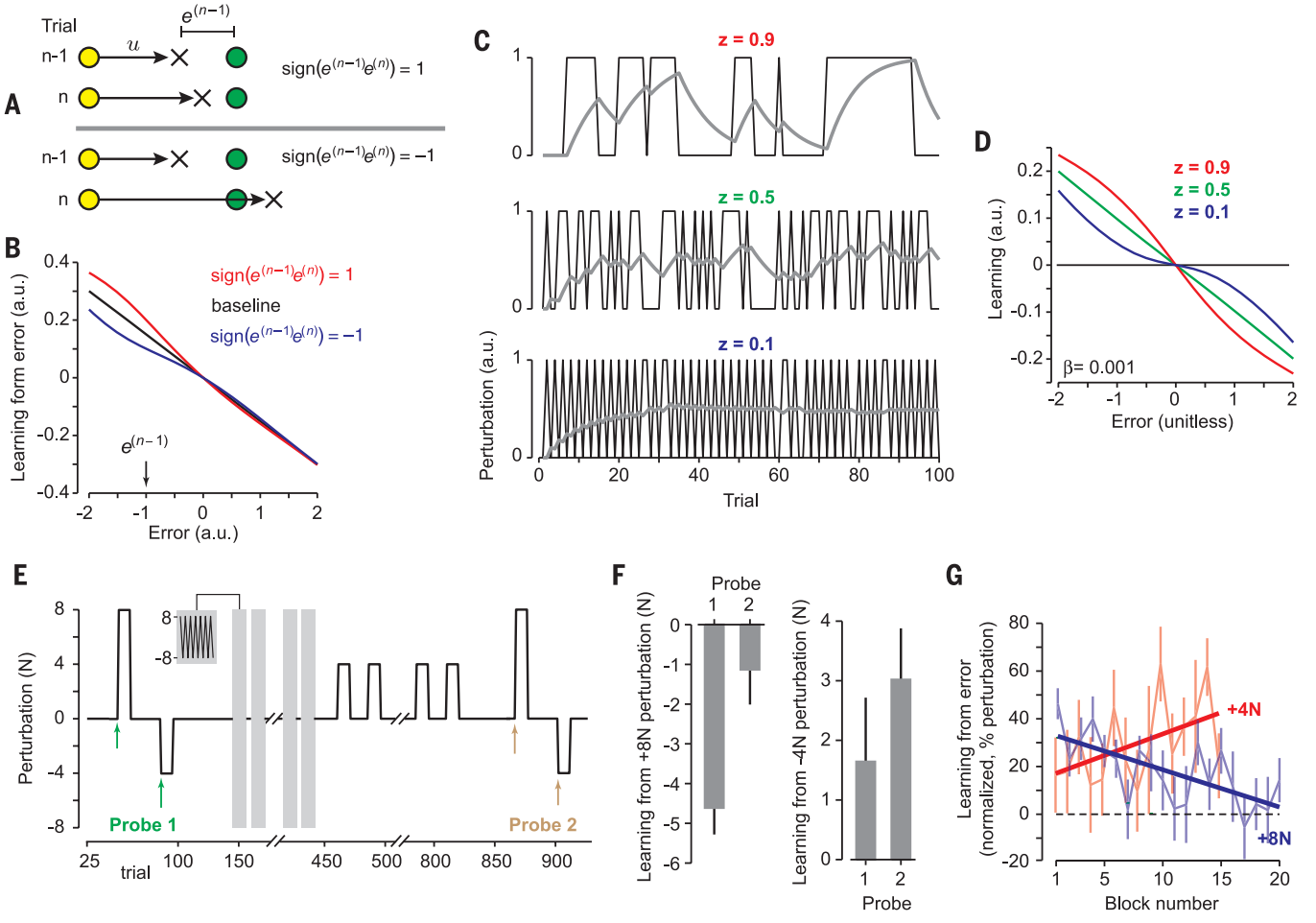

Fig. 3. Theoretical model and experiment 3. (**A**) On trial n − 1, the motor command $u^{(n-1)}$ is generated, resulting in error $e^{(n-1)} = -1$. If the error in trial n is of the same sign as $e^{(n-1)}$, then error sensitivity should increase (top). However, if the error experienced in trial n has a different sign than $e^{(n-1)}$, then error sensitivity should decrease (bottom). (**B**) Learning from error after experience of two consecutive errors from (A). Error sensitivity around $e^{(n-1)}$ increases if sign$(e^{(n-1)}e^{(n)}) = 1$ and decreases otherwise. (**C**) Model performance for slow, medium, and rapidly switching environments (gray line represents $\hat{x}^{(n)}$). Learning from error (**D**) is increased in the slowly switching environment and decreased in the rapidly switching environment. (**E**) Experiment 3 perturbation protocol. (**F**) Single-trial learning from a +8 N perturbation and a −4 N perturbation in probes 1 and 2. Learning is increased for the −4 N perturbation, while simultaneously decreased for a +8 N perturbation. (**G**) Learning from error normalized by the perturbation magnitude (4 or 8 N) in the first trial of each repetition of the rapidly (blue) and slowly switching (red) environments. Learning increased in the slowly switching (4 N) environment but decreased when the perturbation was rapidly switching (8 N). Error bars are SEM.

interaction, $P < 0.01$]. The critical question, however, was whether both of these changes in sensitivity were simultaneously present. After the slowly switching block of perturbations, we again probed sensitivity to +8 N and −4 N errors (probe 2, Fig. 3E). Compared to the baseline block (probe 1), learning from a +8 N error had decreased ($P < 0.005$), while simultaneously, learning from a −4 N error had increased ($P < 0.05$) (Fig. 3F). When we ran our model on the same sequence of errors that participants had experienced, the change in error sensitivity predicted by the model was highly correlated with the change observed in our participants ($R^2 = 0.65$; $P < 10^{-8}$, fig. S1), suggesting that history of error induced changes in error sensitivity in the region of the experienced errors.

This new model of learning provided insights on a wide range of puzzling experiments, including the phenomena of savings and meta-learning (fig. S2). It predicted that when one is better at a task than before, it is not because the brain recalled the motor commands, but because it recognized the errors—the errors for which error sensitivity had been altered. In addition, the model predicted that savings and meta-learning could be blocked by controlling the errors that are experienced during learning.

In experiment 4, volunteers participated in a visuomotor rotation experiment (Fig. 4A and fig. S4, n = 10 per group). The control group (ANA) experienced a +30° perturbation followed by extended washout and then relearning of +30°, a protocol that should produce savings (*13*). According to our model, savings occurs because during the initial exposure to (A), the stable sequence of perturbations increase error sensitivity, and these errors are revisited in the subsequent test of (A). If so, we should be able to block savings by presenting (A) gradually (GNA group, fig. S4), preventing prior exposure to errors that are visited at the onset of (A).

Furthermore, we should be able to produce savings in a very different way: Expose participants to perturbation (B) and then present sudden washout (Fig. 4A, BNA). During washout, they are exposed to a sequence of stable errors, which increase error sensitivity for those errors. Notably, the washout-induced aftereffects are errors that are also experienced during subsequent test of (A). If the meta-learning in BNA is due to errors that are experienced during washout of (B), we should be able to eliminate it by reducing the washout-induced errors. In $B_{wait}NA$, a wait period was inserted between −30° training and washout, reducing the size of aftereffects associated with the transition from −30° to washout (Fig. 4E). We also tested this idea in a different way: gradual washout (B) (group BGNA, fig. S4). In summary, the model predicted

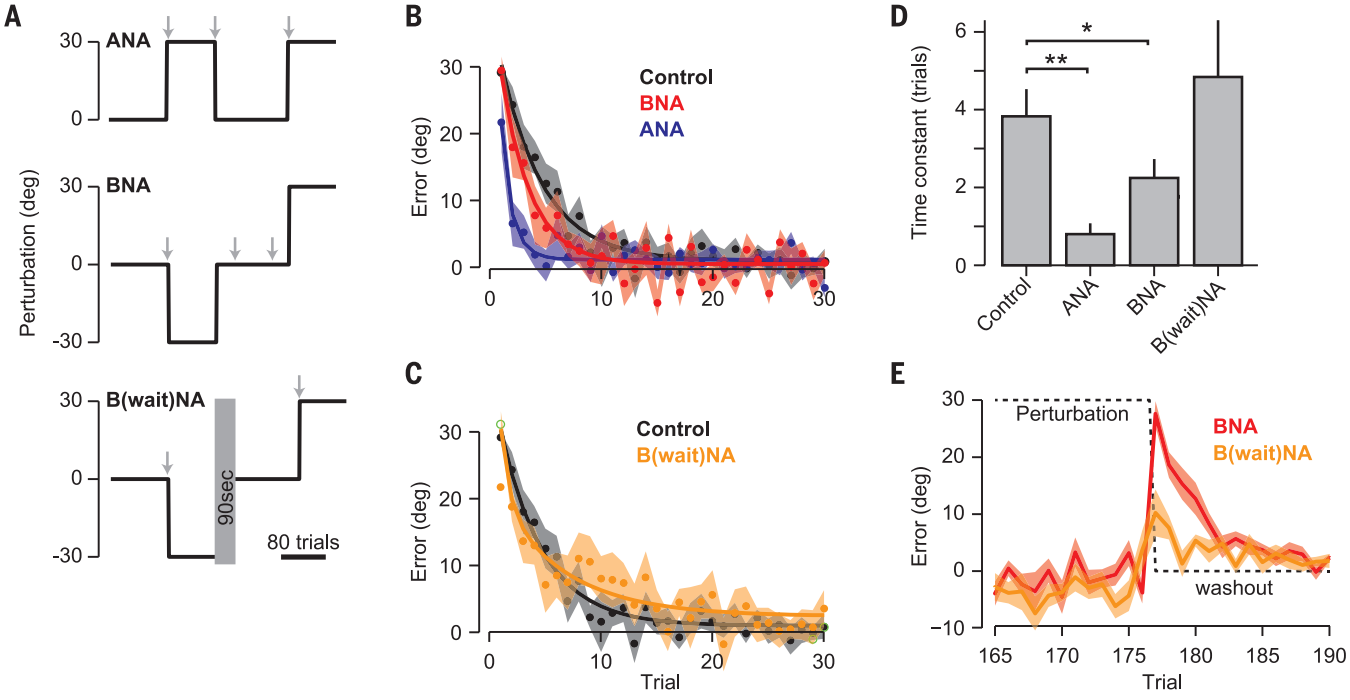

Fig. 4. Saving occurs only when previously experienced errors are revisited. (**A**) A visuomotor perturbation experiment. Gray arrows indicate 1- to 2-min set breaks. (**B**) Performance in the final +30° perturbation. ANA and BNA groups show savings, i.e., faster learning of the perturbation compared to control (naïve). Exponential fits are shown for the group data. (**C**) The B_{wait}NA group does not exhibit savings. (**D**) Exponential time constants are compared to controls (*$P < 0.05$, **$P < 0.01$). A smaller time constant indicates faster learning. (**E**) Comparison of the errors (i.e., aftereffects) experienced by the BNA and B_{wait}NA groups. The B_{wait}NA group experienced smaller errors owing to the presence of the set break.

savings in ANA but not GNA, and meta-learning in BNA but not B_{wait}NA and BGNA. Our experimental results confirmed these predictions (Fig. 4, C and D, and fig. S4).

We found that during learning, the brain controlled error sensitivity in a principled way: learning more from error when perturbations were likely to persist, and less when perturbations were likely to change. Error-sensitivity modulation was specific to the experienced errors, suggesting that training produced a memory of errors. This idea accounted for a host of puzzling observations, including saturation of error sensitivity (*5*, *6*, *29*), the phenomenon of meta-learning (*16*), examples of savings (*10*–*12*), and reinforced repetition (*15*).

The model predicted that meta-learning vanishes when a small delay or gradual washout alters the history of errors (Fig. 4A), demonstrating that savings depends crucially on the memory of errors that is accumulated during training. This memory of errors likely exists in parallel with the two traditional forms of motor memory, memory of perturbations (*23*) and memory of actions (*30*).

In our model, we chose to describe the learner as a process with a single time scale. However, data suggest that learning from error depends on a fast and a slow process with different error sensitivities (*23*, *25*, *31*). We speculate that the memory of errors exerts its influence through the error sensitivity of the fast process, and its manipulation through history of errors may be a useful strategy to speed recovery during rehabilitation (*32*).

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ACKNOWLEDGMENTS

This work was supported by NIH grants T32EB003383, T32GM007057, R01NS078311, and 1F31NS079121. We thank J. Krakauer, J.-J. Orban de Xivry, and A. A. Ahmed for comments. D.J.H., P.A.V., M.K.M., and R.S. conceived and designed the experiments. D.J.H. performed the experiments, analyzed the data, and performed the simulations. R.S. and D.J.H. wrote the paper. The data reported in this paper and in the supplementary materials are available from the corresponding author.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1349/suppl/DC1
Supplementary Text
Figs. S1 to S4
References (*33*–*40*)

10 March 2014; accepted 24 July 2014
Published online 14 August 2014;
10.1126/science.1253138

ICE SHEETS

Boundary condition of grounding lines prior to collapse, Larsen-B Ice Shelf, Antarctica

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Grounding zones, where ice sheets transition between resting on bedrock to full floatation, help regulate ice flow. Exposure of the sea floor by the 2002 Larsen-B Ice Shelf collapse allowed detailed morphologic mapping and sampling of the embayment sea floor. Marine geophysical data collected in 2006 reveal a large, arcuate, complex grounding zone sediment system at the front of Crane Fjord. Radiocarbon-constrained chronologies from marine sediment cores indicate loss of ice contact with the bed at this site about 12,000 years ago. Previous studies and morphologic mapping of the fjord suggest that the Crane Glacier grounding zone was well within the fjord before 2002 and did not retreat further until after the ice shelf collapse. This implies that the 2002 Larsen-B Ice Shelf collapse likely was a response to surface warming rather than to grounding zone instability, strengthening the idea that surface processes controlled the disintegration of the Larsen Ice Shelf.

Our ability to model or predict changes in marine-based glaciers or ice sheets due to warming temperatures or rising sea level is limited by an inadequate understanding of the character of ice shelf/ice sheet grounding zone systems (GZSs) (*1*–*3*). Atmosphere and seawater temperature variation force changes in ice mass transfer to the oceans (*4*); however, the removal of ice shelf buttressing is also critical in regulating glacier response (*5*–*7*). In February to March of 2002, the Larsen-B Ice shelf (LIS-B) catastrophically collapsed, with a loss of ~3250 km^2 of floating ice (*8*). Tributary glaciers that had fed into the ice shelf system—including the Crane and Hektoria Glaciers—accelerated considerably after the collapse (*5*–*7*). After this was a rapid recession of the ice-calving fronts of the glaciers and transition to a tidewater glacier character (*9*). The events revived the thinking that linkages between grounded glaciers and ice shelves are indeed critical, more so than recently assumed (*10*), but underscored the need for detailed knowledge of the characteristics of GZSs and GZS transitions in ice-shelf and tidewater glacier systems.

A swath bathymetry survey in 2006 imaged the inner-shelf sea floor of the Larsen-B embayment that was exposed after the ice shelf collapse (Fig. 1A). The extensive arcuate GZS at the entrance to Crane Fjord is a large sedimentary prism seaward of a local bathymetric divide, which has a variable depth between 690 and 660 m. The grounding zone to the south appears to run parallel to a terrace at 600 m depth, whereas its expression in the drainage of the Jorum/Punchbowl system is less clear because of the steep crystalline bedrock slope that dominates the intervening headland (*11*). The GZS prism tapers relatively gently seaward and is steeper landward. Elongated ridges subparallel to the axis of the Crane Fjord, similar to subglacial lineations observed elsewhere on the Antarctic margin (*12*, *13*), are interpreted as fluted subglacial bedforms [such as mega-scale glacial lineations (MSGLs)]. A series of furrows curve toward the southeast as a result of the ice flow from the Jorum/Punchbowl system, and a pad of fluted sediment marks the central axis of the Crane Fjord. A set of relict channels (in an irregular pattern of salients and reentrants) also appears to crosscut the arcuate furrows on both the lee and stoss side of the GZS. These features collectively mark episodes of decoupling from the GZS, beginning with the formation of the arcuate ridges, followed by cross-cutting of channels and then deposition of the central fluted pad of sediment.

Sediment cores from the Larsen B embayment address the question of whether this GZS served to anchor the LIS-B before its 2002 collapse. Core NBP0603 KC-10 was recovered from the top of the GZS. The boundary between the diamicton (Unit 1) and the overlying sediments dates back at least 11,000 years (Fig. 2 and supplementary materials), indicating that this GZS was abandoned at termination I. The timing of glacial ice liftoff is supported further by a radiocarbon-based chronology from three other sediment cores, where the same stratigraphy was observed (Figs. 2 and 3). These observations of the GZS do not agree with the location of the grounding zone as inferred from satellite images (*14*), and the sediment core data reveal that glacial ice was not grounded here immediately before the 2002 LIS-B collapse. The reason for the misplacement of the grounding line by (*14*) is unclear, but thick floating ice inside a relatively narrow channel would exhibit reduced tidal bending, as would have been the case with the Crane Glacier at this time. It is possible that a rift and/or structural arch in this area would mimic the appearance of a grounding line. The abandonment of the large GZS relatively early (before 11.7 thousand years ago) with respect to Holocene events suggests that retreat of grounded ice within the LIS-B embayment was driven by processes similar to those that drove shelf-wide recession across the NW Weddell Sea continental shelf (*15*). We suggest that this innermost retreat into the deep troughs of the Nordenskӧld Coast was tied to late stages of global eustatic forcing coupled to retreat of global glacial cover. The sedimentology and facies relationships of sediment cores just seaward of the Crane Trough (in Exasperation Inlet) demonstrate a rapid recession of the ice sheet system across the inner shelf, which occurred 12.5 thousand years ago (Figs. 2 and 3 and fig. S1). The chronologic data we present (table S1), combined with existing published data further seaward (*11*), establish recessional rates of the grounding line within the main axis of the LIS-B embayement of ~100 m/year during the period from 12.3 thousand to 11.7 thousand years ago, comparing favorably with ice sheet/shelf recessional rates obtained for the same period in the western Ross Sea (*16*).

Further, geophysical data, combined with Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) imagery, can be used to determine the location of the LIS-B pre-collapse grounding line in the Crane Fjord (Fig. 1). The Crane Trough consists of three very deep (>1000 m) and narrow (~1 km wide) elongated basins separated by more elevated crests, each representing the threshold of the upslope basin. The floor of the basins shows a flat morphology, which is typical of a subsequent sedimentary filling under subaqueous conditions. The upper basin is not fully charted and shows the presence of MSGLs subparallel to the axis of the fjord. We interpret this morphology as the result of deposition of till beneath grounded ice. On Arctic continental margins, the same interpretation is given to similar associations of thresholds and MSGLs (*17*). The fact that the ice was grounded in the fjord is confirmed by the single-channel seismic data, whose landward overdeepening and stairway

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geometry between its three basins can be explained best by the carving action of ice (*18*). The 2001 ASTER Digital Elevation Model (DEM) profile locates the pre-collapse grounding zone of the Crane Glacier portion of the LIS-B at the threshold between the upper and the middle basin, >13 km from the large GZS described above (Fig. 1B). The stability of the LIS-B through the Holocene (*10*) thus was not dependent on grounding at a large GZS; instead, collapse was forced by surface melt processes (*6*).

Well-layered, undisturbed sediments extend for at least 40 m below the lower (outermost) basin (Fig. 4). This thickness is exceptional for an inner-shelf deposystem that has been covered by an ice shelf until only a few years ago (*19*). Seismic data show that the layered fill is underlain by at least two other seismic units of variable thickness and complexity (Fig. 4). In dip profile, this wedge-shaped unit slopes rapidly landward, beneath the middle basin, and subsequently dips seaward beneath the layered fill of the lower basin. Internally, it shows mounded, low-amplitude reflectors terminating landward with marked downlap and seaward progradation. In strike, the sediments at the base of this unit have a relatively high sound velocity (~1700 m/s), as indicated by the analysis of the diffraction hyperbolas. The lowest identified unit is more complex, poorly defined, and characterized by hummocky, irregular, high-amplitude reflectors. Its top shows alternating ridges and troughs in strike profile, which match the morphology of the thresholds between each basin.

The above observations allow us to reconstruct the relative succession of events that generated the sedimentary deposits within the Crane Fjord. Similar to other overdeepened basins on the Antarctic inner shelf (*20*), this fjord has been carved, likely along structural lines of weakness, by grounded ice. The last time the ice was grounded within the fjord is represented by the top of the lowest identified unit (Fig. 4). Its geometry of alternating troughs and ridges matches subglacial bedforms exposed across adjacent

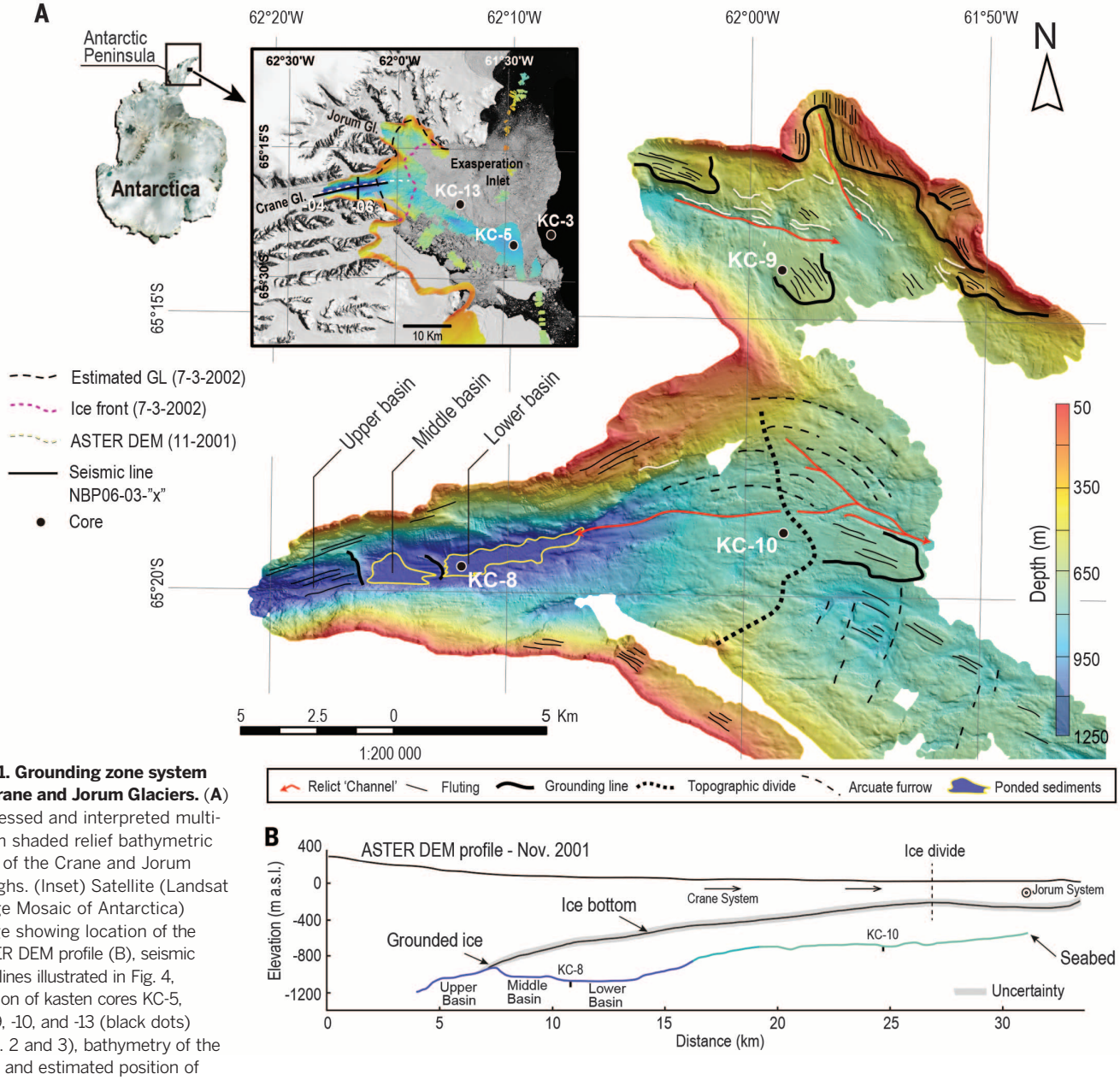

Fig. 1. Grounding zone system of Crane and Jorum Glaciers. (**A**) Processed and interpreted multibeam shaded relief bathymetric map of the Crane and Jorum Troughs. (Inset) Satellite (Landsat Image Mosaic of Antarctica) image showing location of the ASTER DEM profile (B), seismic tracklines illustrated in Fig. 4, location of kasten cores KC-5, -8, -9, -10, and -13 (black dots) (Figs. 2 and 3), bathymetry of the area, and estimated position of grounding line (dashed black line) after (*14*). (**B**) ASTER DEM profile of the LIS-B just before its breakup, and the underlying bathymetry derived from the multibeam data. Before collapse of the ice shelf in 2002, the ice is grounded just landward of the middle ponded basin.

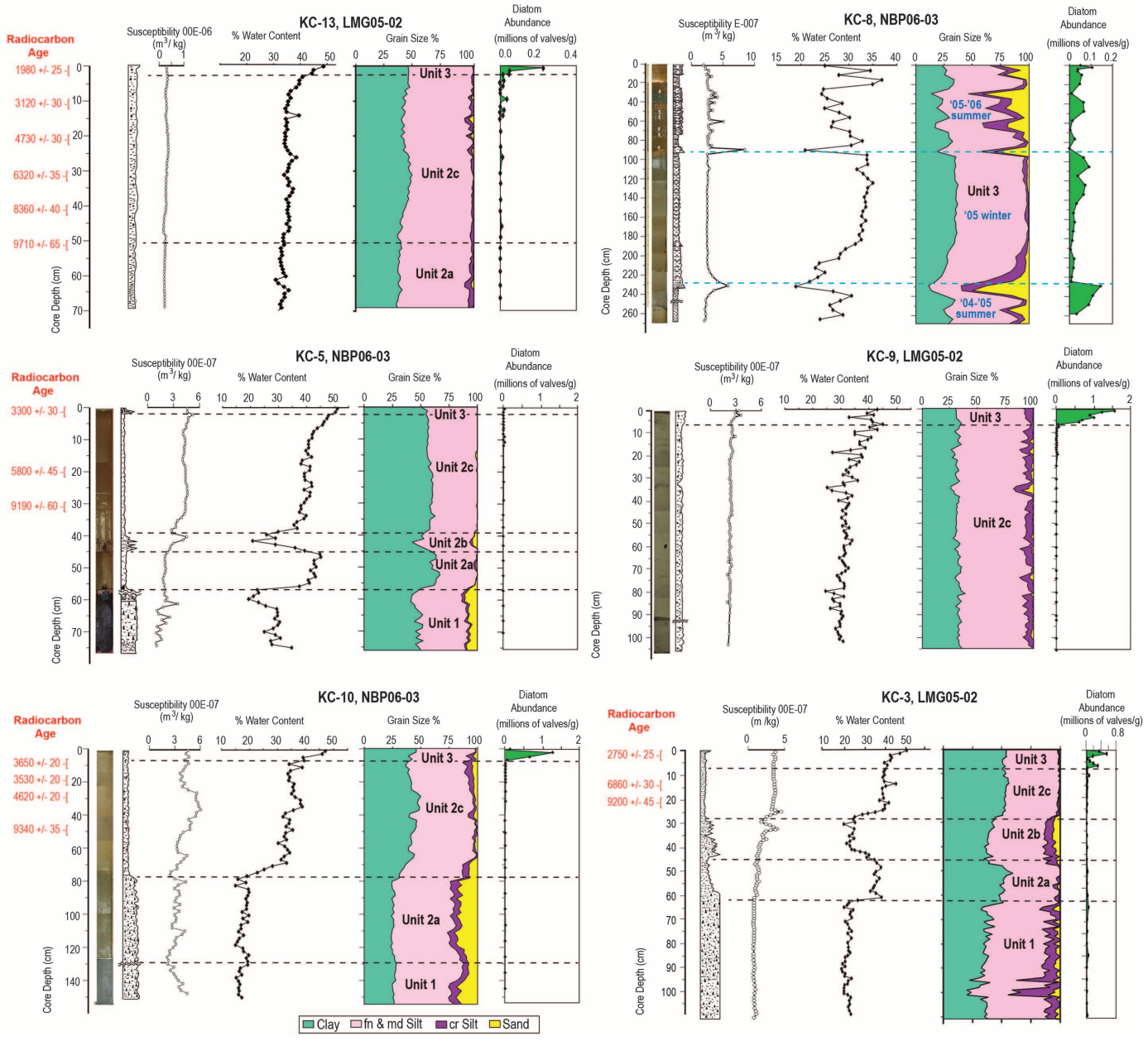

Fig. 2. Photographs, lithofacies, physical properties, and diatom abundance for six kasten cores collected in the vicinity of the Crane Trough. The locations of the cores are depicted in Fig. 1. Unit designations are as follows: Unit 1 is glacial diamicton (till) related to ice sheet (ice stream) grounding upon sea floor; Unit 2 is sub–ice shelf sediment related to near (proximal) grounding line (2a), distal to grounding line (2c) and an, as yet, undetermined coarse interval (2b); and Unit 3 represents the seasonal open marine facies formed since the break up of the LIS-B. Uncorrected radiocarbon dates on foraminiferal calcite (table S1) are indicated in red.

thresholds. Furthermore, the hummocky character of underlying reflectors is consistent with deposition of subglacial till at the glacial bed. Conversely, the overlying wedge-shaped unit with its top sloping rapidly landward suggests the development of a grounding zone, beyond which a body of water separated the base of the ice from the sea floor, such as a "subglacial lake" (*21*). Its geometry and the internal prograding reflectors resemble those of subglacial deltas identified elsewhere along Antarctic grounding zones (*20*). This delta may have developed in a subglacial lake setting similar to that inferred for features in the Northern Prince Gustav Channel (*13*) and the Palmer Deep basin (*19*, *22*).

The overlying layered seismic unit was clearly deposited subaqueously. Core KC-8 collected near the intersection of profiles NBP0603-04 and -06 (Figs. 1 and 3) suggests that the uppermost sediments resulted from the accelerated ice discharge (rafting) from the Crane Glacier after the breakup of LIS-B and seasonal slumping of debris along the flanks of the Crane Fjord (fig. S2). This 2.7-m core shows clear meter-scale grain-size alternation between a fine silt facies (virtually devoid of sand) and a sandy silt facies (sand fraction up to >50%) (Fig. 2). The presence of well-preserved diatoms commonly associated with the sea ice environment suggests that the entire 2.7-m-thick sequence was deposited in open marine conditions with annual sea ice (not sub–ice shelf)—conditions that are known to have been present only since austral

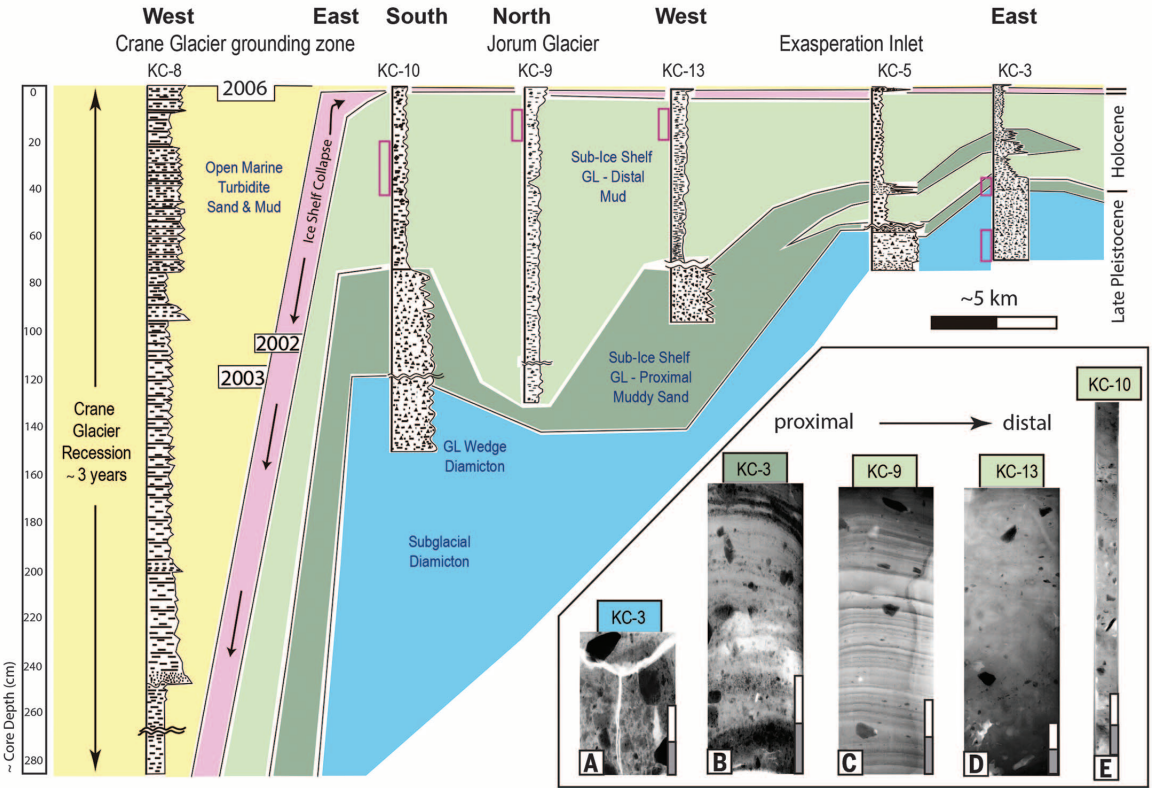

Fig. 3. Fence diagram of core sites and stratigraphic model across the grounding line wedge with sequence of events related to collapse of LIS-B. Facies correlation among cores is shown with color scheme. GL, grounding line. (Inset) X-ray radiograph positives (clasts are opaque) of specific facies intervals in selected cores. Red rectangles next to each core indicate position of each x-ray radiograph. Scale bar, 5 cm. Proximal to distal positions relative to grounded ice are shown for the sequence of x-ray radiographs as left to right, respectively.

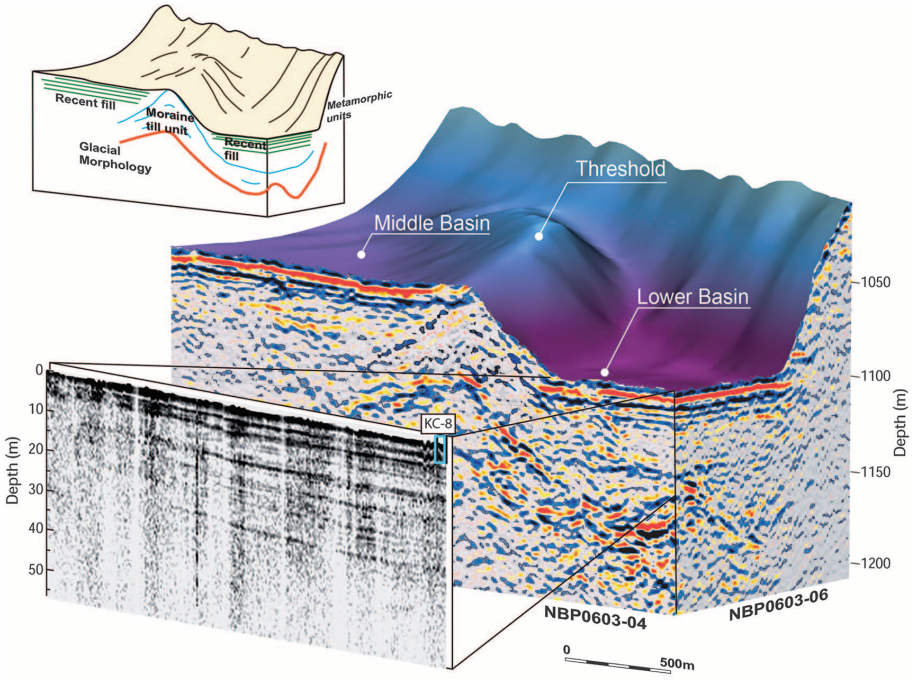

Fig. 4. Three-dimensional view of seismic reflection data and multibeam bathymetry from Crane Fjord. Seismic line locations are shown in Fig. 1, inset. Colored reflectors in block diagram are from Generator Injector Air Gun–seismic reflection, and the grayscale panel is from a 3.5 kHz compressed high-intensity radar pulse subbottom system. (Inset) Line drawing interpretation of layered and chaotic reflectors.

summer 2002–2003 (3 years before core recovery). In general, *Fragilariopsis* comprise >80% of the diatom assemblage, and in half the samples, this genus comprises >90% of the diatom assemblage (tables S2 and S3). Preservation is excellent, with many very lightly silicified specimens occurring in ribbon colonies containing chloroplasts. Although diatoms can be advected underneath ice shelves (*23*), data indicate that advection of diatoms has not been observed beneath the LIS-B (*11*, *24*). Our observations as well suggest the absence of advection of diatoms underneath the LIS-B because these cores also document the opening of the Larsen B embayment, the associated increase in diatom abundance, and the lack of diatoms below the uppermost part of the cores, except in NBP0603 KC-8 (Fig. 2). The pristine condition of the frustules coincides with high water content with generally finer grain size, pointing to the very recent and rapid deposition of the valves at the sea floor and very limited transport. This core is interpreted to represent just over 1 year of deposition (from austral summer 2004–2005 to the end of the austral summer 2005–2006), with an extraordinary sedimentation rate of about 2 m/year (Fig. 3 and fig. S2). The sandy intervals are interpreted as summer turbidites, deposited when open water and surface melt would contribute to slope instabilities along the walls of the fjord and within the glacier terminus system (Figs. 2 and 3 and fig. S2).

Downward extrapolation of the ~2 m/year sedimentation rate estimated from core KC-8 suggests that only the uppermost ~6 m of sediment in the lower basin were deposited after LIS-B breakup, corresponding to the layered reflectors ("recent fill") identified in the seismic reflection data (Figs. 3 and 4). The remainder of the sediments imaged in the lower basin,

~35 m thick, may correspond to a sequence of turbidites, as observed in other grounding zone proximal sites (*25*). The ponded morphology and acoustic stratification of the sediments support this interpretation (Figs. 1 and 4). The middle basin likely contains a sediment sequence similar to that observed in the lower basin. In its position landward of the 2001 grounding zone (Fig. 1), the deep upper basin may have existed as a subglacial lake through the Holocene (*21*).

As estimates for projected sea-level rise over the next century continue to be revised from recent Intergovernmental Panel on Climate Change forecasts, the stability of GZSs need further evaluation. Relict GZSs now observed from several offshore troughs on the Antarctic continental shelf record a time of extreme rates of sea-level change in the Quaternary (*19*, *26*, *27*). Yet, "modern" GZSs found near critical regions are interpreted to have been abandoned within recent decades, which is coincident with feedbacks in ice-shelf basal melting, grounding line recession, and ice-sheet thinning (*28*). The stability of the LIS-B with its GZS through the Holocene and their recent rapid collapse suggests strong sensitivity to surface warming. This adds to the scenario of instability now facing Antarctic glacial masses and must invigorate continued examination of GZS in spite of difficulty in access, logistical risk, and competing resources.

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ACKNOWLEDGMENTS

We thank the shipboard participants, captain, and crew of cruises NBP0603 and LMG0502 and the staff at the Antarctic Marine Geology Research Facility, Florida State University. This project was supported by grants from the NSF–Office of Polar Programs (0732467 to E.D.; 0732625 to A.L.; 0732605 to S.B.) and from Italian Programma Nazionale di Ricerche in Antartide project ULISSE. The data reported in this paper are tabulated in the supplementary materials. M.R. collected and interpreted seismic reflection data aboard cruise NBP0603 and assisted with writing major portions of the manuscript. E.D. supervised collection of field data and sedimentologic core data for cruises LMG0502 and NBP0603, wrote major portions of the paper, and drafted Figs. 2 and 3. F.Z. collected, processed, and interpreted seismic reflection data aboard cruise NBP0603 and wrote sections of the paper dealing with interpretation of the reflection data. C.L. processed multibeam data, drafted Fig. 1, and wrote sections of the paper dealing with multibeam data. A.L. helped collect data aboard LMG0502 and NBP0603, analyzed diatom abundance data, and wrote sections of the paper dealing with diatom abundance and interpretation. S.B. helped collect data aboard NBP0603, collected and analyzed magnetic susceptibility data, and wrote sections of the paper dealing with physical properties of sediment cores. V.W. assisted in collecting sediment cores during LMG0502, collected color images of sediment cores during this cruise, and helped to draft portions of Fig. 2. G.H. assisted in collecting sediment cores during NBP0603, provided color images of sediment cores collected in this cruise, and helped with editing the manuscript. M.T. imported air geophysical data for interpretation of longitudinal profile of the Crane Glacier and provided editing of the manuscript. T.S. imported remote sensing data and interpretation of grounding line fluctuation before and after ice shelf collapse and assisted in writing and editing the paper. J.S. participated in collecting sediment cores and other data during LMG0502 and assisted in writing and editing the paper. E.P. was instrumental in the extensive discussion related to supplemental material dealing with ice terraces and morainal debris.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1354/suppl/DC1
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29 May 2014; accepted 1 August 2014
10.1126/science.1256697

FISH PIGMENTATION

Thyroid hormone–dependent adult pigment cell lineage and pattern in zebrafish

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Pigment patterns are useful for elucidating fundamental mechanisms of pattern formation and how these mechanisms evolve. In zebrafish, several pigment cell classes interact to generate stripes, yet the developmental requirements and origins of these cells remain poorly understood. Using zebrafish and a related species, we identified roles for thyroid hormone (TH) in pigment cell development and patterning, and in postembryonic development more generally. We show that adult pigment cells arise from distinct lineages having distinct requirements for TH and that differential TH dependence can evolve within lineages. Our findings demonstrate critical functions for TH in determining pigment pattern phenotype and highlight the potential for evolutionary diversification at the intersection of developmental and endocrine mechanisms.

Vertebrates exhibit a stunning variety of pigment patterns, yet the mechanisms underlying pattern development and evolution are only beginning to be discovered. Among the most conspicuous and elaborate patterns are those of teleost fishes, which function in mate choice, shoaling, camouflage, and speciation (*1*–*3*). In the zebrafish *Danio rerio*, the adult pattern comprises dark stripes of black melanophores and a few iridescent iridophores, alternating with light "interstripes" of yellow/orange xanthophores and abundant iridophores, all within the hypodermis, between the epidermis and myotome (*4*) (Fig. 1A). Short-range and

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long-range interactions among pigment cells are critical for patterning (*5*–*9*), and the dynamics of some of these interactions resemble those predicted by Turing models of pattern formation (*10*, *11*). Despite the emphasis on "local" pigment cell autonomous interactions, tissue-specific positional information (*7*, *8*) and "global" endocrine factors are likely required as well.

Adult pattern development involves postembryonic differentiation and morphogenesis, potentially subject to hormonal control. By ~4 days post-fertilization (dpf), an embryonic/early larval (EL) pattern has formed with melanophore stripes and widespread, autofluorescing xanthophores (*12*) that subsequently disappear (fig. S1).

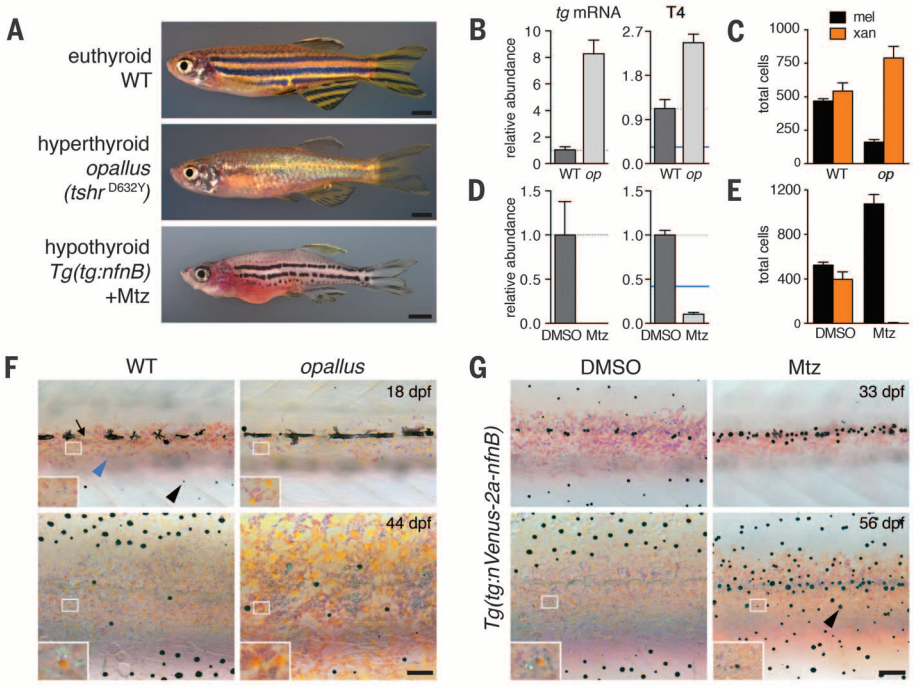

Fig. 1. TH is required for xanthophore development and melanophore repression. (**A**) Wild-type (WT), hyperthyroid, and hypothyroid phenotypes. (**B** and **C**) In *opallus*, *tg* transcript and T4 were increased [$F_{1,4} = 46$, $P < 0.005$; $F_{1,6} = 36$, $P < 0.001$; blue line, ELISA (enzyme-linked immunosorbent assay) detection limit] and there were more xanthophores but fewer melanophores [$F_{1,4} = 46$, $P < 0.005$; $F_{1,6} = 36$, $P < 0.001$; standard length (SL) 17 to 22 mm, stage J++ (*13*)]. (**D** and **E**) In thyroid-ablated fish, *tg* mRNA and T4 were undetectable ($F_{1,4} = 13$, $P < 0.05$; $F_{1,6} = 100$, $P < 0.0001$); xanthophores were missing but there were extra melanophores ($F_{1,14} = 29$, $P < 0.0001$; $F_{1,14} = 184$, $P < 0.0001$; SL 17 to 18 mm). (**F**) At 18 dpf (top), interstripe xanthophores had not yet differentiated in WT but were abundant in *opallus* (insets). Blue arrowhead, iridophores; black arrowhead, adult melanophore. By 44 dpf (bottom), *opallus* exhibited a gross xanthophore excess and severe melanophore deficiency. Fish were treated with epinephrine to contract pigment granules and facilitate counting. (**G**) Hypothyroid fish lacked xanthophores (insets) and had ectopic melanophores (black arrowhead). Note that development rate and xanthophore numbers were decreased even in dimethyl sulfoxide (DMSO) controls owing to a TH-free diet. Scale bars, 2 mm (A), 100 μm [(F) and (G)].

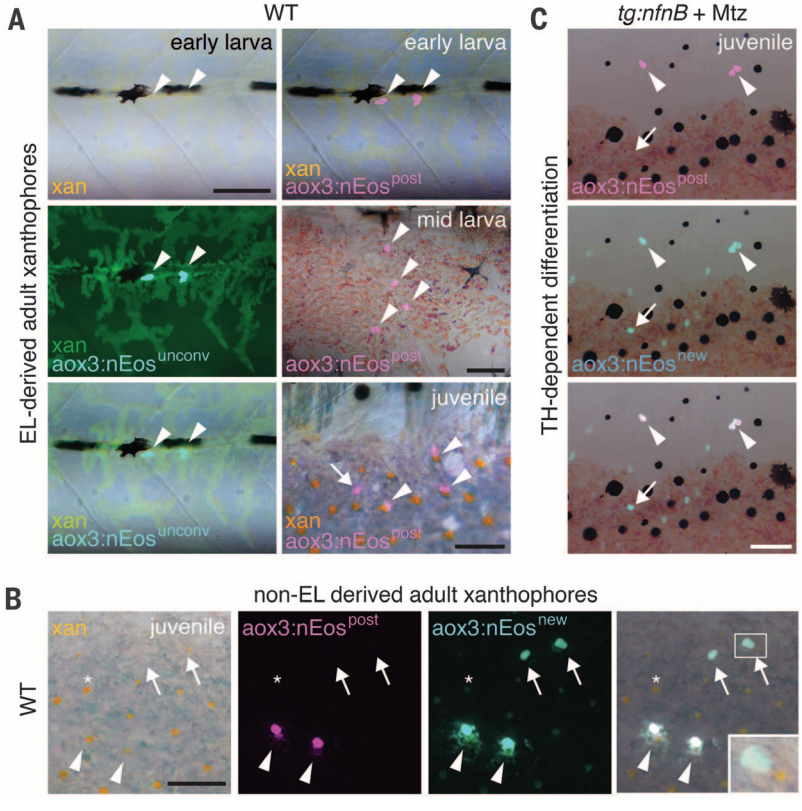

Fig. 2. Hypodermal xanthophore origins and TH dependence. (**A**) Autofluorescing *aox3*:nEos$^+$ EL xanthophores (left panels; post-photoconversion, upper right). Later, yellow pigment was absent and each cell had divided (middle right). In the juvenile, three cells redifferentiated (arrowheads) and one remained unpigmented (arrow). Overall, 86 photoconverted EL xanthophores generated 71 adult xanthophores and 78 unpigmented cells ($n = 44$ larvae); including all stages examined, marked cells had a 59% probability of dividing ($n = 82$ larvae, 190 EL xanthophores, 283 cells later). (**B**) In a juvenile in which all *aox3*$^+$ cells had been photoconverted at 5 dpf, two photoconverted EL-derived xanthophores were visible (arrowheads), but so were cells expressing only unconverted nEos (arrows), one of which had differentiated (inset); 9 of 15 juveniles had unconverted cells that developed after 5 dpf (5.8 ± 1.9, range 1 to 19 cells), of which 54% had differentiated as xanthophores. (**C**) In thyroid-ablated fish, the likelihood of photoconverted EL xanthophores (arrowheads) persisting into the juvenile did not differ from controls ($\chi^2 = 0.01$, df = 1, $P = 0.9$; Mtz, $n = 18$ larvae; DMSO, $n = 13$ larvae, 20 EL xanthophores); some cells initiated *aox3*:nEos expression only after 5 dpf (e.g., arrow). Scale bars, 60 μm.

Then, over ~4 weeks the pattern transforms into that of the adult (*13*) (movie S1). Adult melanophores and iridophores arise from "extrahypodermal" peripheral nerve–associated precursors that migrate to the hypodermis (*14*–*16*). Iridophores differentiate in the interstripe, establishing the adult pattern; melanophores initiate their differentiation more widely over the flank, and adult interstripe xanthophores appear later, in association with iridophores. Stripes gradually become more distinct as adult and residual EL melanophores in the interstripe die, are covered by iridophores, or join adult stripes, and as remaining melanophores expand and align at stripe edges (*7*, *14*, *16*–*20*). We hypothesized that the morphogenesis and differentiation of pigment cells during adult stripe development requires thyroid hormone (TH), which regulates metabolism, has cell type–specific effects, and is essential for the abrupt metamorphoses of amphibians and flatfishes (*21*–*25*).

To test TH responsiveness of pigment cells, we reared larvae in T4 (the tetra-iodinated form of TH) and observed a marked xanthophore excess and melanophore deficiency (fig. S2). These phenotypes resembled the mutant *opallus*^{b1071}, which we identified as harboring a missense mutation (Asp^{632} → Tyr) in *thyroid stimulating hormone receptor* (*tshr*) identical to a human mutation causing constitutive Tshr activity and hyperthyroidism (*26*) (Fig. 1A and fig. S3). *opallus* mutants had elevated expression of the TH precursor gene *thyroglobulin* (*tg*) and T4, more xanthophores, and fewer melanophores (Fig. 1, B, C, and F, and movies S1 and S2). Although xanthophores were the last adult pigment cell type to differentiate on the lateral flank of wild-type fish, they were the first to differentiate in *opallus* mutants (Fig. 1F and fig. S4).

To determine whether TH is required for pattern development, we sought to abolish TH production and so generated a transgenic line, *Tg(tg:nVenus-2a-nfnB)*$^{wp.rt8}$, to ablate the thyroid by metronidazole (Mtz) treatment (*27*). Fish thyroid-ablated at 4 dpf exhibited no *tg* mRNA or T4 expression as juveniles, lacked adult body xanthophores, and had excess melanophores (Fig. 1, A, D, E, and G, and movies S3 and S4). Although some xanthophores developed eventually, they were delayed relative to stage progression, which was itself retarded (figs. S4 and S5). Hypothyroid fish also had craniofacial and other defects, resembling the mutant *manet*$^{wp.r23e1}$, which we identified as a hypomorphic allele of *tshr* expressing *tg* mRNA at levels 7% of the wild type (fig. S6).

To better understand the TH dependence of xanthophores, we first identified their origins. Adult fin xanthophores and melanophores arise from stem cells (*28*), whereas adult body melanophores and iridophores arise from extrahypodermal, peripheral nerve–associated precursors (*14*–*16*); fate mapping has not revealed an extrahypodermal source of body xanthophores. We hypothesized that xanthophore precursors are already present in the hypodermis, and specifically that EL xanthophores give rise to adult xanthophores. Consistent with this idea, EL xanthophore ablation resulted in adult xanthophore deficiency (as well as defects in adult melanophore patterning; fig. S7).

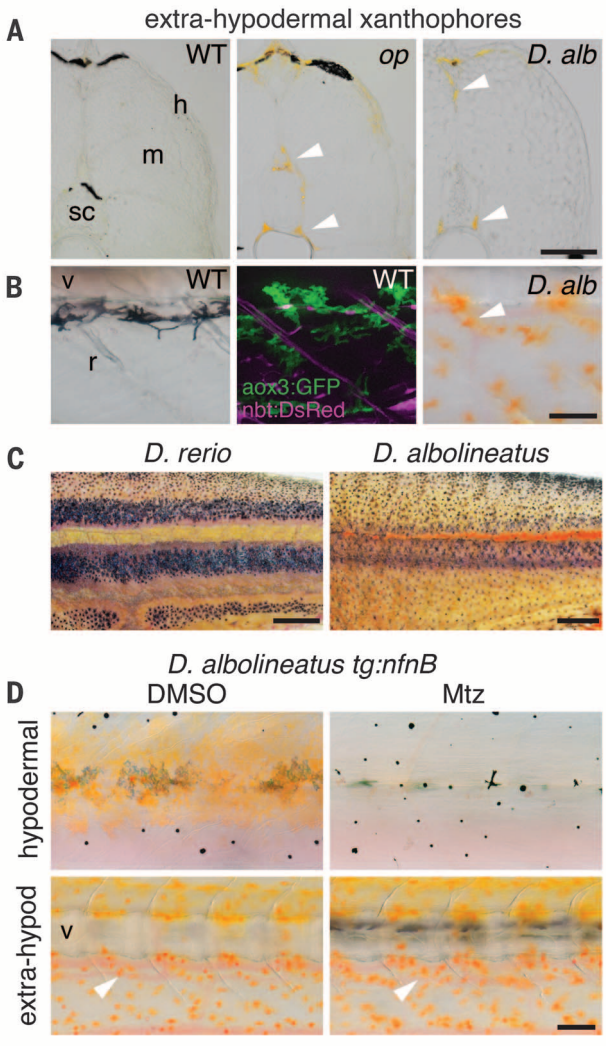

Fig. 3. Extrahypodermal xanthophores and evolution of TH dependence. (**A**) Hyperthyroid *D. rerio* (*op*) had extrahypodermal xanthophores (arrowheads). These cells were present in wild-type *D. albolineatus* (*D. alb*) as well (stage AR). sc, spinal cord; m, myotome; h, hypodermis. (**B**) Medial *aox3*: GFP$^+$ cells relative to *nbt*: DsRed$^+$ peripheral nerves in WT *D. rerio* (left and middle) and xanthophores (right, arrowhead) in *D. albolineatus*. v, vertebral column; r, rib. (**C**) Adult *D. rerio* and *D. albolineatus*. (**D**) Thyroid-ablated *D. albolineatus* retained extrahypodermal xanthophores (arrowheads) despite lacking most hypodermal xanthophores (fig. S12). Scale bars, 60 μm [(A) and (B)], 1 mm (C), 100 μm (D).

To test whether EL xanthophores are a source of adult xanthophores, we fate-mapped these cells with nuclear localizing photoconvertible Eos (nEos) driven mosaically by the promoter of the xanthophore pigment synthesis gene *aox3* (*17*). EL xanthophores over the lateral myotomes lost their pigment by ~8 dpf, yet persisted and proliferated (Fig. 2A, fig. S8, and movie S5). Later, photoconverted cells were found within the interstripe, where many reacquired pigment; others, in the interstripe or in melanophore stripes, failed to reacquire pigment. Thus, some adult xanthophores originate directly from neural crest–derived EL xanthophores: These cells enter a cryptic period, in which they lose pigment and proliferate; some of these cells, localizing in the interstripe, then redifferentiate during adult pattern formation. Additional cells initiated *aox3* expression only after early larval stages and thus exhibited only unconverted nEos in the juvenile, indicating a source of adult xanthophores independent of EL xanthophores as well (Fig. 2B).

In thyroid-ablated fish, EL xanthophores persisted and new *aox3*$^+$ cells developed, but none acquired pigment by juvenile stages (Fig. 2C). Thus, TH is not essential for survival of xanthophore lineage cells but instead promotes their differentiation. In hyperthyroid *opallus* mutants, xanthophores were more likely to divide as compared to the wild type (fig. S9 and movie S6), and differentiated not only in the hypodermis but also extrahypodermally (Fig. 3A). Stable *aox3:GFP* (green fluorescent protein) reporter lines confirmed the presence of unpigmented precursors in hypothyroid larvae and the presence of these cells within melanophore stripes of euthyroid larvae (fig. S10); *aox3*:GFP$^+$ cells were also observed extrahypodermally even in the wild type (Fig. 3B). Thus, although interstripe xanthophores are the last adult pigment cell to differentiate (*7*) (fig. S4), xanthophore precursors are present throughout pattern formation, which suggests that interactions between melanophores and

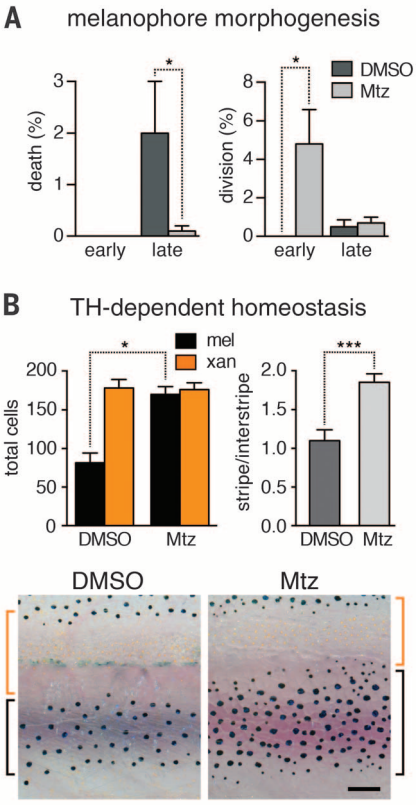

Fig. 4. TH repression of melanophores. (**A**) Melanophores of thyroid-ablated fish were less likely to die at a late stage of pattern formation (PB+; χ^2 = 35.7, df = 1, $P < 0.0001$) and were more likely to divide at an early stage (DR; genotype, χ^2 = 10.5, df = 1, $P < 0.005$; N = 45 larvae, 2510 melanophores). *$P < 0.05$, Tukey-Kramer post hoc test. (**B**) Thyroid ablation of juveniles resulted in more melanophores ($F_{1,19}$ = 50, $P < 0.0001$; *$P < 0.05$, Tukey-Kramer post hoc test) and wider stripes ($F_{1,19}$ = 19, $P <$ 0.0001, ***). Scale bar, 200 μm.

xanthophores that contribute to stripe patterning (*5*, *6*, *11*) depend on the differentiative states of the interacting cells. Additionally, because xanthophore precursors were widely distributed but differentiated principally in the interstripe—where their densities increased relative to stripes (fig. S10)—a close interplay of differentiation and migration is likely important for establishing and maintaining pattern.

Effects of TH on zebrafish xanthophores led us to ask whether xanthophores of other species are TH-dependent as well. Thus, we examined *D. albolineatus*, which has an evolutionarily derived pattern in which differentiated xanthophores are intermingled with melanophores (Fig. 3C) (*29*). In this species, we found extrahypodermal xanthophores similar to those of hyperthyroid *D. rerio* (Fig. 3, A and B, and fig. S11). To see whether TH requirements are conserved, we ablated thyroids of *D. albolineatus*. These fish had hypothyroid phenotypes, yet still developed extrahypodermal xanthophores and some hypodermal xanthophores (Fig. 3D and fig. S12); this finding suggests a partial evolutionary decoupling of xanthophore development from TH in *D. albolineatus*.

In contrast to xanthophores, melanophores were more numerous in hypothyroid fish of both species, and this was particularly evident in the interstripe of *D. rerio* (Fig. 1G). To test whether TH represses proliferation or survival of these cells, we treated fish with N-phenylthiourea to retard melanization of new melanophores and facilitate tracking of previously melanized cells (*28*) (fig. S13). In euthyroid larvae, melanophores rarely divided and often died in the interstripe. In hypothyroid larvae, these cells divided frequently and were more likely to survive, whereas in hyperthyroid larvae, melanophores differentiated but many died (Fig. 4A and movies S3, S4, S7, and S8). To assess roles for TH in earlier melanophore lineage development, we examined *mitfa:GFP*^{w47}-expressing presumptive melanophore precursors (*14*). *mitfa*:GFP$^+$ cells of hypothyroid larvae were less likely to differentiate, arrive at the hypodermis, or survive (fig. S14). These defects may indicate a metabolically compromised state in the absence of TH. Thus, TH promotes hypodermal melanophore precursor abundance and differentiation while simultaneously having a greater, repressive effect on melanophore proliferation and survival.

The preceding analyses focused on pattern development, but mechanisms are also needed for pattern maintenance (*5*, *10*, *11*), and patterning mechanisms at one stage may or may not be relevant at another. To test whether TH is required for homeostasis, we ablated thyroids after the adult pattern had formed. Six months later, these fish had extra melanophores, wider stripes, and craniofacial defects, yet xanthophore numbers were unchanged (Fig. 4B and fig. S15). Thus, TH limits melanophore population expansion homeostatically but is not required by xanthophores once they have differentiated. Roles for TH in amniote melanocytes have not been well studied, but our finding that TH limits melanophore numbers may be translationally relevant, given that hypothyroidism is especially prevalent among melanoma patients (*30*, *31*).

We have identified origins of adult xanthophores distinct from those of melanophores and iridophores, revealing a previously unappreciated diversity in postembryonic neural crest lineages underlying adult pigment pattern (fig. S16A). We also found that TH represses melanophore numbers and promotes xanthophore development, yet this latter dependency has been reduced in *D. albolineatus*, raising the possibility of a new compensatory factor in this species (fig. S16B). Our results indicate that responsiveness to "global" factors such as TH should be considered along with modifications to "local" interactions in attempting to understand pattern development and evolution. The different TH dependencies of these variously colored pigment cells (fig. S5C) further suggest the potential for complex selection involving behavior, endocrine mechanisms, and diverse pigment cell lineages arising from different sources and having different fate restrictions.

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ACKNOWLEDGMENTS

Supported by NIH grants R01 GM096906, R01 GM096906, and R03 HD074787 (D.M.P.), NIH grant P01 HD22486 (J.S.E.), and NIH grants F32 GM090362 and K99 GM105874 (S.K.M.). For assistance we thank O. Blackstone, T. Larson, J. Lewis, H. Majeski, E. Melancon, S. Palekha, T. Pham, A. Pruett, S. Redmond, J. Spiewak, and A. Wagner.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1358/suppl/DC1
Materials and Methods
Figs. S1 to S16
Movies S1 to S8
References (*32*–*37*)

19 May 2014; accepted 6 August 2014
Published online 28 August 2014;
10.1126/science.1256251

FISH PIGMENTATION

Local reorganization of xanthophores fine-tunes and colors the striped pattern of zebrafish

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The pattern of alternating blue and golden stripes displayed by adult zebrafish is composed of three kinds of pigment cells: black melanophores, yellow xanthophores, and silvery-blue iridophores. We analyzed the dynamics of xanthophores during stripe morphogenesis in vivo with long-term time-lapse imaging. Larval xanthophores start to proliferate at the onset of metamorphosis and give rise to adult xanthophores covering the flank before the arrival of stem-cell–derived iridophores and melanophores. Xanthophores compact to densely cover the iridophores forming the interstripe, and they acquire a loose stellate shape over the melanophores in the stripes. Thus, xanthophores, attracted by iridophores and repelling melanophores, sharpen and color the pattern. Variations on these cell behaviors may contribute to the generation of color pattern diversity in fish.

Many animals have evolved diversity in their color patterns, which serve as an essential component of their survival strategy. Replacing a much simpler larval pattern (Fig. 1, A and B), the zebrafish acquires a stereotypic pattern of four to five longitudinal dark stripes and four light interstripes (Fig. 1C) during the metamorphic period [20 to 45 days postfertilization (dpf)]. The stripes consist of melanophores covered by a thin layer of iridophores and xanthophores, whereas interstripes are composed of iridophores covered by xanthophores (*1*–*4*). Iridophores come in two morphologically distinct shapes that are clonally related: dense iridophores in interstripes and loose blue iridophores in stripes. The first light interstripe is formed by dense iridophores emerging along the horizontal myoseptum that serves as a morphological prepattern (*3*). At the margins of the first interstripe, iridophores switch shape and spread dorsally and ventrally as loose iridophores in the skin of the juvenile fish by proliferation and migration. They form a coherent net of cells covering the flank of the fish and generate additional light stripes at a distance from the horizontal myoseptum by patterned aggregation into dense iridophores (*5*). Melanoblasts emerge at their future position between these interstripes along peripheral neurons innervating the skin; they differentiate and expand in size to form the compact dark stripes (*5*–*7*). Thus, the dark and light stripes are formed by different cellular routes. Xanthophores are thinly distributed over the dark stripes and densely cover the iridophores in light stripes giving them a yellow tinge (*1*, *2*, *4*). Adult iridophores and melanophores originate from a small set of stem cells located at the segmentally reiterated dorsal root ganglia (*5*, *7*), but the origin of adult xanthophores has remained unknown.

Analysis of mutants lacking one or two of the three pigment cell types has revealed that interactions between all the three cell types are absolutely necessary for emergence of the final striped coloration (*3*, *8*). Iridophores play a leading role in stripe formation and mutants lacking iridophores do not add stripes to a basic pattern, whereas mutants lacking xanthophores (*pfeffer/fms/csl1a*) or melanophores (*nacre/mitfa*) exhibit a residual striped pattern, albeit an irregular one (fig. S1) (*3*, *8*–*10*). *pfeffer (pfe)* encodes *csf1a/fms* that is expressed and required specifically in xanthophores (*3*, *8*, *11*, *12*). In adult *pfe* mutants, a number of stripes and interstripes, although irregular, are discernible: The

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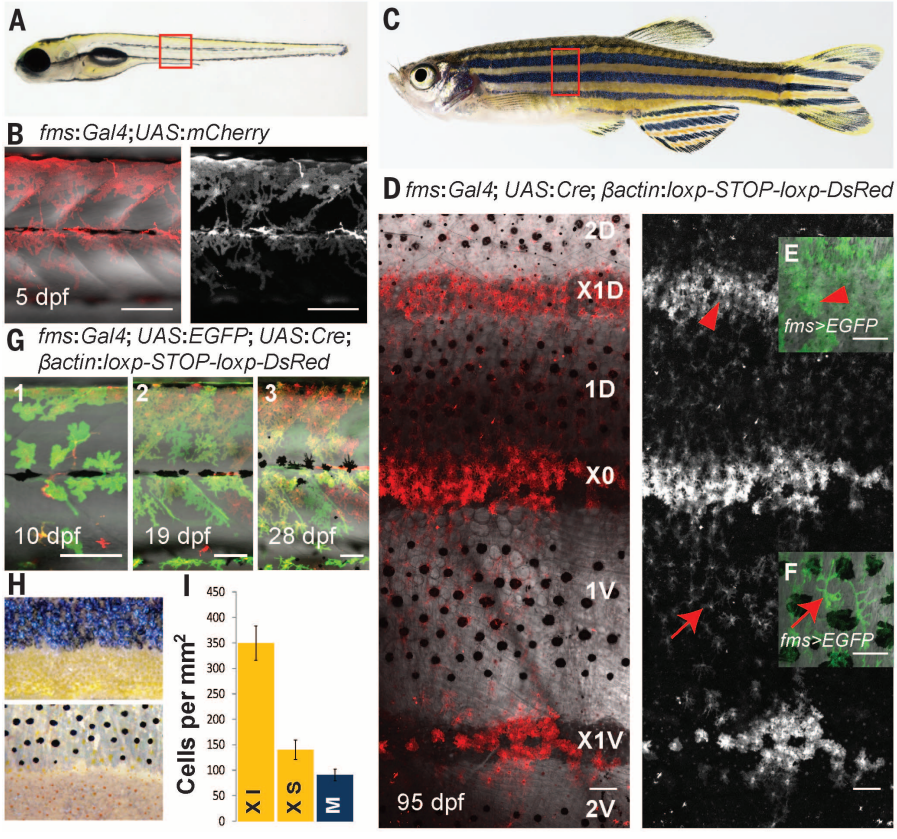

Fig. 1. Distribution of xanthophores in larval, metamorphic, and adult zebrafish. Pigmentation pattern and xanthophores of the (**A** and **B**) larva and (**C** and **D**) adult. Arrowhead, dense interstripe xanthophores; arrow, loose stripe xanthophores. Red boxes in (A) and (C) indicate approximate areas highlighted in (B) and (D), respectively. (**E** and **F**) Xanthophores at higher magnifications. EGFP, enhanced green fluorescent protein. (**G**, 1 to 3) Continuous development of xanthophores from larval to postmetamorphic stages. (**H**) Normal (top) and epinephrine-treated (bottom) zebrafish adult skin. (**I**) Density of interstripe xanthophores (XI), stripe xanthophores (XS), and melanophores (M) in adult zebrafish. Error bars denote SD. Scale bars, 100 μm.

melanophore stripes break up into spots; dense iridophore regions invade the stripes; and further, ectopic melanophores are observed in the interstripe regions. Introduction of xanthophore progenitor cells into *pfe* mutant embryos by blastomere transplantations restores a normal pattern indicating the importance of xanthophores in stripe sharpening and coloration (*8*, *11*). We examined xanthophore origin and behavior in vivo to establish their role in color pattern formation.

We used *Tg(fms:Gal4.VP16)* (*13*) in combination with appropriate UAS-reporter lines or *Tg(Pax7:GFP)* (GFP, green fluorescent protein) (see supplementary materials and methods) to label xanthophores from larval stages throughout metamorphosis (Fig. 1). Labeled cells were identified on the dorsal side of the embryo at ~24 hours postfertilization (movie S1). These cells migrate while dividing along the dorsolateral path of neural crest migration, differentiate into xanthophores and spread over the larval skin, ventrally decreasing in density (*1*) (Fig. 1, A and B). The larva at 5 dpf displays six to eight xanthophores per segment. In the adult fish, xanthophores are distributed over the stripes and interstripes all along the skin (Fig. 1, C and D). The xanthophores associated with the interstripe region were densely packed, expanded, possessed small filopodial protrusions, and displayed bright orange pigment (arrowheads in Fig. 1, D, E, and H, and fig. S2). In contrast, xanthophores in the stripe region displayed a netlike, loose organization; they were arborized, had long filopodial protrusions, and displayed faint yellow pigment (arrows in Fig. 1, D, F, H, and fig. S2). Xanthophore density was ~2.5 times higher in the interstripe region as compared with the stripe region (Fig. 1I and fig. S2). This indicates two distinct types of xanthophores: (i) compact and bright in the interstripes and (ii) stellate and faint in the stripes.

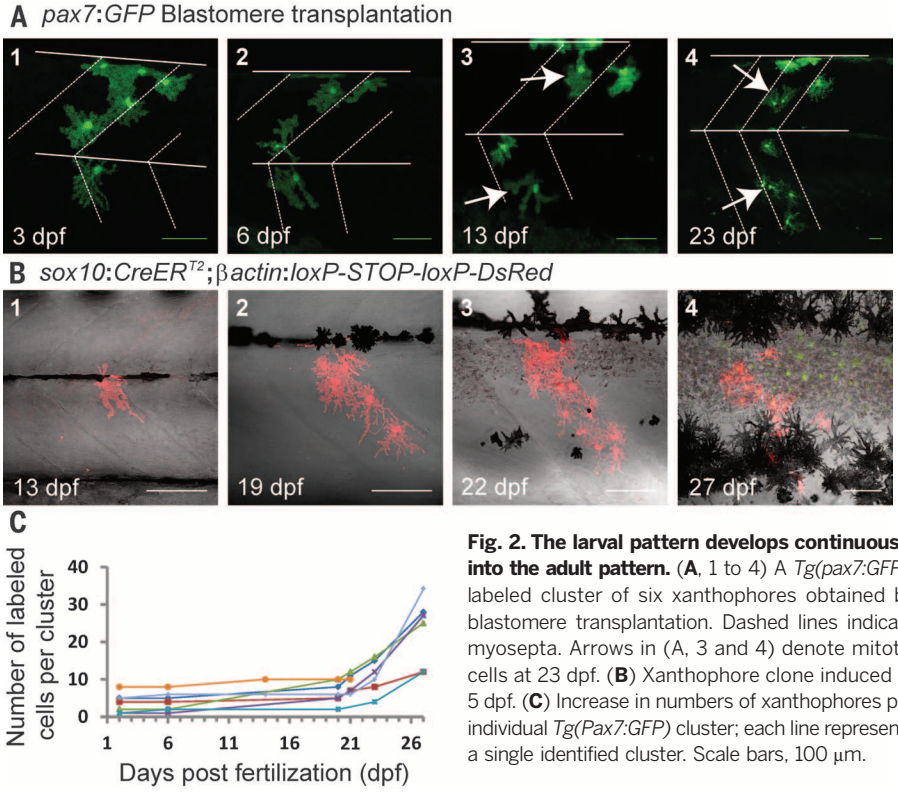

Fig. 2. The larval pattern develops continuously into the adult pattern. (**A**, 1 to 4) A *Tg(pax7:GFP)*-labeled cluster of six xanthophores obtained by blastomere transplantation. Dashed lines indicate myosepta. Arrows in (A, 3 and 4) denote mitotic cells at 23 dpf. (**B**) Xanthophore clone induced at 5 dpf. (**C**) Increase in numbers of xanthophores per individual *Tg(Pax7:GFP)* cluster; each line represents a single identified cluster. Scale bars, 100 μm.

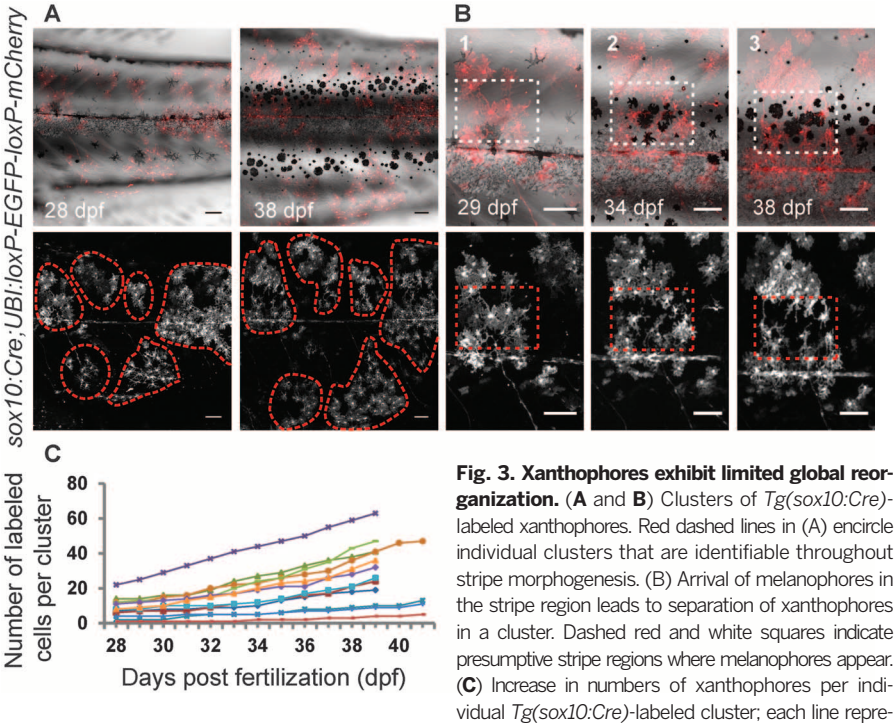

Fig. 3. Xanthophores exhibit limited global reorganization. (**A** and **B**) Clusters of *Tg(sox10:Cre)*-labeled xanthophores. Red dashed lines in (A) encircle individual clusters that are identifiable throughout stripe morphogenesis. (B) Arrival of melanophores in the stripe region leads to separation of xanthophores in a cluster. Dashed red and white squares indicate presumptive stripe regions where melanophores appear. (**C**) Increase in numbers of xanthophores per individual *Tg(sox10:Cre)*-labeled cluster; each line represents a single identified cluster. Scale bars, 100 μm.

Time-course analysis of xanthophores revealed that the larval xanthophores persisted, increased in number at the onset of metamorphosis, and finally covered the entire dorsolateral skin (Fig. 1G). To obtain labeled clusters of xanthophores for lineage analysis, we transplanted cells of *Tg(pax7:GFP)* embryos into wild-type hosts at the blastula stage (Fig. 2A). The xanthophore number remained constant until the onset of metamorphosis (~16 to 20 dpf), when xanthophores begin to divide and increase in number continuously with a doubling time of about 1 week (five fish, seven clusters of xanthophores) (Fig. 2C). Second, we analyzed xanthophore clones induced in *Tg(sox10:ER^{T2}-Cre)* larvae in which tamoxifen-inducible Cre was expressed under the *sox10* promoter (*14*). DsRed-labeled larval xanthophores in such clones gave rise to metamorphic xanthophores (six clones) (Fig. 2B). In contrast to previous studies (*10*, *15*), our in vivo analysis suggests that xanthophores, albeit barely visible by pigment content, are the first pigment cell type to cover the trunk skin before the appearance of metamorphic iridophores and melanophores (Figs. 1G and 2B and figs. S3 and S4).

We further analyzed the behavior of clusters (coherent groups of clones) of labeled xanthophores in *Tg(sox10:Cre)* (*16*) individuals throughout metamorphosis (Fig. 3A and fig. S5). The xanthophore clusters maintained their relative positions during the formation of the stripes and interstripes, whereas numbers of xanthophores in each cluster continuously increased throughout metamorphosis (Fig. 3C and fig. S6) by proliferation (fig. S7). Thus, there is no global reorganization of xanthophores during stripe morphogenesis (fig. S9). However, arrival of the iridophores during metamorphosis lead to a compaction of the overlaying xanthophores (fig. S4). Furthermore, upon arrival of melanophores in the stripe region, the xanthophores underwent

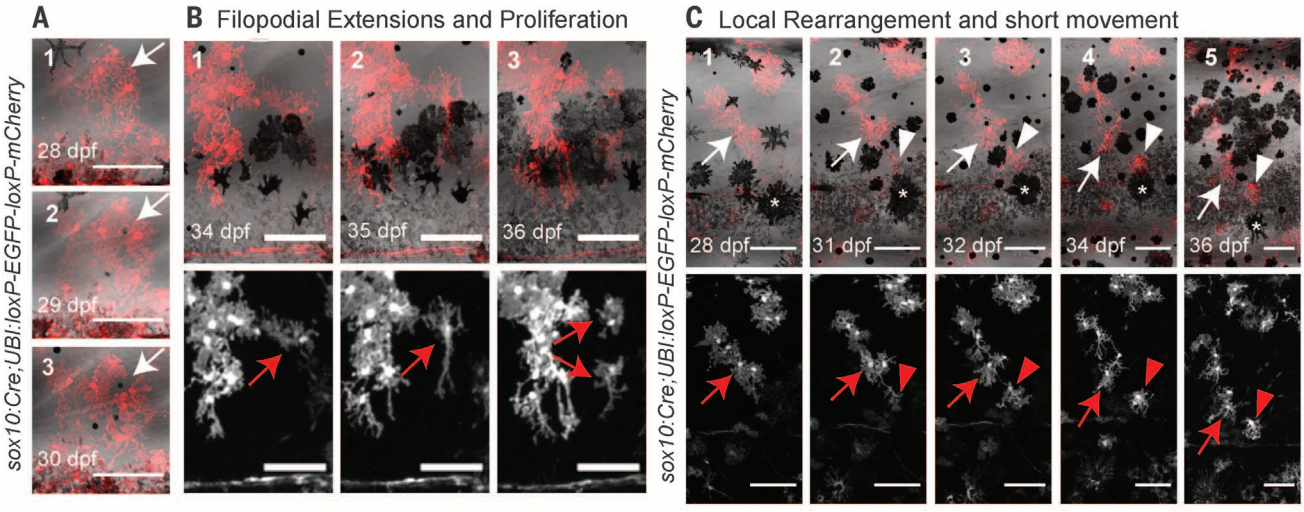

Fig. 4. Context-dependent changes in xanthophore behavior during stripe pattern formation. Time-lapse imaging of labeled xanthophores during metamorphosis. (**A**, 1-3) Xanthophores present in the presumptive stripe region give space (arrows) to an arriving melanophore. (**B**, 1-3) A xanthophore near the stripe-interstripe boundary extends filopodial protrusions (arrows) directed toward the interstripe and undergoes cell division. (**C**, 1-5) A xanthophore (arrows) locally reorganizes upon arrival of melanophores in the stripe region. Arrowheads indicate short movement of the xanthophore into the interstripe region. Asterisks indicate a melanophore that moves away from the interstripe upon encountering a xanthophore (arrowheads). Scale bars, 100 μm.

local rearrangement to accommodate the increasing number of melanophores (Figs. 3B and 4). Over the period of metamorphosis, more melanophores appeared in the skin and expanded, forming a compact stripe (*5*). This resulted in cellular reorganization and separation of the xanthophores and a decrease in xanthophore density (Figs. 1, D to I, and 3B and fig. S6B). Xanthophores of the stripe region reorganized their filopodia to give way to melanophores that appeared in their vicinity (Fig. 4A). The xanthophores spread out and became stellate with long filopodial extensions (arrows in Fig. 4, B and C) forming a netlike organization in the stripe region (figs. S8 and S9).

Xanthophores at the boundary of the developing stripes and interstripes often exhibited filopodial extensions directed toward the interstripe region, leading to cell division followed by integration of the proximal xanthophore into the interstripe (arrows in Fig. 4B) or a short-scale movement of the xanthophore into the interstripe region (arrowheads in Fig. 4C, and fig. S10). Limited local proliferation and context-specific local reorganization was the most prominent behavior exhibited by xanthophores (Fig. 4C). This is in contrast to iridophores, which exhibit extensive local proliferation combined with long-range dispersal along the dorsoventral axis and prefigure the stripe organization by patterned aggregation (*5*). Genetic analyses have indicated a role for xanthophores in sustaining melanophore numbers but also in sharpening the stripe-interstripe boundary by mutual repulsion (*3*, *8*, *11*). Xanthophores present in the first interstripe take part in elimination of the larval lateral stripe melanophores, as well as metamorphic melanophores that get trapped in the interstripe. In contrast, the stellate xanthophores of the stripes might be required for survival of melanophores and for compact stripe formation and maintenance. In summary, we found that the striped organization of pigment cells involves proliferation, local rearrangements, and short-scale movements of xanthophores in the skin of the fish.

Our observations do not support Turing-type models in their current form, which assume that stripe formation starts with a random distribution of melanophores and xanthophores that sort out by repulsion and attraction involving extensive cell movements (*15*, *17*–*19*). These models may hold for patterning in the fins [however, see (*20*)] but would require substantial modification for the body stripes to accommodate the cellular behaviors of xanthophores and melanophores observed in the present study, the *pfe* mutant phenotype, and the involvement of iridophores (*3*, *5*, *8*). Long-term imaging indicates that stripe melanophores barely move, whereas xanthophore movement is restricted to the boundary region between stripe and interstripe. The interactions between all the three kinds of pigment cells and their environment are crucial for stripe pattern formation (*3*, *8*, *10*). Xanthophores play a permissive role in the formation of the striped pattern by covering the skin of metamorphic fish, into which metamorphic iridophores and melanophores get integrated (schematic in fig. S11). The striped pattern is achieved by context-dependent cell shape changes occurring in both iridophores and xanthophores, which switch between loose and dense shapes. In both cases, the two forms are clonally related and depend on local cell-to-cell interactions. Melanophores in the stripes exist in only one shape. The precise superposition of the dense form of iridophores and xanthophores in the interstripe and the loose iridophores and xanthophores superimposed over the stripe melanophores produces a marked contrast between the golden and blue coloration of the pattern.

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ACKNOWLEDGMENTS

We thank A. Mongera and S. Alsheimer for providing transgenic lines before publication; U. Irion, C. Söllner, and P. Müller for comments on the manuscript; and S. Perathoner, H.-G. Frohnhöfer, A. Fadeev, U. Schach, H. Heth, and our fish facility, as well as C. Liebig and our light microscopy facility, for support. This work was funded by the Max-Planck Society. A.P.S. received a postdoctoral fellowship (EMBO-LTF) from the European Molecular Biology Organization.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1362/suppl/DC1
Materials and Methods
Figs. S1 to S11
References (*21*–*31*)
Movie S1

15 April 2014; accepted 6 August 2014
Published online 28 August 2014;
10.1126/science.1254837

ORIGIN OF NOTOCHORD

Development of the annelid axochord: Insights into notochord evolution

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The origin of chordates has been debated for more than a century, with one key issue being the emergence of the notochord. In vertebrates, the notochord develops by convergence and extension of the chordamesoderm, a population of midline cells of unique molecular identity. We identify a population of mesodermal cells in a developing invertebrate, the marine annelid *Platynereis dumerilii*, that converges and extends toward the midline and expresses a notochord-specific combination of genes. These cells differentiate into a longitudinal muscle, the axochord, that is positioned between central nervous system and axial blood vessel and secretes a strong collagenous extracellular matrix. Ancestral state reconstruction suggests that contractile mesodermal midline cells existed in bilaterian ancestors. We propose that these cells, via vacuolization and stiffening, gave rise to the chordate notochord.

Defining the properties and characteristics of the last common ancestor of bilaterian animals, Urbilateria, is a key question of the evolution and development field (*1*). In an attempt to infer a possible urbilaterian precursor for the chordate notochord (*2*, *3*), we reasoned that this structure should occupy a similar position with regard to overall morphology and molecular topography during development and in the adult body plan of living descendants (Fig. 1, A and B); that it should express, during its development, a suite of genes that have proven specific and indispensable for notochord formation in the chordates; and that it should be of widespread occurrence in bilaterian body plans (Fig. 1C). We focused our search on the model annelid *Platynereis dumerilii*, which is amenable to molecular studies and has retained more ancestral features than *Drosophila melanogaster* or *Caenorhabditis elegans* (*4*). By looking for cell populations that would resemble the vertebrate chordamesoderm (a population of mesodermal midline cells that converge medially to give rise to the notochord; red in Fig. 1A), we identified segmental pairs of mesodermal cells on the non–bone morphogenetic protein (BMP) body side (*5*) that stood out by early and continuous expression of *colA1*, encoding collagen type A (Fig. 2, A to D). SiMView light sheet microscopy (*6*) revealed that these cells moved underneath the neuroectoderm toward the midline until they contacted their bilateral counterpart (movie S1 and Fig. 2E). Subsequently, these cells narrowed and elongated without a net increase in cell surface (fig. S1), and additional adaxial mesodermal cells were observed to intercalate between the elongating pairs (Fig. 2E), reminiscent of the processes by which the chordamesoderm converges and extends (table S1) (*7*). Lineage tracking by targeted photoconversion of the fluorescent protein kikGR confirmed the origin of these cells from the mesodermal bands (fig. S2).

The unique location, large size, and specific arrangement of the *Platynereis* mesodermal midline cells allowed their unambiguous identification after whole-mount in situ hybridization (WMISH) and thus expression profiling by confocal imaging. To test a possible homology of these cells with the chordamesoderm, we chose a chordamesoderm-specific gene set according to the following criteria: (i) specificity—their combined expression uniquely defines the chordamesoderm; (ii) conservation—their chordamesoderm expression is conserved in at least three of four vertebrate species; and (iii) function—they have proven essential for chordamesoderm development or signaling. We thus investigated expression of seven transcription factors (*brachyury*, *foxA*, *foxD*, *twist*, *not*, *soxD*, and *soxE*), the signaling molecules *noggin* and *hedgehog* [*chordin* appears absent from annelid genomes (*8*)], and the guidance factors *netrin* and *slit* (table S2 for references). Transcripts for all but one [the *not* gene (*2*)] were detected (figs. S3 to S5) in accordance with previously reported *brachyury* expression (*9*), and their coexpression confirmed by double WMISH (Fig. 2, F to L). Although none of the genes were exclusively expressed in the annelid mesodermal midline, their combined coexpression was unique to these cells (implying that mesodermal midline in annelids and chordamesoderm in vertebrates are more similar to each other than to any other tissue). It is unlikely that the molecular similarity between annelid and vertebrate mesodermal midline is due to independent co-option of a conserved gene cassette, because this would require either that this cassette was active elsewhere in the body (which is not the case) or that multiple identical independent events of co-option occurred (which is unparsimonious). As in vertebrates, the mesodermal midline resembles the neuroectodermal midline, which expresses *foxD*, *foxA*, *netrin*, *slit*, and *noggin* (figs. S6 and S7) but not *brachyury* or *twist*. However, unlike in chicken (*10*), the annelid mesodermal and ectodermal midline populations are not directly related by lineage (fig. S2). Last, the *Platynereis* mesodermal midline is devoid of *paraxis*, which is exclusively expressed in laterally adjacent mesoderm (fig. S8), in line with its vertebrate ortholog demarcating paraxial mesoderm (*11*). In vertebrates, this segregation depends on canonical Wnt signaling, with β-catenin–positive cells preferentially adopting a paraxial fate and position (*12*). Consistently, we observed nuclear localization of β-catenin in the more-lateral mesoderm only, and β-catenin overactivation converted the mesodermal midline toward a more lateral fate and position (fig. S8).

We next compared the developmental fate of annelid and vertebrate mesodermal midline cells. Phalloidin staining and expression analysis of muscle markers (fig. S9) revealed that, after elongation, the *Platynereis* mesodermal midline cells differentiate into the previously described "medial ventral longitudinal muscle" (*13*) (Fig. 3A). Given the ropelike appearance and axial position of this muscle, we propose to call it "axochord." A muscular nature of a putative invertebrate counterpart of the chordate notochord is consistent with the observation that in the most basal chordate, amphioxus, the notochord is composed of specialized muscle cells (*14*) and expresses the same muscle markers (*15*). We further observed segmental sets of transverse muscles connecting to the axochord ("ventral oblique muscles") (*13*) (Fig. 3, A and B, and fig. S3). Scanning electron microscopy revealed that, in adult worms, the axochord is deeply embedded in the fibrous sheath of the ventral nerve cord (*16*) and remains connected to the transverse muscles (Fig. 3, C and D). Immunostainings confirmed its axial position between neuropil and blood vessel (fig. S12; similar to the notochord; Fig. 1, A and B). Axochord contractility was evident from live imaging (fig. S9, E to G, and movie S2) and occurred in alternation with the transverse muscles (movie S3). Electron micrographs confirmed the muscular nature of axochordal cells and revealed a tight physical connection to transverse muscles (Fig. 3, E to I). Laser ablation of the axochord impaired crawling (fig. S10 and movie S4) and confirmed anchoring of the transverse musculature. Additionally, we found that the

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Platynereis transverse muscles share a specific molecular profile (*en+foxD*; fig. S11) with the vertebrate pioneer myocytes flanking the notochord (*17*).

We next examined whether an axochord is also present in other annelids, lophotrochozoans, or protostomes (Fig. 1C). Phalloidin stainings had revealed ventral midline muscles in almost all annelid families (table S3), yet in some cases only pairs of ventral muscles had been reported (*18*, *19*). One such example is *Capitella teleta*, which belongs to the second big clade of annelids, the Sedentaria (beside Errantia, to which *Platynereis* belongs). We investigated development and molecular identity of ventral muscle fibers in *Capitella* (*18*) and found that (i), preceding metamorphosis, these converge and fuse into an axochord (Fig. 4, A to D, and fig. S13); (ii) the expression patterns of *foxA*, *noggin*, *brachyury*, *netrin* (Fig. 4, B and D, and fig. S13, B and C); *twist2* (*20*); and *hedgehog* (*21*) are consistent with coexpression in the axochord; and (iii) pairs of transverse muscles connect to the *Capitella* axochord (Fig. 4E) as in *Platynereis*. We also investigated the annelid *Owenia fusiformis* that belongs to the most basal annelid family (*22*) and likewise found an axochord connected to transverse muscle fibers (Fig. 4F). A similar arrangement also occurs in mollusk (*23*) and brachiopod larvae (*24*), and ventral midline muscles are observed in most lophotrochozoan phyla (table S3), suggesting that an axochord is ancestral for lophotrochozoa. The lophotrochozoan axochord is a genuinely paired structure, composed of left and right adjacent muscle strands that often bifurcate anteriorly and/or posteriorly, as also observed in chaetognaths (Fig. 4, G and H), a possible protostome outgroup (*25*, *26*) (Fig. 1C).

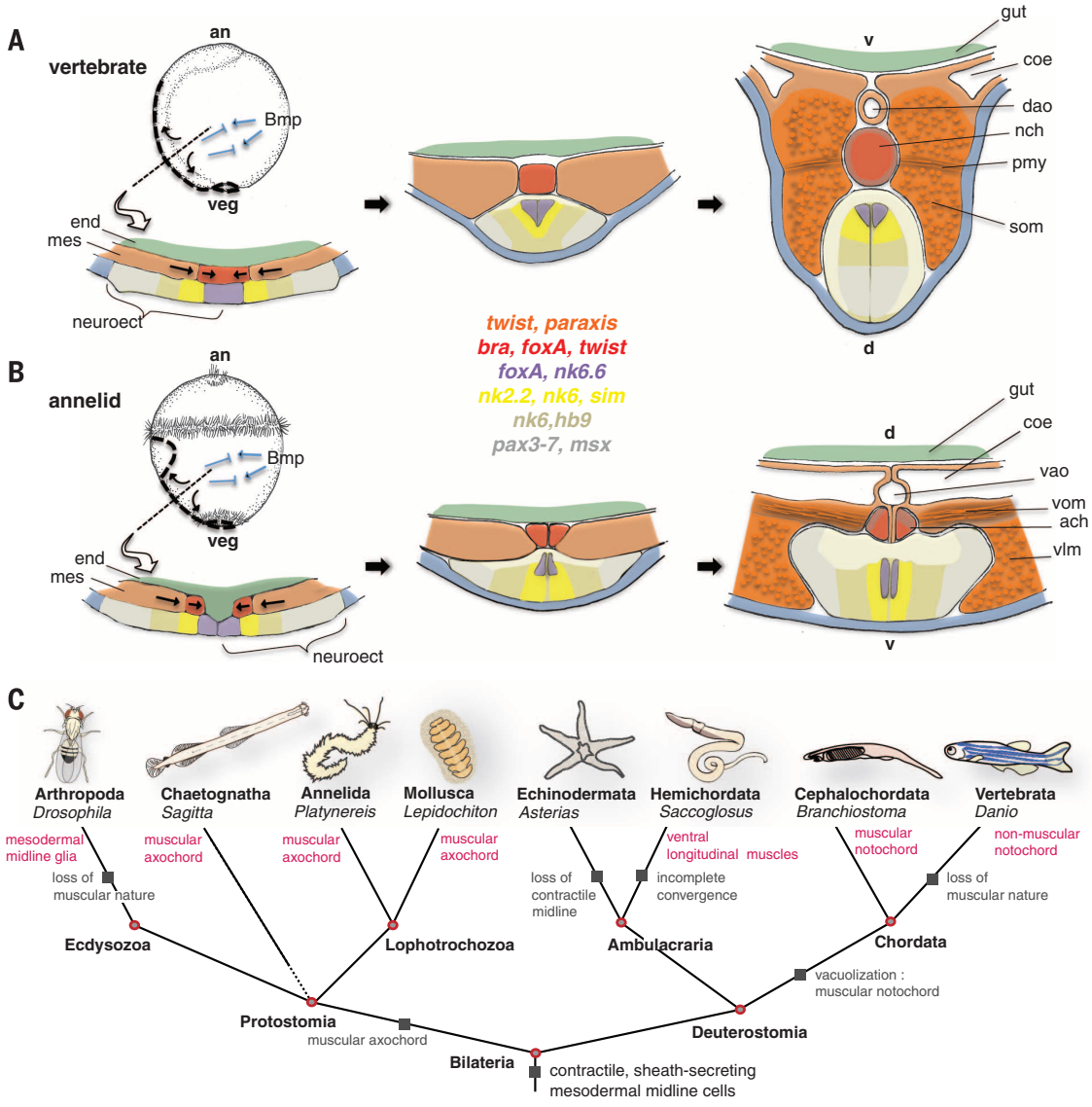

Fig. 1. Comparison of notochord and axochord development, gene expression, and anatomy. (**A**) Notochord development schematized for zebrafish at 9 hours post fertilization (hpf) or 90% epiboly, 14 hpf/neural keel, and 30 hpf/organogenesis stages. (**B**) Axochord development schematized for *Platynereis* at 34 hpf, 72 hpf, and 2 months of development. Top left images are in similar orientation with regard to the animal (an)–vegetal (veg) axis. Bold dashed lines represent lines of convergence of neuroectodermal and mesodermal cells, opposite to BMP signaling (blue arrows). The non-BMP body side will be dorsal (d) in vertebrate and ventral (v) in annelid, reflecting inversion of body posture in early chordate evolution (*35*). Thin black arrows indicate convergence and extension. Thin black dashed lines indicate positions of transverse sections (bottom left). Red, notochord (nch) or axochord (ach); orange, mesoderm (mes); purple, neuroectodermal midline; yellow, medial interneuron column; faint yellow, motor neuron column; gray, sensory interneuron column; blue, epidermis; green, endoderm (end)/gut. Transcription factors defining the respective tissues are written in corresponding colors. Bold black arrows indicate developmental progression. neuroect, neuroectoderm; coe, coelom; dao, dorsal aorta; pmy, primary myocyte; som, somite; vao, ventral aorta; vom, ventral oblique muscle; vlm, ventral longitudinal muscle. (**C**) Simplified phylogenetic tree. Black boxes illustrate the proposed evolutionary transition from ventral midline contractile cells to notochord.

An ancestral state reconstruction based on our data and on a survey of bilaterian musculature (table S3) favored the presence of an axochord in protostome ancestors (fig. S14).

Regarding deuterostomes, previous studies on the origin of the notochord focused on the hemichordate stomochord, an unpaired chordoid outpocketing of the pharynx, as a possible notochord homolog (*27*). Speaking against this hypothesis is its very anterior position and the absence of *brachyury*, *foxA*, and *noggin* expression (*27*, *28*). *goosecoid*, *hedgehog*, and *colA* expression rather suggest homology to the vertebrate prechordal plate (*29*–*33*). The pygochord, a stiff vacuolated rod in the posterior trunk of ptychoderid hemichordates (*34*), lies dorsal to the ventral blood vessel in the ventral mesentery. This stands in contrast to both axochord and

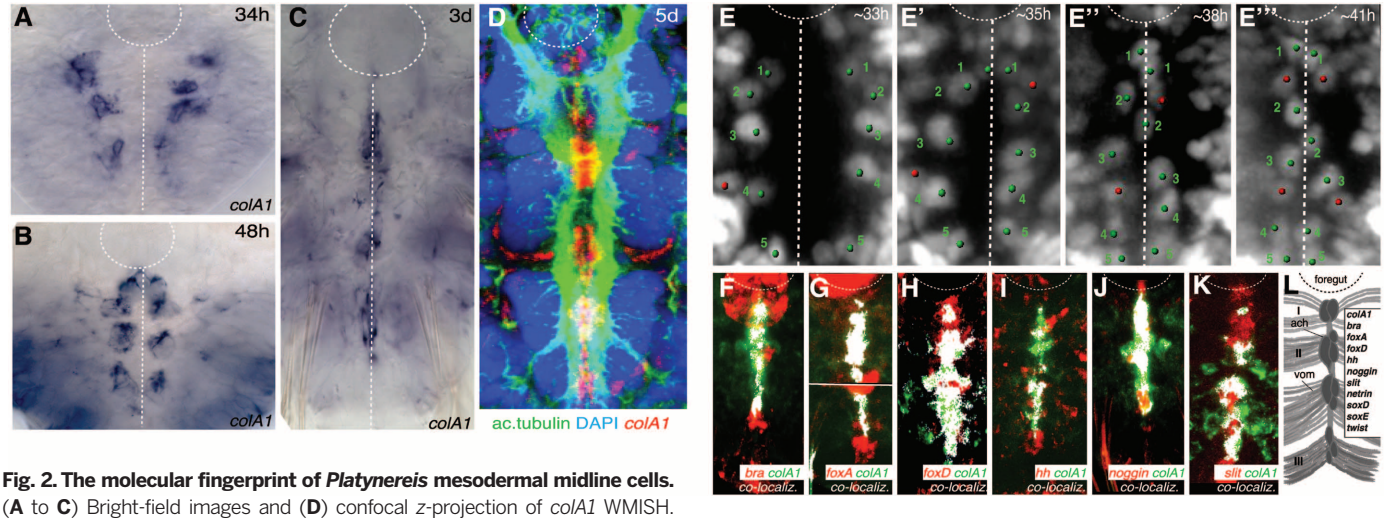

Fig. 2. The molecular fingerprint of *Platynereis* mesodermal midline cells. (**A** to **C**) Bright-field images and (**D**) confocal *z*-projection of *colA1* WMISH. Dotted white circle and line indicate foregut and midline, respectively. DAPI, 4′,6-diamidino-2-phenylindole. (**E** to **E'''**) Snapshots of SiMView time lapse of a live larva with fluorescently labeled nuclei showing ventrally converging (green dots) and intercalating (red dots) axochordal cells. Time interval, 2 to 3 hours. (**F** to **K**) Confocal *z*-projections of double WMISH 3-dpf larvae, ventral views, anterior up. (**L**) Explanatory scheme. vom also weakly express *colA* and *foxD*. Foregut also expresses *bra* and *foxA*.

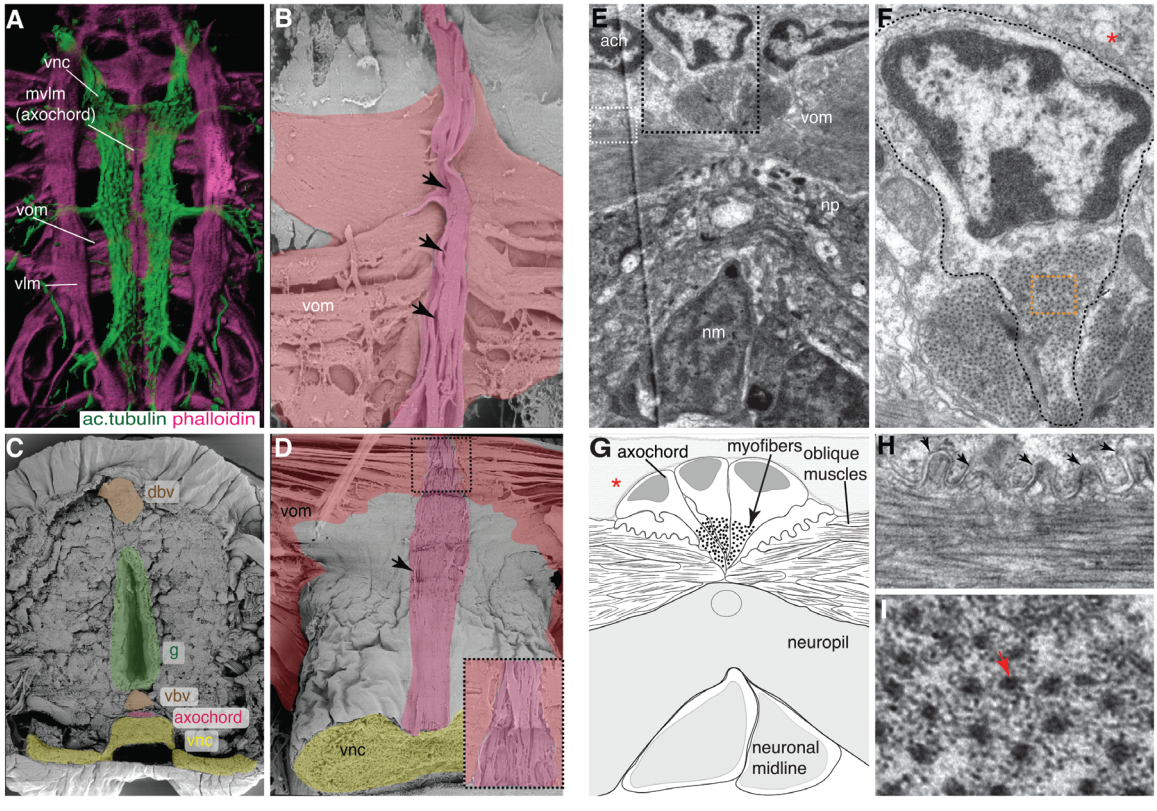

Fig. 3. The axochord is a ventral medial longitudinal muscle. (**A**) Ventral view of *Platynereis* immunolabeled musculature and nervous system, *z*-projection of confocal stack. Vnc, ventral nerve cord; mvlm, median ventral longitudinal muscle (axochord). (**B**) Pseudocolored scanning electron micrograph of *Platynereis* juvenile trunk, dorsal view. Axochord (deep pink) and attached oblique muscles (light pink). (**C** and **D**) Pseudocolored scanning electron micrographs. (C) Adult cross section. g, gut; vbv/dbv, ventral/dorsal blood vessel. (D) Dissected specimen showing axochord and oblique muscles embedded in the vnc sheath. Closeup illustrates axochord cell morphology. (**E**) Transmission electron micrograph showing axochordal cells, ventral oblique muscles, neuronal midline (nm), and the neuropil (np). (**F**) Closeup of area in black square in (E). One axochordal cell is outlined with dashed black line; asterisk indicates extracellular matrix. (**G**) Schematic drawing of (E). (**H**) Closeup of are in white square in (E), showing interdigitations between axochordal cells and oblique muscles. (**I**) Closeup of (F) (orange square) showing cross-sectioned myofibers (red arrow).

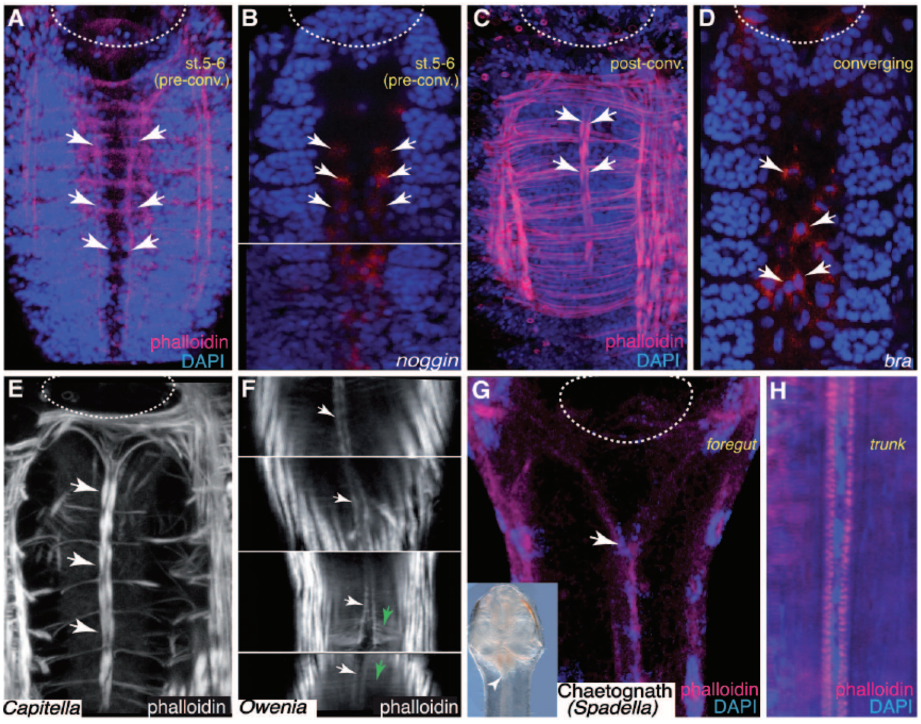

Fig. 4. An axochord is widespread in protostomes. (**A** to **D**) Convergence of ventral muscles into an axochord (white arrows) in *Capitella*, illustrated by phalloidin stainings (A and C) and WMISH (red in B and D) of selected axochord markers. (**E**) Axochord and transverse musculature in a stage-9 *Capitella* larva. Axochord bifurcation at the level of the foregut. (**F**) Axochord (white arrows) and transverse muscle fibers (green arrows) in juvenile *Owenia*, below the circumferential layer of longitudinal muscles. (**G**) Axochord (white arrow) in adult chaetognath (inset) bifurcating at the level of foregut. (**H**) Closeup of the axochord in the same specimen. Note elongated median nuclei and the two parallel strands of striated myofibers. All panels are *z*-projections of confocal stacks, ventral view, anterior up, white dashed line outlining foregut.

notochord, which are positioned between blood vessel and nerve cord (Fig. 1). Thus, vacuolization in the hemichordate stomochord and pygochord might have occurred independent to that of the chordate notochord. Unfortunately, no data are available for the specification and developmental fate of ventral mesodermal midline cells in hemichordates or larval echinoderms; except for *Protoglossus*, no ventromedian musculature has yet been observed (table S3).

Our study of annelid development reveals a population of mesodermal cells that converge and extend along the ventral midline and express a combination of transcription factors, signaling molecules, and guidance factors that closely matches that of the vertebrate chordamesoderm. These comparative data suggest that a similar population of mesodermal midline cells already existed in urbilaterian ancestors but leave open its ancient developmental fate. In annelids, these cells differentiate into an axochord; our investigation of chaetognath musculature and an ancestral state reconstruction based on comparative anatomy (fig. S14) suggest that a similar paired longitudinal muscle existed in protostome ancestors. Yet, in the absence of detailed investigations of expression profile and developmental fate of mesodermal midline cells in basal ecdysozoans and deuterostome ambulacrarians, the nature of ventral midline tissue in urbilaterians remains undecided. It might have constituted sheath-secreting mesenchyme that was independently converted into muscular axochord and notochord in lophotrochozoans and chordates, respectively; alternatively, this tissue might have been contractile already and transformed into axochord in protostomes and notochord in chordates (Fig. 1C). Regardless of its nature, dorsoventral axis inversion (*35*) would have brought the ventral midline tissue into a dorsal position in the chordate lineage, and the appearance of incompressible vacuoles (*14*) would have gradually transformed it into a stiff rod of constant length; the amphioxus notochord could then be regarded a vestige of a contractile-cartilaginous transition. This transition could have involved the co-option of *not* expression (which is absent from the axochord, see above) given that zebrafish *not* null mutants form muscle tissue instead of notochord (*36*). Last, in vertebrates, the notochord was complemented by a rigid backbone that crucially contributed to the success of our phylum.

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ACKNOWLEDGMENTS

We thank A. Miyawaki for kikGR, R. Renkawitz-Pohl for the antibody against β-tubulin; P. McCrea for the antibody against β-catenin, A. Altenburger for brachiopod data; S. Kaul-Strehlow, G. Mayer, M. V. Sørensen, and K. Worsaae for insightful discussions; I. Haußer-Siller at the Electron Microscopy Core Facility (EMCF) (BioQuant, Heidelberg University); and the EMBL EMCF. The work was supported by the European Union's Seventh Framework Programme project "Evolution of gene regulatory networks in animal development (EVONET)" [215781-2] (A.L.), the Zoonet EU-Marie Curie early training network [005624] (A.F. and R.T.), the European Molecular Biology Laboratory (A.L., T.B., M.T.H., A.H.L.F., O.S.,P.R.H.S., and R.T.), the EMBL International Ph.D. Programme (A.L., T.B., A.H.L.F., P.R.H.S., R.T.), the Boehringer Ingelheim Foundation (O.S.), the Howard Hughes Medical Institute (P.J.K.), and the Visiting Scientist Program at the Janelia Farm Research Campus (M.H.T., R.T., D.A., and P.J.K.). A.L. and T.B. designed, performed, and documented most of the experiments; developed protocols; and wrote the manuscript. R.T. initiated and, together with M.H.T. and P.J.K., developed the *Platynereis* SiMView live imaging assay, and M.H.T. acquired the SiMView data. A.H.L.F. investigated musculature development and gene expression in *Platynereis*. M.H.T. contributed to the expression analysis. O.S. did transmission electron microscopy with T.B. and A.L. P.R.H.S. cloned and characterized *twist*. R.T. developed the injection protocol with N. Kegel and B. Backfisch. D.A. coordinated the study, wrote the manuscript, and drew the summary figure. The plasmid encoding the kikGR construct is covered by a material transfer agreement provided by the RIKEN Brain Science Institute. Alignments used for tree building are deposited on Dryad (10.5061/dryad.1ct82).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1365/suppl/DC1
Materials and Methods
Figs. S1 to S16
Tables S1 to S3
References (*37*–*152*)
Movies S1 to S4

14 March 2014; accepted 6 August 2014
10.1126/science.1253396

VIRAL EVOLUTION

Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak

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In its largest outbreak, Ebola virus disease is spreading through Guinea, Liberia, Sierra Leone, and Nigeria. We sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone to ~2000× coverage. We observed a rapid accumulation of interhost and intrahost genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African lineages around 2004, crossed from Guinea to Sierra Leone in May 2014, and has exhibited sustained human-to-human transmission subsequently, with no evidence of additional zoonotic sources. Because many of the mutations alter protein sequences and other biologically meaningful targets, they should be monitored for impact on diagnostics, vaccines, and therapies critical to outbreak response.

Ebola virus (EBOV; formerly Zaire ebolavirus), one of five ebolaviruses, is a lethal human pathogen, causing Ebola virus disease (EVD) with an average case fatality rate of 78% (*1*). Previous EVD outbreaks were confined to remote regions of central Africa; the largest, in 1976, had 318 cases (*2*) (Fig. 1A). The current outbreak started in February 2014 in Guinea, West Africa (*3*) and spread into Liberia in March, Sierra Leone in May, and Nigeria in late July. It is the largest known EVD outbreak and is expanding exponentially, with a doubling period of 34.8 days (Fig. 1B). As of 19 August 2014, 2240 cases and 1229 deaths have been documented (*4*, *5*). Its emergence in the major cities of Conakry (Guinea), Freetown (Sierra Leone), Monrovia (Liberia), and Lagos (Nigeria) raises the specter of increasing local and international dissemination.

In an ongoing public health crisis, where accurate and timely information is crucial, new genomic technologies can provide near–real-time insights into the pathogen's origin, transmission dynamics, and evolution. We used massively parallel viral sequencing to understand how and when EBOV entered human populations in the 2014 West African outbreak, whether the outbreak is continuing to be fed by new transmissions from its natural reservoir, and how the virus changed, both before and after its recent jump to humans.

In March 2014, Kenema Government Hospital (KGH) established EBOV surveillance in Kenema, Sierra Leone, near the origin of the 2014 outbreak (Fig. 1C and fig. S1) (*6*). Following standards for field-based tests in previous (*7*) and current (*3*) outbreaks, KGH performed conventional polymerase chain reaction (PCR)–based EBOV diagnostics (*8*) (fig. S2); all tests were negative through early May. On 25 May, KGH scientists confirmed the first case of EVD in Sierra Leone. Investigation by the Ministry of Health and Sanitation (MoHS) uncovered an epidemiological link between this case and the burial of a traditional healer who had treated EVD patients from Guinea. Tracing led to 13 additional cases—all females who attended the burial. We obtained ethical approval from MoHS, the Sierra Leone Ethics and Scientific Review Committee, and our U.S. institutions to sequence patient samples in the United States according to approved safety standards (*6*).

We evaluated four independent library preparation methods and two sequencing platforms

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‡Deceased. §These authors jointly supervised this work.

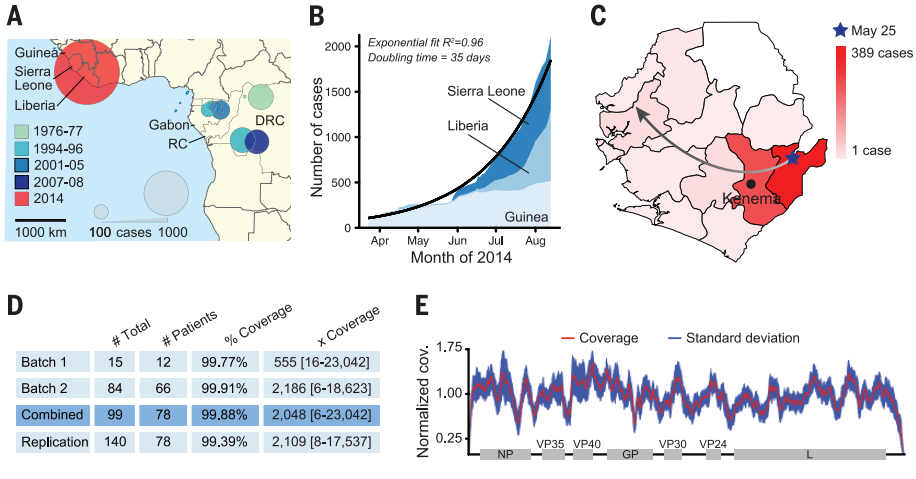

Fig. 1. Ebola outbreaks, historical and current. (**A**) Historical EVD outbreaks, colored by decade. Circle area represents total number of cases (RC = Republic of the Congo; DRC = Democratic Republic of Congo). (**B**) 2014 outbreak growth (confirmed, probable, and suspected cases). (**C**) Spread of EVD in Sierra Leone by district. The gradient denotes number of cases; the arrow depicts likely direction. (**D**) EBOV samples from 78 patients were sequenced in two batches, totaling 99 viral genomes [replication = technical replicates (*6*)]. Mean coverage and median depth of coverage with range are shown. (**E**) Combined coverage (normalized to the sample average) across sequenced EBOV genomes.

(*9*) (table S1) for our first batch of 15 inactivated EVD samples from 12 patients. Nextera library construction and Illumina sequencing provided the most complete genome assembly and reliable intrahost single-nucleotide variant (iSNV, frequency >0.5%) identification (*6*). We used this combination for a second batch of 84 samples from 66 additional patients, performing two independent replicates from each sample (Fig. 1D).

We also sequenced 35 samples from suspected EVD cases that tested negative for EBOV; genomic analysis identified other known pathogens, including Lassa virus, HIV-1, enterovirus A, and malaria parasites (fig. S3).

In total, we generated 99 EBOV genome sequences from 78 confirmed EVD patients, representing more than 70% of the EVD patients diagnosed in Sierra Leone from late May to mid-June; we used multiple extraction methods or time points for 13 patients (table S2). Median coverage was >2000×, spanning more than 99.9% of EBOV coding regions (Fig. 1, D and E, and table S2).

We combined the 78 Sierra Leonean sequences with three published Guinean samples (*3*) [correcting 21 likely sequencing errors in the latter (*6*)] to obtain a data set of 81 sequences. They

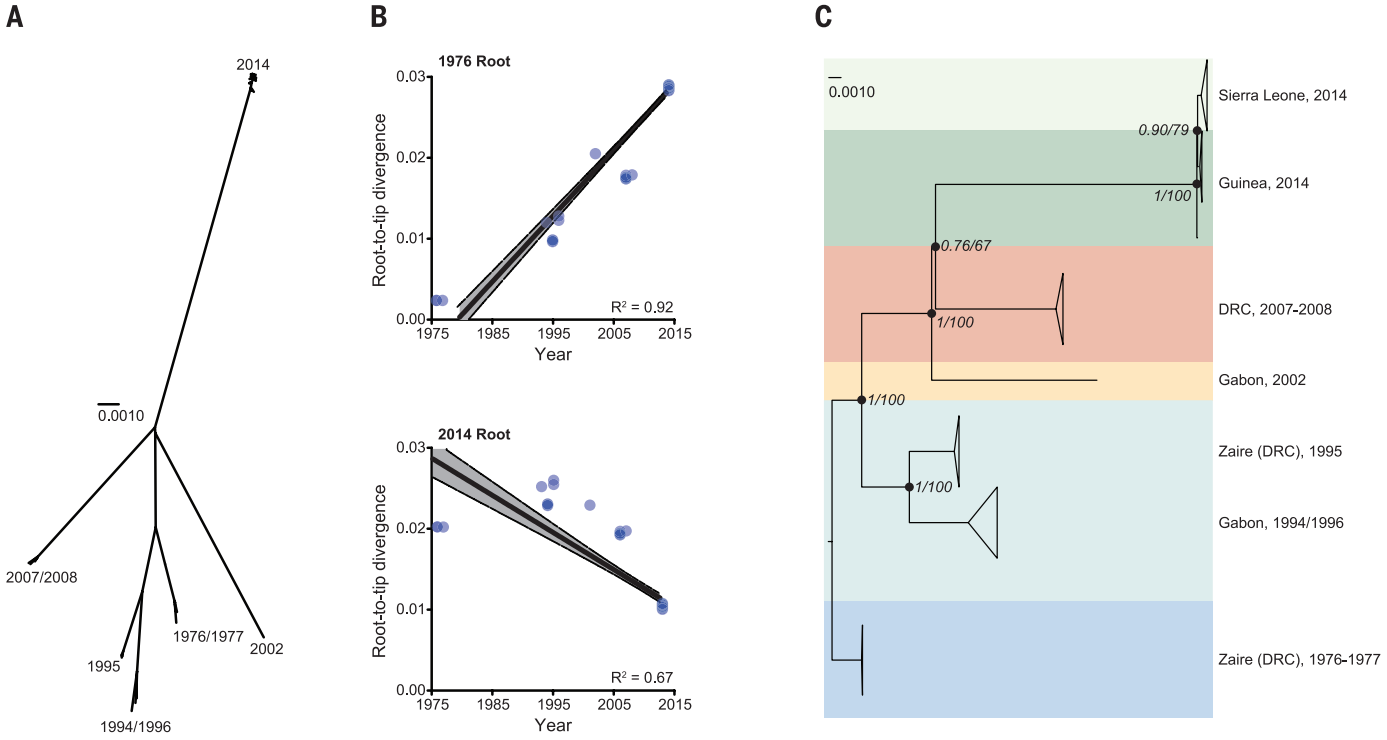

Fig. 2. Relationship between outbreaks. (**A**) Unrooted phylogenetic tree of EBOV samples; each major clade corresponds to a distinct outbreak (scale bar = nucleotide substitutions per site). (**B**) Root-to-tip distance correlates better with sample date when rooting on the 1976 branch (R^2 = 0.92, top) than on the 2014 branch (R^2 = 0.67, bottom). (**C**) Temporally rooted tree from (A).

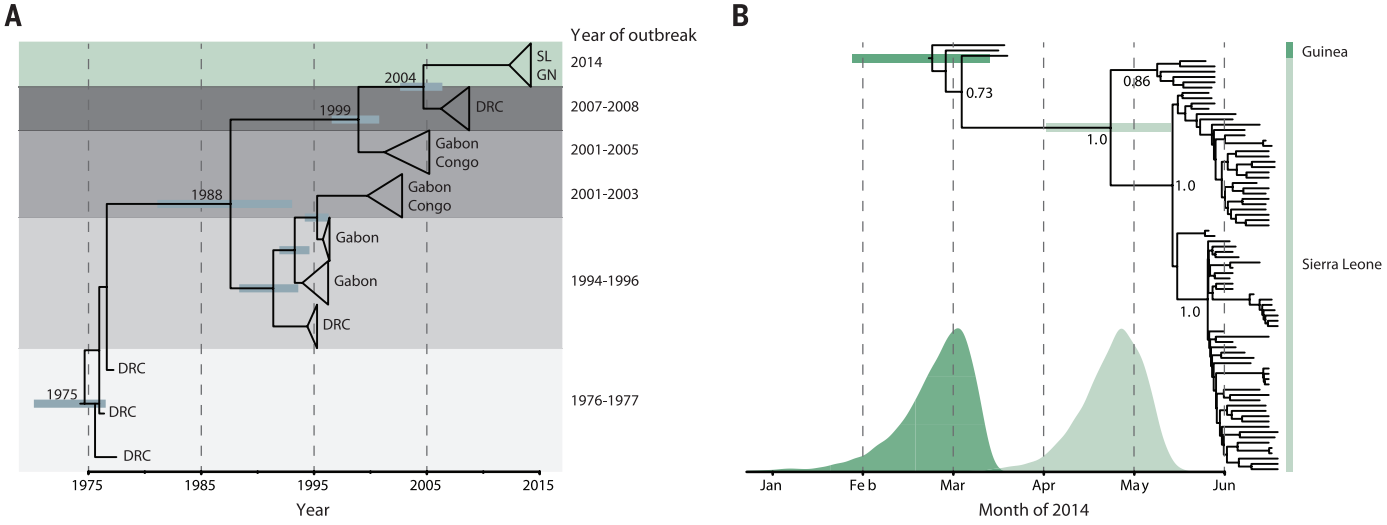

Fig. 3. Molecular dating of the 2014 outbreak. (**A**) BEAST dating of the separation of the 2014 lineage from central African lineages [SL, Sierra Leone; GN, Guinea; DRC, Democratic Republic of Congo; time of most recent common ancestor (tMRCA), September 2004; 95% highest posterior density (HPD), October 2002 to May 2006]. (**B**) BEAST dating of the tMRCA of the 2014 West African outbreak (23 February; 95% HPD, 27 January to 14 March) and the tMRCA of the Sierra Leone lineages (23 April; 95% HPD, 2 April to 13 May). Probability distributions for both 2014 divergence events are overlaid below. Posterior support for major nodes is shown.

reveal 341 fixed substitutions (35 nonsynonymous, 173 synonymous, and 133 noncoding) between the 2014 EBOV and all previously published EBOV sequences, with an additional 55 single-nucleotide polymorphisms (SNPs; 15 nonsynonymous, 25 synonymous, and 15 noncoding), fixed within individual patients, within the West African outbreak. Notably, the Sierra Leonean genomes differ from PCR probes for four separate assays used for EBOV and pan-filovirus diagnostics (table S3).

Deep-sequence coverage allowed identification of 263 iSNVs (73 nonsynonymous, 108 synonymous, 70 noncoding, and 12 frameshift) in the Sierra Leone patients (*6*). For all patients with multiple time points, consensus sequences were identical and iSNV frequencies remained stable (fig. S4). One notable intrahost variation is the RNA editing site of the glycoprotein (GP) gene (fig. S5A) (*10*–*12*), which we characterized in patients (*6*).

Phylogenetic comparison to all 20 genomes from earlier outbreaks suggests that the 2014 West African virus likely spread from central Africa within the past decade. Rooting the phylogeny using divergence from other ebolavirus genomes is problematic (Fig. 2A and fig. S6) (*6*, *13*).

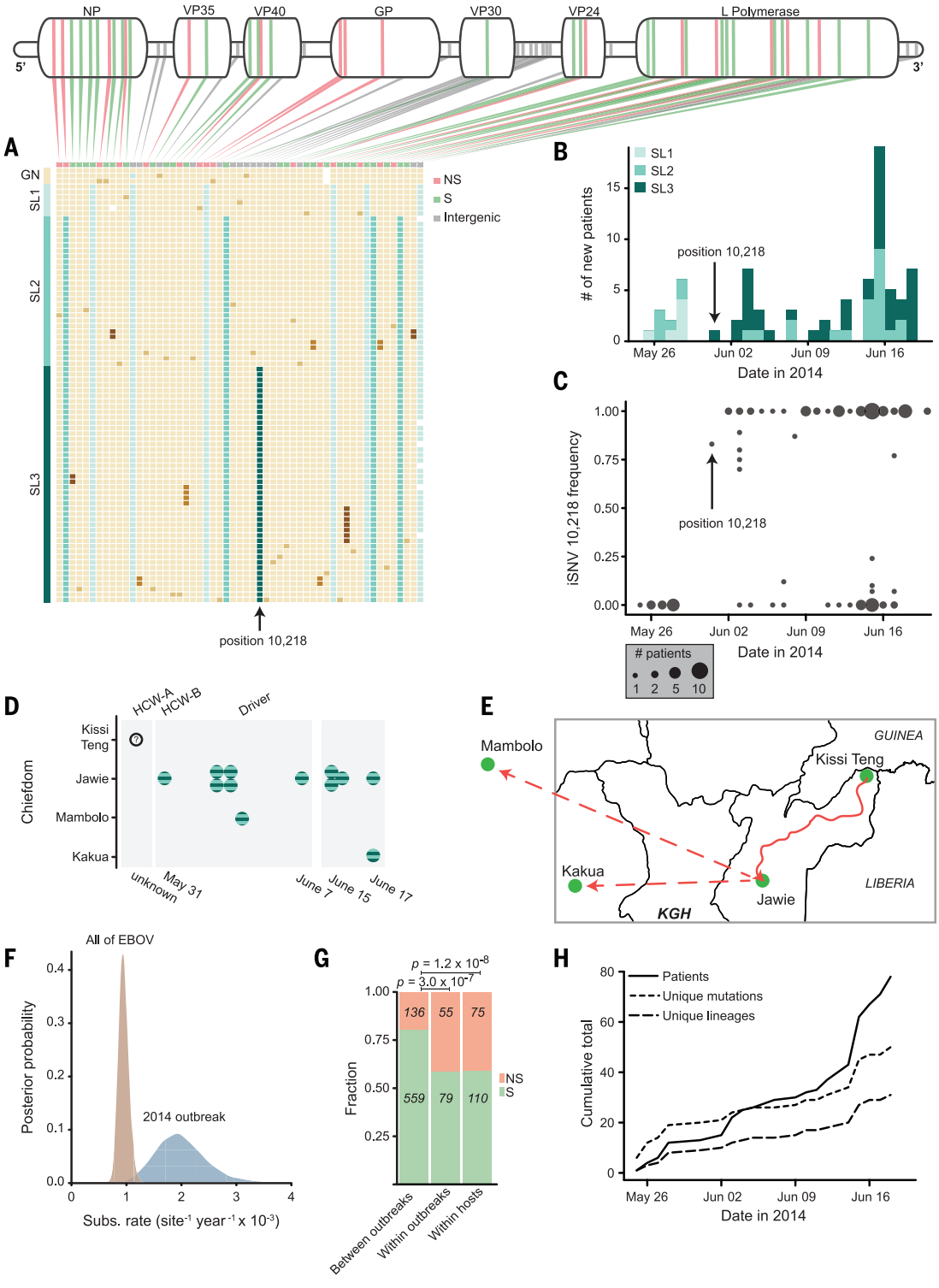

Fig. 4. Viral dynamics during the 2014 outbreak. (**A**) Mutations, one patient sample per row; beige blocks indicate identity with the Kissidougou Guinean sequence (GenBank accession KJ660346). The top row shows the type of mutation (green, synonymous; pink, nonsynonymous; gray, intergenic), with genomic locations indicated above. Cluster assignments are shown at the left. (**B**) Number of EVD-confirmed patients per day, colored by cluster. Arrow indicates the first appearance of the derived allele at position 10,218, distinguishing clusters 2 and 3. (**C**) Intrahost frequency of SNP 10,218 in all 78 patients (absent in 28 patients, polymorphic in 12, fixed in 38). (**D** and **E**) Twelve patients carrying iSNV 10,218 cluster geographically and temporally (HCW-A = unsequenced health care worker; Driver drove HCW-A from Kissi Teng to Jawie, then continued alone to Mambolo; HCW-B treated HCW-A). KGH = location of Kenema Government Hospital. (**F**) Substitution rates within the 2014 outbreak and between all EVD outbreaks. (**G**) Proportion of nonsynonymous changes observed on different time scales (green, synonymous; pink, nonsynonymous). (**H**) Acquisition of genetic variation over time. Fifty mutational events (short dashes) and 29 new viral lineages (long dashes) were observed (intrahost variants not included).

However, rooting the tree on the oldest outbreak reveals a strong correlation between sample date and root-to-tip distance, with a substitution rate of 8×10^{-4} per site per year (Fig. 2B and fig. S7) (*13*). This suggests that the lineages of the three most recent outbreaks all diverged from a common ancestor at roughly the same time, around 2004 (Fig. 2C and Fig. 3A), which supports the hypothesis that each outbreak represents an independent zoonotic event from the same genetically diverse viral population in its natural reservoir.

Genetic similarity across the sequenced 2014 samples suggests a single transmission from the natural reservoir, followed by human-to-human transmission during the outbreak. Molecular dating places the common ancestor of all sequenced Guinea and Sierra Leone lineages around late February 2014 (Fig. 3B), 3 months after the earliest suspected cases in Guinea (*3*); this coalescence would be unlikely had there been multiple transmissions from the natural reservoir. Thus, in contrast to some previous EVD outbreaks (*14*), continued human-reservoir exposure is unlikely to have contributed to the growth of this epidemic in areas represented by available sequence data.

Our data suggest that the Sierra Leone outbreak stemmed from the introduction of two genetically distinct viruses from Guinea around the same time. Samples from 12 of the first EVD patients in Sierra Leone, all believed to have attended the funeral of an EVD case from Guinea, fall into two distinct clusters (clusters 1 and 2) (Fig. 4A and fig. S8). Molecular dating places the divergence of these two lineages in late April (Fig. 3B), predating their co-appearance in Sierra Leone in late May (Fig. 4B); this finding suggests that the funeral attendees were most likely infected by two lineages then circulating in Guinea, possibly at the funeral (fig. S9). All subsequent diversity in Sierra Leone accumulated on the background of those two lineages (Fig. 4A), consistent with epidemiological information from tracing contacts.

Patterns in observed intrahost and interhost variation provide important insight about transmission and epidemiology. Groups of patients with identical viruses or with shared intrahost variation show temporal patterns suggesting transmission links (fig. S10). One iSNV (position 10,218) shared by 12 patients is later observed as fixed within 38 patients, becoming the majority allele in the population (Fig. 4C) and defining a third Sierra Leone cluster (Fig. 4, A and D, and fig. S8). Repeated propagation at intermediate frequency suggests that transmission of multiple viral haplotypes may be common. Geographic, temporal, and epidemiological metadata support the transmission clustering inferred from genetic data (Fig. 4, D and E, and fig. S11) (*6*).

The observed substitution rate is roughly twice as high within the 2014 outbreak as between outbreaks (Fig. 4F). Mutations are also more frequently nonsynonymous during the outbreak (Fig. 4G). Similar findings have been seen previously (*15*) and are consistent with expectations from incomplete purifying selection (*16*–*18*). Determining whether individual mutations are deleterious, or even adaptive, would require functional analysis; however, the rate of nonsynonymous mutations suggests that continued progression of this epidemic could afford an opportunity for viral adaptation (Fig. 4H), underscoring the need for rapid containment.

As in every EVD outbreak, the 2014 EBOV variant carries a number of genetic changes distinct to this lineage; our data do not address whether these differences are related to the severity of the outbreak. However, the catalog of 395 mutations, including 50 fixed nonsynonymous changes with 8 at positions with high levels of conservation across ebolaviruses, provides a starting point for such studies (table S4).

To aid in relief efforts and facilitate rapid global research, we have immediately released all sequence data as it is generated. Ongoing epidemiological and genomic surveillance is imperative to identify viral determinants of transmission dynamics, monitor viral changes and adaptation, ensure accurate diagnosis, guide research on therapeutic targets, and refine public health strategies. It is our hope that this work will aid the multidisciplinary international efforts to understand and contain this expanding epidemic.

In memoriam: Tragically, five co-authors, who contributed greatly to public health and research efforts in Sierra Leone, contracted EVD and lost their battle with the disease before this manuscript could be published: Mohamed Fullah, Mbalu Fonnie, Alex Moigboi, Alice Kovoma, and S. Humarr Khan. We wish to honor their memory.

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ACKNOWLEDGMENTS

We thank the Office of the President of Sierra Leone (President E. Koroma, M. Jones, S. Blyden), the Sierra Leone Ministry of Health and Sanitation (Minister M. Kargbo, B. Kargbo, M. A. Vandi, A. Jambai), the Kenema District Health Management Team, and the Lassa fever program for their efforts in outbreak response. We thank P. Cingolani, Y.-C. Wu, M. Lipsitch, S. Günther, S. Baize, N. Wauquier, J. Bangura, V. Lungay, L. Hensley, J. Johnson, M. Voorhees, A. O'Hearn, R. Schoepp, L. Gaffney, J. Kuhn, S. C. Sealfon, J. B. Shapiro, C. Edwards, and Sabeti lab members for technical support and feedback. Supported by the NSF Graduate Research Fellowship Program (R.S.G.S.), NIH grant GM080177 (S. Wohl), NIH grant 1U01HG007480-01 and the World Bank (C.H.), European Union grant FP7/2007-2013 278433-PREDEMICS and European Research Council grant 260864 (A.R.), Natural Environment Research Council grant D76739X (G.D.), NIH grant 1DP2OD006514-01, and National Institute of Allergy and Infectious Diseases grant HHSN272200900049C. Sequence data are available at NCBI (NCBI BioGroup: PRJNA257197). Sharing of RNA samples used in this study requires approval from the Sierra Leone Ministry of Health and Sanitation.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/345/6202/1369/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S11
Tables S1 to S4
Supplementary files S1 to S4
References (*19*–*44*)

5 August 2014; accepted 21 August 2014
Published online 28 August 2014;
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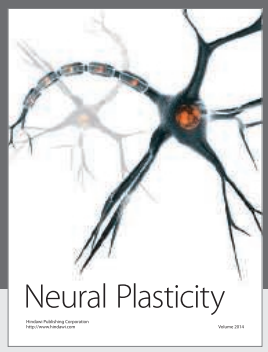

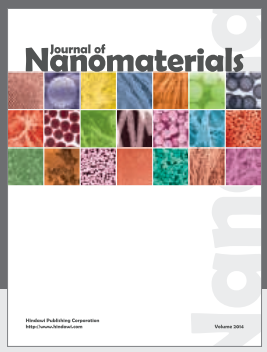

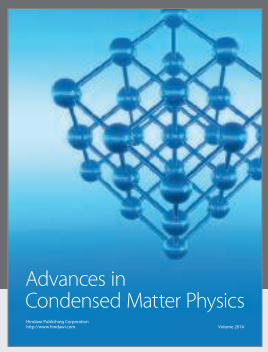

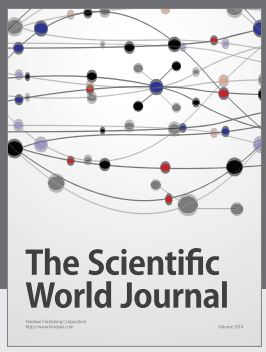

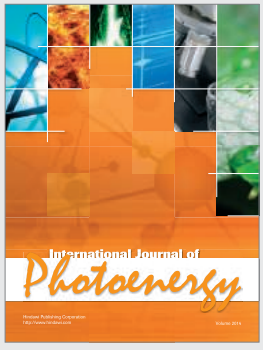

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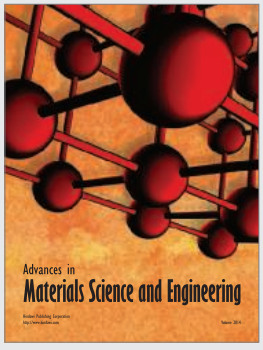

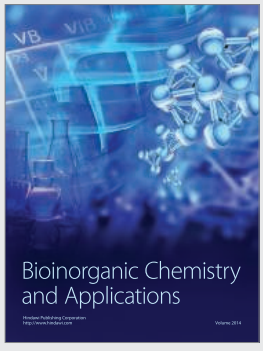

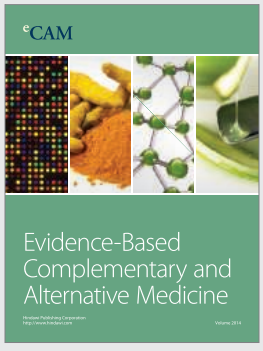

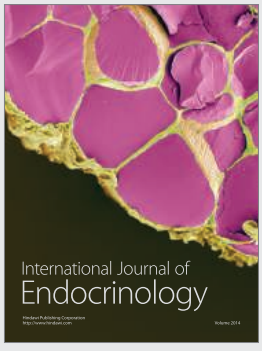

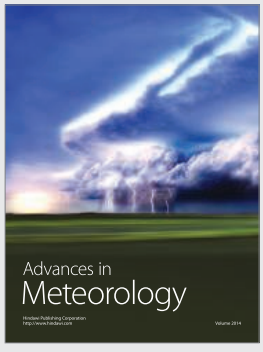

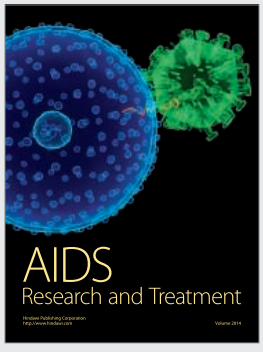

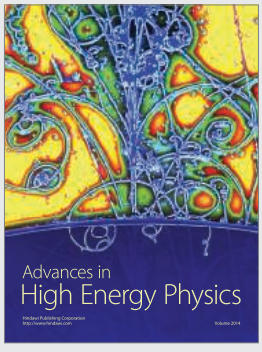

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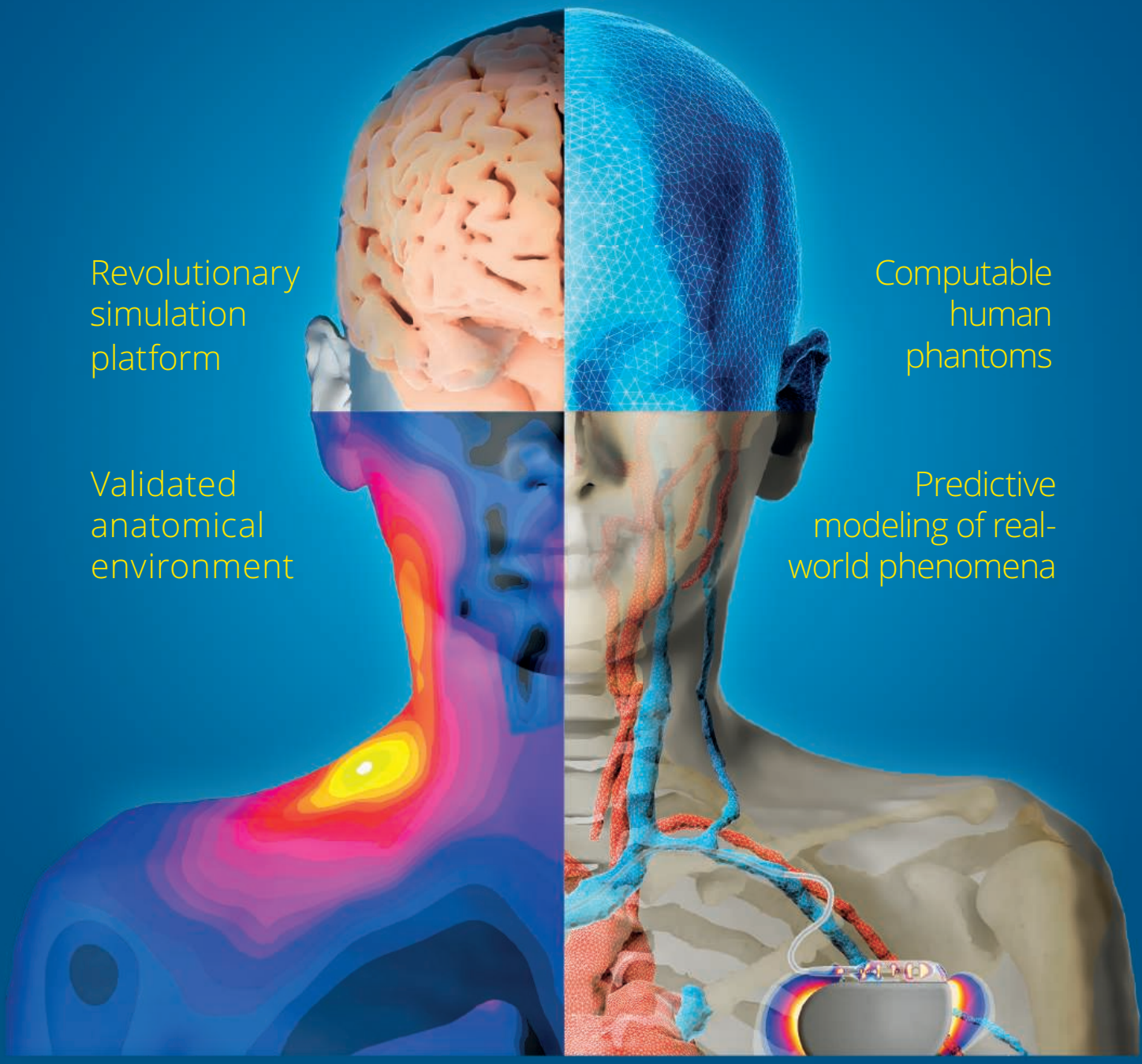

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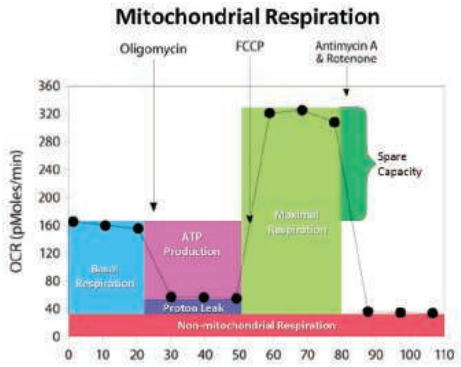

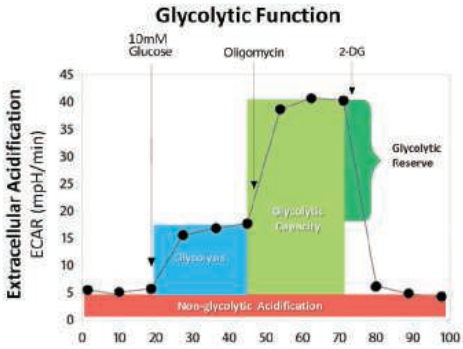

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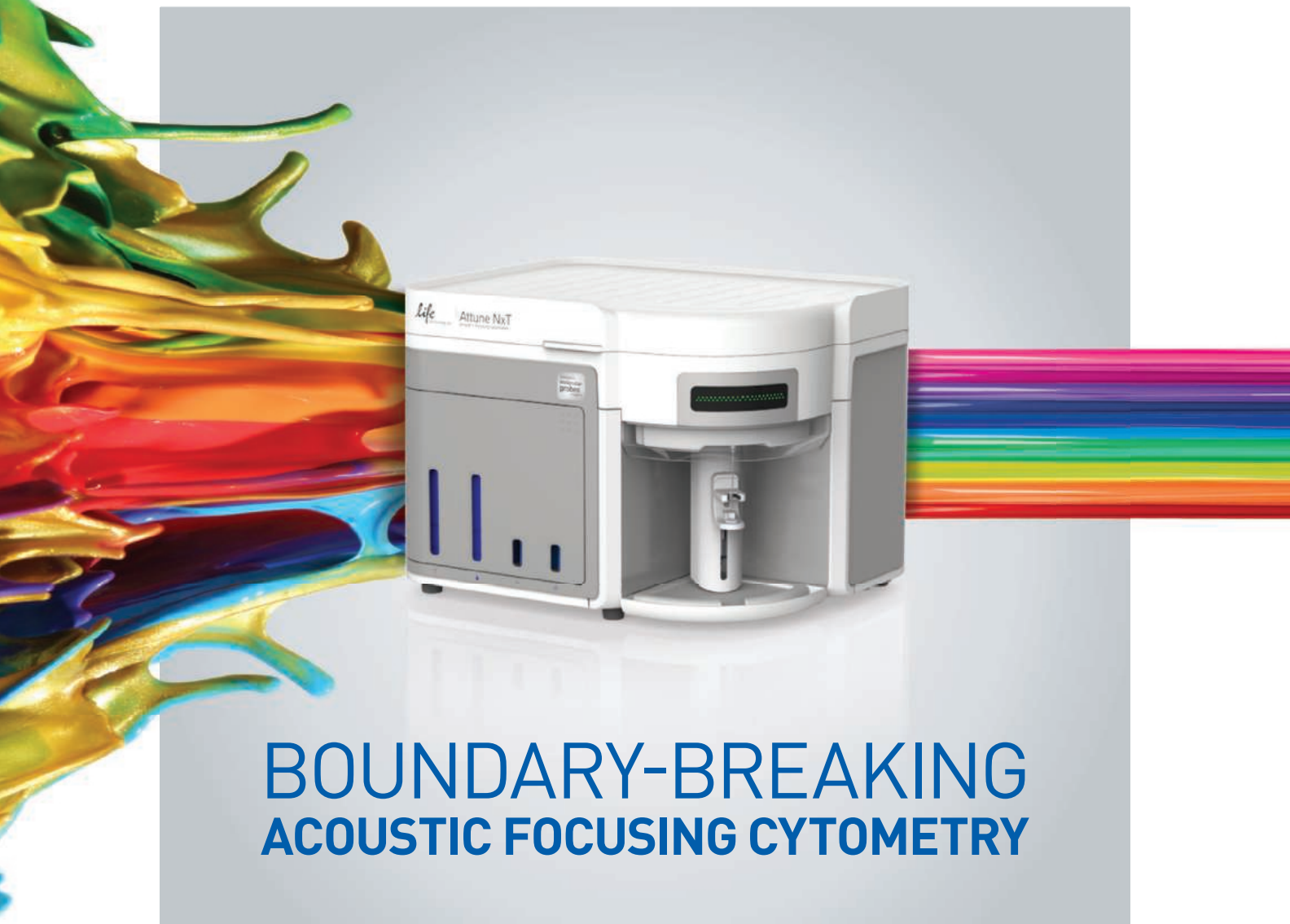

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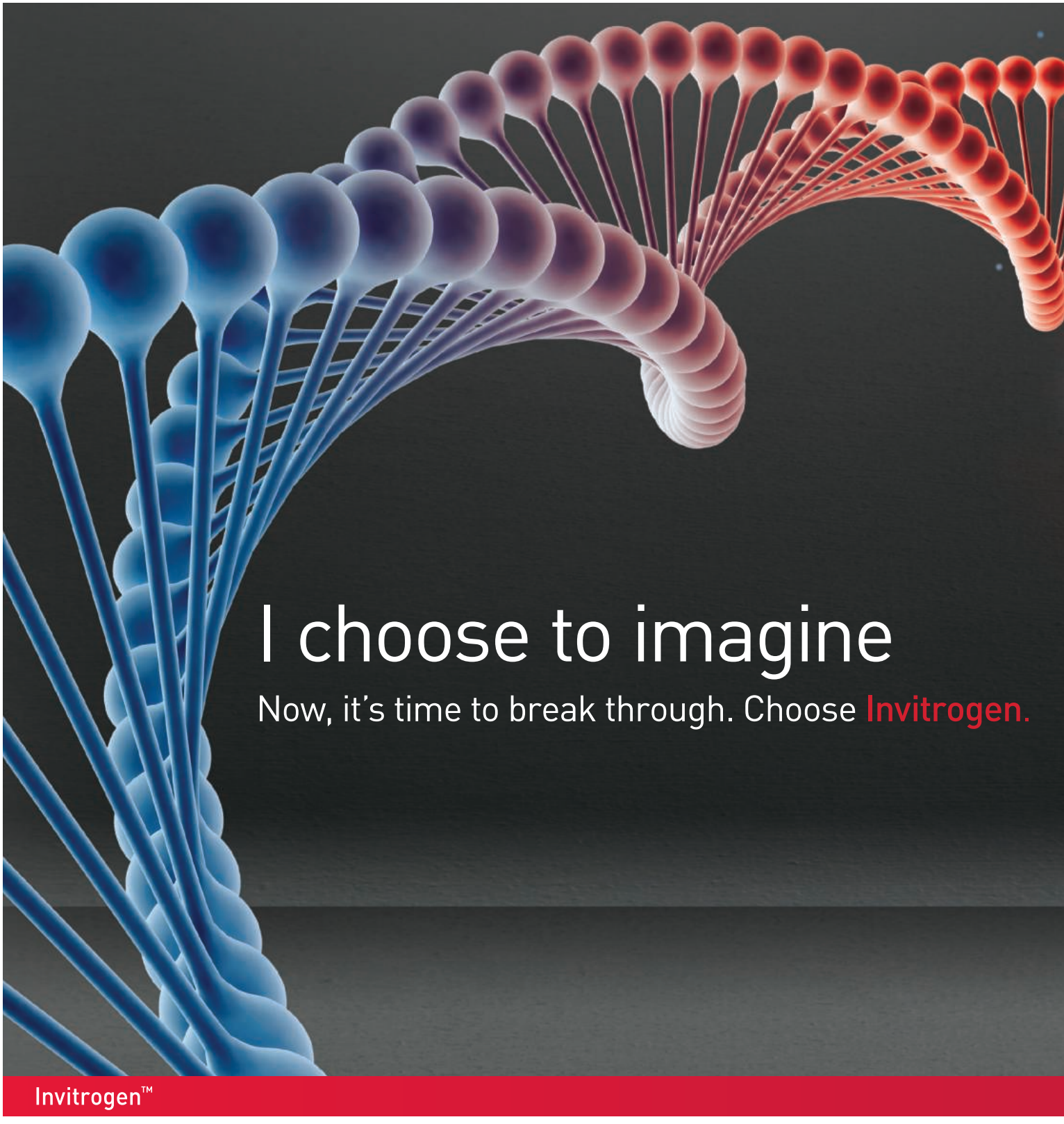

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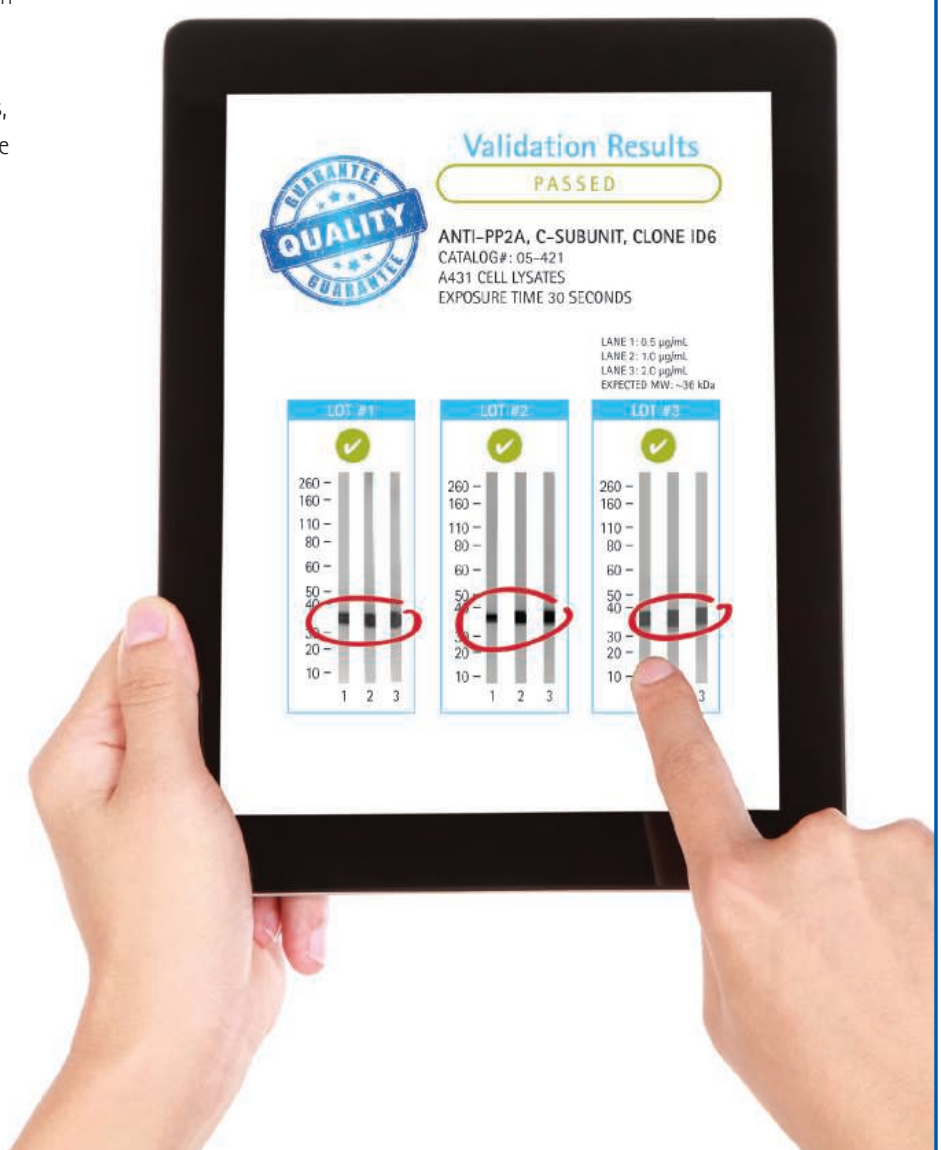

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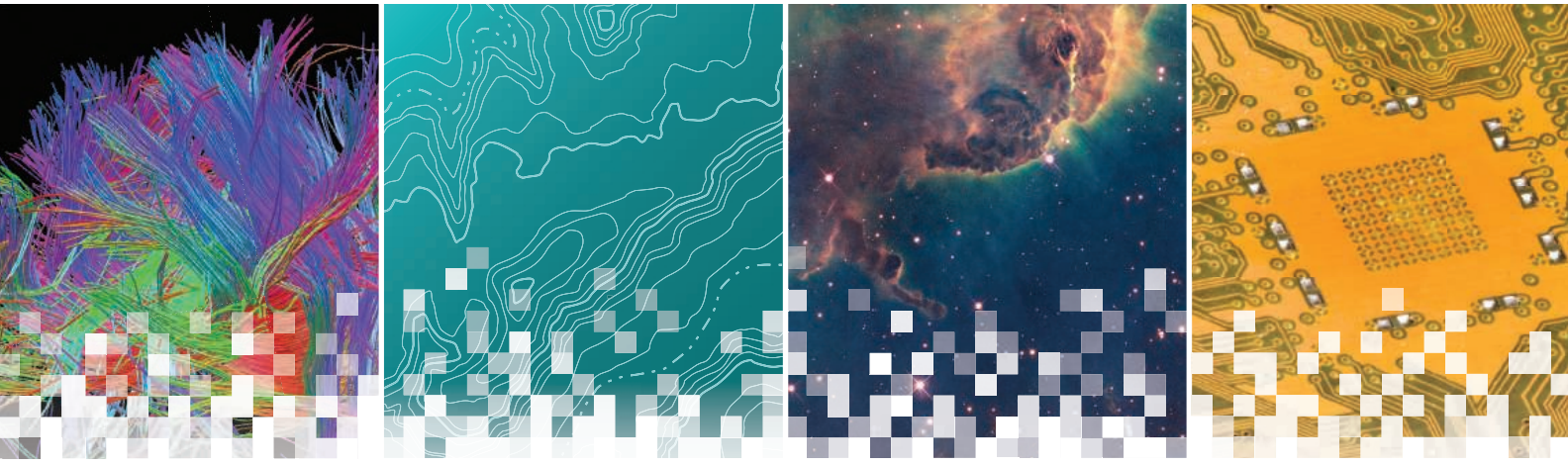

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The Children's Health Research Center (CHRC, Sioux Falls, SD **http://www.sanfordresearch.org/researchcenters/childrenshealth/**), invites applications from researchers for full time faculty at the rank of Associate Scientist within Sanford Research (**http://www.sanfordresearch.org/**) with commensurate rank of Assistant Professor in the Department of Pediatrics of the Sanford School of Medicine at The University of South Dakota. An historic $400 million gift by philanthropist Denny Sanford has allowed for expansion of Sanford Research and development of the CHRC, an energetic and collegial research community focused on pediatric research.

We seek outstanding scientists with research programs on the underlying mechanisms and/or treatment of congenital defects, developmental disorders, and pediatric diseases. Applicants should hold a PhD, MD or MD/PhD degree and complement the existing strengths and the interdisciplinary and collaborative nature of the CHRC. Candidates will be expected to develop independent research programs with extramural funding.

Significant institutional support, including modern laboratory space and state-of-the-art facilities, will be provided at the Sanford Center. In addition, a comprehensive compensation package will be tailored to the individual's qualifications.

Sanford Health is an Equal Opportunity/Affirmative Action Employer. Candidates should submit a single PDF including a detailed *curriculum vitae*, description of research experience and future research plans with specific details on the relevance of their research to pediatric research. Candidates should also submit at least three letters of recommendation. Incomplete candidate packages will not be accepted.

All application materials should be sent by email to:

David A. Pearce, Ph.D.
Director, Children's Health Research Center
Sanford Research
Professor, Department of Pediatrics
Sanford School of Medicine of The University of South Dakota
2301 E. 60th Street North, Sioux Falls, SD 57104
Telephone: 605-312-6004 FAX: 605-312-6071
Email: researchrecruitment@sanfordhealth.org

IOWA STATE UNIVERSITY
Helping you become your best.

Biophysics: Physical Biology of the Cell Faculty Search

As part of a major interdisciplinary hiring initiative (www.las.iastate.edu/faculty-careers) in the College of Liberal Arts and Sciences (www.las.iastate.edu/) at Iowa State University (ISU), a new joint initiative by the Department of Physics and Astronomy (http://www.physastro.iastate.edu) and the Department of Genetics, Development and Cell Biology (http://www.gdcb.iastate.edu/) aims to chart a Quantitative Roadmap of the Living Cell. Multiple new hires in the field of Physical Biology are planned for the next 2-3 years and should expect to benefit from and contribute to the interaction and collaboration among these and other departments.

As part of this initiative, the Department of Physics and Astronomy invites candidates to apply for a tenure-track faculty position in experimental cellular biophysics at the Assistant Professor level. Areas of interest include, but are not limited to, techniques for characterizing the architecture and dynamics of cells and their components, with an emphasis on X-ray based approaches. The appointment is expected to begin in Fall 2015. The hire will have a primary appointment in the Department of Physics and Astronomy, with the option of having a secondary appointment in the Department of Genetics, Development and Cell Biology. In addition, teaching at the undergraduate level and graduate level will be required.

Qualified applicants must have a Ph.D. in the physical sciences, publications in well-regarded journals, and be prepared to establish a program of externally funded research and teach undergraduate and graduate courses. Application instructions are at **http://www.iastatejobs.com** (*Vacancy #140661*). Applicants should include: a cover letter, curriculum vitae, summary of prior research, concise description of future research plans and a statement of teaching experience and philosophy. Submission of three confidential letters of recommendation should be arranged as per instructions in the on-line application system. Review of applications will begin **November 1, 2014**.

ISU is an EO/AA Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability, or protected Vet status.

ISU is an AAU-member comprehensive, land grant, Carnegie Doctoral/Research Extensive University with an enrollment of over 33,000 students. The university is located in Ames, IA, one of the nation's most highly rated metropolitan areas of its size. ISU is committed to achieving inclusive excellence through a diverse workforce and is dedicated to supporting work-life balance through an array of flexible policies.

Multicultural Relationships: Working Across Industries

Academic researchers are increasingly venturing out of the laboratory and into unfamiliar professional territory. The draw might be a multidisciplinary project, industry collaboration, or consulting. The new field might be government, law, or even the military. To ease interactions with colleagues in other fields with different work environments, seven researchers share what they've learned about productive, professional cross-disciplinary relationships. By Chris Tachibana

Academic scientists have more opportunities than ever to collaborate across disciplines through industry open innovation programs, translational science grants, and joint projects between scientists and educators. But these interactions pull science professors away from campus, with its student-oriented culture, tolerance of eccentricity, and relaxed dress code. Here we provide guidance on expectations and norms in work environments outside of academic science, from education to government to industry.

Learning from education

Universities are places of learning, so academic scientists might be inspired to contribute their knowledge and skills to improving general education. However, they might not know where to begin. A good starting point is an educational outreach program, where researchers get an introduction to K–12 education from experts like **Jennifer Eklund**, who has a science Ph.D. and postdoctoral training in education. At the Institute for Systems Biology in Seattle, Eklund works as a liaison between researchers and educators working together to improve a science curriculum. The researchers also benefit from the partnership, says Eklund: "They get exposed to education theory and methods that can help with their own teaching."

At the beginning of a collaboration with educators, academic researchers "want to share both big ideas and minute details," says Eklund, "and often come in wanting to tell teachers what to do." She helps scientists have an impact by showing them how their contributions can fit into a particular grade level and match curriculum considerations such as the Next Generation Science Standards that guide U.S. science education. Eklund also offers scientists guidance about how to work with educators, who tend use positive reinforcement in their interactions with each other. "Teachers are used to a school environment with students," she says, "so they can sometimes be taken aback by the direct criticism that is a part of the culture of scientists."

Lessons in law

Another department now common on campus is the technology transfer office. Commercializing results is a complex process and researchers need the guidance of navigators like **Blaine Bettinger**, who specializes in intellectual property at Bond, Schoeneck & King in Syracuse, New York. Like Eklund, Bettinger has a science Ph.D. and is a liaison between two fields, in this case, science and law. Although an advanced science degree generally doesn't mean much to attorneys, Bettinger says his biochemistry Ph.D. provides him with a bond with his scientist clients. "They recognize me as someone who went through the same trial by fire," he says, "although I jumped out and they stayed in."

Bettinger says that scientists initially encountering intellectual property law are struck by the formality, not only in dress but also in language, compared with the laid-back atmosphere of academia. Scientists who are used to openly and casually sharing their ideas must learn to communicate them in legalese, which can be daunting. "Don't be afraid to ask questions," says Bettinger. "A good patent attorney will be more than happy to answer them." Over time, Bettinger has learned to anticipate the confusion and concerns of his inventor clients. Fortunately, he says, "scientists are always excited about their ideas," and that enthusiasm can power them through the complicated patent process. continued>

"You will find it a shock to be in an environment where science isn't the most important thing."
— Muffy Calder

PHOTOS (FROM TOP): AFRICASTUDIO/SHUTTERSTOCK.COM; UNIVERSITY OF GLASGOW

Upcoming Features

Faculty: Professionalism 2—Oct. 3 ■ *Top Employers Survey—October 17th* ■ *Neuroscience—October 31*

Vikram Jandhyala recalls his first encounter with research commercialization as, "like going back to school for a few days—a great learning experience." Jandhyala is a former chair of electrical engineering and the incoming vice provost for innovation at the University of Washington. In 2006, he commercialized cloud-based software for electromagnetic simulations that was developed by his research group. Jandhyala worked with experts from the university commercialization center, who taught his group best practices for intellectual property protection including how to present and publish their results. Incorporating this new work style had a steep learning curve, he says, but several factors helped. His postdocs and students were eager to become entrepreneurs. Also, the initial research had an applied orientation because it was funded by DARPA, which Jandhyala says, "drives projects in startup mode, with people in teams and providing monthly project updates."

For researchers preparing to work with patent attorneys and especially venture capitalists, Jandhyala advises being prepared for a cold, hard evaluation of your potential product. "With funding as it is, faculty members are used to people saying no to them," he says. "It's the same when you raise funds for a startup. What's different is the directness of potential investors telling you your idea is not market ready. But these people are doing you a favor, telling you to change your technology early so you develop the right product for your customers."

Blaine Bettinger says that scientists initially encountering intellectual property law are struck by the formality, not only in dress but also in language, compared with the laid-back atmosphere of academia.

Inside industry

Scientists who get beyond the patent stage might find themselves in yet another professional culture: the science-based company. Expect a goal-oriented atmosphere with stricter deadlines than academia, say industry researchers. Companies ultimately must deliver products to customers and that bottom line influences the work culture, even at firms with strong research departments. In addition, says **Joan Greve**, academic scientists entering industry partnerships should be aware of how much work is required to bring a product to market and that it might never happen.

Greve worked for the biotech company Genentech for many years, before and after earning a bioengineering Ph.D., and was a scientific program manager at the Allen Institute for Brain Science, a nonprofit organization supported in part by Microsoft cofounder Paul Allen. Greve is now applying this broad professional experience to setting up her own research group. She is a new biomedical engineering faculty member at the University of Michigan where she is studying preclinical medical imaging.

"Experiencing different work environments is a great way to develop your own management style," she says. "I learned not from any one environment but from several: academic, for-profit, nonprofit, and flat and hierarchical structures." Greve's personal management philosophy boils down to stating expectations up front, which helps people be prepared and efficient. "The golden rule of management," she says, "is no surprises. Give clear expectations and be committed to open communication that goes both ways. This is something that can be applied to any environment or workplace." The website for Greve's research group illustrates this principle, telling potential members that she expects individual initiative from them and pledging "ample encouragement" in return.

Clear expectations are also the foundation of a solid professional relationship between academic and industry scientists, says Greve. Especially important is setting up realistic timelines and milestones from the start. This gives everyone a sense of the project lifespan, she says, especially university researchers who might not realize how long it can take to develop a potential medical device or drug. A clear-cut plan can also help academic scientists understand how crucial it is to stay on track by producing agreed-upon deliverables on time.

Even for academic scientists who are not considering industry collaborations, Greve has advice from her diverse professional background. Training in leadership, budgeting, and other practical skills should be a standard part of a scientist's training, she says. Faculty advisors can prepare their students and postdocs for any career, says Greve, by training them to "think like a leader. What is your mission and how can you arrange day-to-day operations to achieve it?"

Within the military perimeter

Lior Weizman echoes Greve's points about the long timelines of product development and the importance of good team leadership, but his industry experience is from a place that most scientists never experience: the military. Weizman is an electrical engineering postdoctoral fellow at the Israel Institute of Technology (Technion) who did his conscripted service at Rafael Advanced Defense Systems. First, he would like to correct a common but incorrect mental image: he did not wear fatigues and carry firearms at work. "I did my military service sitting in an office at a computer," he says, "and going to meetings in which everyone freely expressed their opinions, just like in any industry."

Working for a military-related industry means adhering to some absolute rules, though. Military scientists can't work from home, says Weizman, because classified materials must stay onsite. Deadlines are strict, with no extensions. "In academic research, if you miss a submission deadline, you can find another journal or conference or grant," he says. "In the army, other units are relying on your work so it must be done on time." The high level of professional cooperation and coordination, he says, is similar to any industry in which many groups contribute to a joint project.

Weizman worked on imaging applications for Rafael and now studies medical imaging. He finds that this dual background helps him explain the motivation behind his research to students and the public because at Rafael, he had to think about how scientific theory could be applied to real-world problems. Industry work also taught him the importance of a well-functioning team. Now in a position to recruit students, Weizman says that although he considers their grades, the most important quality he wants in a coworker is the ability to get along with others: "knowing how to negotiate, share ideas, and work side-by-side with other people." continued>

PHOTO: COURTESY OF GRZEGORZ WICHE

FEATURED EMPLOYER Science Careers online @sciencecareers.org

UC San Diego

Division of Biological Sciences

The Division of Biological Sciences at the University of California San Diego (**www.biology.ucsd.edu**) invites applications from outstanding candidates for multiple tenure-track or tenured faculty positions. All candidates must have earned a Ph.D. or equivalent degree, and be committed to teaching at the undergraduate and graduate levels. Successful candidates will have demonstrated excellence and creativity in research, scholarship, and a commitment to equity and inclusion in higher education.

Host/Microbe Interactions (10-814, 10-816): The Section of Cell and Developmental Biology and the Section of Molecular Biology invite applications for two tenure-track Assistant Professor positions. Candidates focusing on interactions between microbes and their host organism at the cellular and molecular levels in pathogenic and/or symbiotic settings are encouraged to apply.

Neurobiology (10-817): The Section of Neurobiology invites applications for a tenured position, particularly at the level of Full Professor, with a strong research emphasis on the development of behavioral circuitry. Candidates at Associate and Assistant level will also be considered. Candidates using any experimental animal are welcome to apply. This highly interactive group is looking for colleagues who will bring novel approaches, either technical or conceptual, to their study of brain function. Although not a requirement, candidates who can establish strong ties to the new Center for Brain Activity Mapping initiative at UCSD will be given particular attention.

Quantitative Evolutionary Biology (10-815): The Section of Ecology, Behavior and Evolution invites applications for a faculty position at the tenure-track Assistant, Associate, or Full Professor level in Quantitative Evolutionary Biology. We are searching broadly for an evolutionary biologist with strong quantitative skills working with any taxonomic group (animal, plant, microbial) or environment (terrestrial or aquatic). Research interests may include, but are not limited to: evolutionary theory and modeling; investigation of how genetic variation affects complex traits and their evolution; experimental evolution; and/or the use of genomic tools and comparative analysis to reconstruct the evolutionary histories of populations and species; with the goal of understanding how traits evolve under different kinds of selection, including those associated with global change.

Teaching Professor (10-813): The Section of Ecology, Behavior and Evolution invites applications for a new tenure-track Assistant Teaching Professor position. Faculty in the Teaching Professor series are Academic Senate members whose expertise and responsibilities center on undergraduate education, as well as the scholarly analysis and improvement of teaching methods.

Review of applications will commence by **October 17, 2014** and will continue until all the positions are filled. Interested applicants must submit a cover letter, curriculum vitae, statement of research, statement of teaching, a statement describing their past experience and leadership in fostering equity and diversity and/or their potential to make future contributions and 3-5 publications. Applicants at the Assistant Professor level need to submit 3-5 references, and applicants at the Associate or Full Professor level need to provide contact information for 3-5 references.

Applications must be submitted through the University of California San Diego's Academic Personnel RECRUIT System at **https://apol- recruit.ucsd.edu/apply**.

UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to excellence and diversity (http://diversity.ucsd.edu/).

Jennifer Eklund helps scientists have an impact by showing them how their contributions can fit into a particular grade level and match curriculum considerations such as the Next Generation Science Standards that guide U.S. science education.

Featured Participants

Allen Institute for Brain Science
www.alleninstitute.org

Blaine Bettinger
www.blainebettinger.com

Genentech
www.gene.com

Institute for Systems Biology
www.systemsbiology.org

Israel Institute of Technology
www.technion.ac.il/en

Rafael Advanced Defense Systems
www.rafael.co.il

Randy Olson
www.randyolsonproductions.com

The Scottish Science Advisory Council
www.scottishscience.org.uk

University of Glasgow
www.gla.ac.uk

University of Michigan
www.umich.edu

University of Washington Center for Commercialization
depts.washington.edu/uwc4c/

In the halls of government

Even scientists who remain strictly within their academic department can't avoid intersecting with the government. For one thing, research funding is driven by national and global priorities about health, energy, climate, and other science-related fields. Some scientists interact even more closely with the government through contract work or as expert consultants. Fortunately, university researchers have a built-in training program for government work: administration.

Muffy Calder is a professor in computing science at the University of Glasgow and chief scientific advisor for Scotland. Serving as chair of her professional association was an excellent introduction to her advisory work, she says: "I learned about the process of government consultations and how to explain the contributions of my discipline to government policies." She was also dean of research for the College of Science and Engineering, which gave her broader exposure to the strengths and challenges in different scientific disciplines. "It was an excellent preparation for my role in government," she says. "It prepared me for being an advocate for all of these areas." Calder will bring her government experience back to academia this year, when she returns to the University of Glasgow full time, as vice principal and head of the College of Science and Engineering.

Researchers often confuse two different aspects of the government-science relationship, says Calder. The government uses science to inform policy and advising in that area is Calder's job. A separate governmental role is supporting the conduct of science, for example through research funding. In dealing with people in government, advises Calder, remember that informing policy and funding research are different concerns.

In approaching policymakers, Calder advises a little mental reorientation. The best advice she received before starting her national position as scientific advisor was to expect a work culture that doesn't revolve around your research. "You will find it a shock to be in an environment where science isn't the most important thing," she was told, "and you won't only talk with scientists." Don't worry, though. Civil servants are exceptionally collegial, she says, and strongly committed to working together.

In government, you're in a politician's world, so adapt to their perspective. "Understand the problems that people in government are trying to solve," says Calder. "Don't just tell them your results, but help them find solutions for their problems." Remember that politicians must justify their positions to voters and stating, "This is supported by scientific evidence," is generally not convincing. Calder says, "We need scientists to not only present evidence to politicians and civil servants, but also help them to explain findings and policies based on that evidence."

The secret to multicultural relationships

For experience in cross-disciplinary relationships, no one can beat **Randy Olson**. In 1994, he left a tenured marine biology position at the University of New Hampshire to become a filmmaker. Now, in addition to making films, he writes books, gives talks, and holds workshops on science communication. Olson's advice for scientists who want to build a solid relationship with people in a different professional field is, of course, that communication is crucial. But science communication is more than just knowing how to market yourself and having a message. Too much science communication advice focuses on the icing and skips the cake, says Olson. The icing is quick tips about being concise and getting people's attention. "The cake," says Olson "is listening. Really listening is the core of communication. The academic world doesn't train us to listen. The academic world trains us to lecture."

In fact, Olson's advice encapsulates the single message that emerged from all the experts in multidisciplinary interactions: Take the focus off yourself. Listen to your colleagues, consider their background and perspective, and recognize and respect their knowledge, skills, and experience. Think about your team, your common goal and how you can contribute to getting stuff done. And don't forget to have fun together, adds Greve: "Science is hard. You have to celebrate success."

Chris Tachibana is a science writer based in Seattle, USA, and Copenhagen, Denmark.

DOI: 10.1126/science.opms.r1400145

Read the sequel on multicultural relationships in the October 3, 2014 issue.

PHOTO: BY BRIAN KRAATZ

HUDSONALPHA
INSTITUTE FOR BIOTECHNOLOGY

science for life

invites applications for new Faculty Investigators and Junior Fellows to join its dynamic team of scientists.

HudsonAlpha opened its doors in 2008 with a mission to translate the promise of genomics into medical, agricultural, educational, and commercial realms. Scientists at HudsonAlpha use genomic and genetic approaches to answer important biological questions, especially those relevant to the genetic and molecular basis of human disease, agriculture, bioenergy, and biodiversity.

HudsonAlpha's 152-acre campus has state-of-the-art buildings and infrastructure for its unique blend of nonprofit research scientists, educators, and commercially-oriented entrepreneurs, who share work-space and know-how across organizational boundaries. The Institute has assembled an impressive concentration of research expertise and infrastructure in high-volume DNA sequencing, genotyping and functional assays that serves its own research scientists, as well as hundreds of partners' needs, generating exomes, genomes, transcriptomes, and functional genomic datasets.

The Institute provides generous support for productive research programs and offers an exciting academic environment thanks to formal partnerships with leading universities and medical centers around the world. Part of a vibrant science and engineering community, HudsonAlpha sits at the heart of Cummings Research Park, one of the world's largest science and technology business parks, located in Huntsville, Alabama.

Junior Fellows

Exceptionally talented **graduating Ph.D. students** who wish to apply to the **3-year Junior Fellow program** should have a well-defined research proposal that will benefit from the intellectual, technological, and collaborative environment at HudsonAlpha. Junior fellows will perform independent research supported by the Institute with a budget commensurate to the plan proposed. Apply to **JuniorFellows@hudsonalpha.org.**

For more information, visit www.hudsonalpha.org.

Faculty Candidates

should have a graduate degree and an outstanding record of research achievement, including an established, internationally competitive, and independent research program.

Faculty candidates with interests and expertise in the following areas are especially encouraged to apply.

- computational biology
- human disease (particularly cancer genomics and neuro-psychological disorders)
- plant genomics

Faculty recruitment will proceed through the 2014-2015 academic year; invitations for interviews will begin in the second half of 2014 in anticipation of start-dates in 2015. HudsonAlpha Institute is an equal opportunity employer and values diversity in its workplace.

Apply to **FacultyCareers@hudsonalpha.org. Please send a curriculum vitae and 3-page research summary and plan.**

FACULTY POSITIONS Science Careers online @sciencecareers.org

The University of Georgia®

Skidaway Institute of Oceanography

TWO FACULTY VACANCIES (ASSISTANT PROFESSOR) IN OCEANOGRAPHY

The Skidaway Institute of Oceanography of the University of Georgia (SkIO), a multidisciplinary research institution located on the Georgia coast near Savannah, and the UGA Department of Marine Sciences within the Franklin College of Arts and Sciences, invite applications for two, nine-month, tenure-track positions resident in Savannah. Successful candidates will be interdisciplinary, self-motivated and interested in pursuing innovative research and education in a highly supportive environment. The successful candidate will enhance existing programs within the Marine Sciences Department at SkIO (http://www.skio.uga.edu) and in Athens (http://www.marsci.uga.edu). Appointments will be made at the Assistant Professor level, but consideration will be given to exceptional applicants seeking more senior appointments. Applicants working in diverse marine settings are encouraged to apply, although experience and a desire to work in estuarine, coastal and shelf environments are preferred as are researchers who focus on the roles of anthropogenic forcing on marine processes.
We are particularly interested in applicants with research expertise in the following oceanographic areas:

Biological Oceanography—We seek candidates who excel in addressing interdisciplinary and large-scale questions. Candidates with expertise in marine phytoplankton, microzooplankton, marine viruses or disease ecology are particularly encouraged to apply.

Chemical Oceanography—We seek a chemist with a desire to engage in interdisciplinary research that advances the understanding of fundamental oceanographic questions. Areas of expertise could include inorganic carbon cycling; biogeochemistry and dynamics of particles; or cycling, transport and/or fate of organic pollutants.

Coastal Oceanography— A successful candidate will have expertise in interactions between the land margin and the coastal ocean. We are particularly interested in scientists who focus on the interface between the environment and anthropogenic activities, including, but not limited to, delivery, transport and fate of materials within estuarine and coastal settings, physico-chemical behavior of materials along the estuarine continuum, nearshore and shelf transport processes and impacts of climate change.

Successful candidates are expected to develop an energetic, externally funded research program and to teach at the undergraduate and graduate level. The recent merger of SkIO with UGA allows for close collaborations in research and education with UGA faculty in Marine Sciences, as well as the College of Engineering and School of Ecology, and with institutions throughout the University System of Georgia. A Ph.D. in Oceanography or other appropriate discipline is required and postdoctoral experience (minimum six months) is preferred.

These positions will be available August 2015. The committee will begin to review applications on October 24, 2014 and will continue until the positions are filled.

We will receive applications for these positions through the University of Georgia, Franklin College Faculty Recruitment website (https://www.franklin.uga.edu/jobs/index.php). To apply use the Application Materials dialog box to upload a single PDF file consisting of the following materials:

- letter of application, including a statement of teaching philosophy and research interests and an explanation of qualifications relative to the responsibilities specified above;
- curriculum vitae;
- examples of current published research/writing.

The Franklin College Recruitment website will prompt applicants to supply the names and contact information for three references, who will be sent a link by which they can submit a letter directly.

Candidates who cannot apply through the website may post hard copy materials to:

Chair, Oceanography Committee
Skidaway Institute of Oceanography
10 Ocean Science Circle
Savannah, GA 31411

The University of Georgia, with over 35,000 students and 2,900 faculty members, is one of the four major units of the University System of Georgia and offers programs of study at the doctoral level in most fields. In addition to its home campus, The University of Georgia maintains and is expanding its list of study-abroad programs around the world. Georgia is well known for its quality of life in regard to both outdoor and urban activities (www.exploregeorgia.org).

The University of Georgia 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, or protected veteran status.

Science Careers online @sciencecareers.org
FACULTY POSITIONS

The Jackson Laboratory

Faculty Positions

Candidates must have a Ph.D., M.D., or D.V.M. degree, and relevant postdoctoral training with an exceptional record of research accomplishment, and the ability to develop a competitive, independently funded research program. Opportunities are available for shared mentorship of trainees, and integration with other campuses of The Jackson Laboratory.

The Jackson Laboratory is an EOE/AA Employer.

MAMMALIAN GENETICS | Bar Harbor, Maine

The Jackson Laboratory for Mammalian Genetics in Bar Harbor, Maine is inviting applications for **Assistant, Associate** and **Full Professors**. Faculty on the Bar Harbor campus use genetic and genomic approaches to conduct basic research on fundamental biological problems and mechanisms of disease with emphasis on the mouse as a model system. Faculty members are being recruited in diverse areas, including:

- Cancer Biology and Cancer Genomics
- Computational Biology and Bioinformatics
- Developmental and Reproductive Biology
- Genomic Editing and Engineering
- Immunology and Infectious Diseases
- Metabolic Disorders and Genetics of Aging
- Neurology and Neurobiology
- Statistical and Systems Genetics
- Trans-generational Inheritance and Epigenetics

For inquiries, please contact **Dr. Robert Braun**, professor and vice president of research of The Jackson Laboratory, at robert.braun@jax.org. Information about The Jackson Laboratory for Mammalian Genetics and its current faculty may be found at www.jax.org.

GENOMIC MEDICINE | Farmington, Conn.

The Jackson Laboratory for Genomic Medicine in Farmington, Connecticut is inviting applications for **Assistant, Associate** and **Full Professors**. The campus is dedicated to advancing precision medicine using genetic and genomic strategies to understand human biology, the complex functional networks underlying health and disease, and the development of novel diagnostics and therapeutics. We are seeking individuals to join our interactive culture of cooperation and program integration. Areas for recruitment include:

- Cancer Biology and Cancer Genomics
- Computational Biology and Bioinformatics
- Functional Genomics and Genomic Technologies
- Genomic Editing and Engineering
- Human Genetics
- Metabolic Disorders and Genetics of Aging
- Microbial Genomics, Microbiome Research and Infectious Diseases
- Neurology and Neurobiology
- Statistical and Systems Genetics

For inquiries, please contact **Dr. Charles Lee**, scientific director and professor of The Jackson Laboratory for Genomic Medicine, at charles.lee@jax.org. Information for The Jackson Laboratory for Genomic Medicine and its current faculty may be found at www.jax.org/ct.

Applicants must apply online. Please submit a curriculum vitae and a concise statement of research interests as one document to www.jax.org/careers, click on Faculty Positions. In addition, please have three letters of reference sent to: facultyjobs@jax.org. The application review process will start November 1, 2014.

The Jackson Laboratory offers a uniquely collaborative scientific research environment. Faculty are supported by outstanding scientific services, unparalleled mouse and genomic resources, postdoctoral and predoctoral training programs, and numerous courses and conferences centered on the mouse as a genetic model for human biology and disease.

Leading the search for tomorrow's cures

Tufts UNIVERSITY

School of Medicine

Faculty Position in Microbiology Specializing in Mycobacteriology

The Department of Molecular Biology and Microbiology at Tufts University School of Medicine (http://medicine.tufts.edu/Education/Academic-Departments/Basic-Science-Departments/Molecular-Biology-and-Microbiology) is committed to developing a strong program focusing on the pathogenesis of Mycobacterium tuberculosis and is seeking candidates at the **Assistant, Associate, or Full Professor levels** with a research focus on tuberculosis and mycobacteriology. An ideal candidate can build on the Department's core strengths in microbial pathogenesis and physiology, molecular genetics, and microbial cell & structural biology. We are particularly interested in candidates taking innovative approaches that include, but are not limited to: analyses of mycobacterial physiology, gene regulation, drug response, and metabolism; and interactions of pathogenic mycobacteria with host cells or in model hosts. We offer generous start up support, newly renovated BSL-2 and BSL-3 laboratory space, and a highly collaborative environment offering opportunities for both basic and translational research. A joint appointment in a clinical department is possible for individuals with the M.D. or M.D./Ph.D. degrees and clinical training.

Applicants should hold a Ph.D. and/or M.D. degree, and have an excellent track record of research productivity. Successful candidates will be expected to establish and direct a thriving, high-quality independent research program and to contribute to graduate and medical education. Please submit electronic applications including a CV, a statement of research interests and the names and contact information for at least three references to **https://academicjobsonline.org/ajo/jobs/4428**. Review of applications will begin **December 1, 2014** and continue on a rolling basis. Inquiries, but not application materials, may be directed to **MBMFacultyRecruitment@tufts.edu**.

Tufts University is an Equal Opportunity Affirmative Action Employer. We are committed to increasing the diversity of our faculty. Applications from women and members of underserved minority groups are encouraged.

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

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder

It takes a team to save a child

…and professionals like you to help make it happen.

FACULTY POSITION IN MEDICINAL CHEMISTRY FOR CANCER DRUG DISCOVERY

The Department of Chemical Biology and Therapeutics at St. Jude Children's Research Hospital invites applications for a faculty position at the level of ASSISTANT MEMBER or ASSOCIATE MEMBER in medicinal chemistry. We are specifically seeking applicants whose research program will be focused on the discovery and development of novel therapies for the treatment of cancer. The successful applicant will have a demonstrated track record in medicinal chemistry and cancer drug discovery working in a team environment.

The Department of Chemical Biology and Therapeutics is a multidisciplinary, thematically integrated department focused on the discovery and development of small molecules for perturbing cellular functions – particularly in systems relevant to pediatric oncology, infectious disease, hematology and immunology. Departmental faculty work in all areas of drug discovery, including medicinal chemistry, chemical biology, cheminformatics, high throughput and high content screening, in vivo small molecule screening and analytical chemistry. The department also houses a substantial infrastructure for the discovery and rapid development of novel small molecules that affect biological systems.

Individuals will be expected to contribute to one of the programs in the National Cancer Institute-supported Comprehensive Cancer Center at St. Jude, the only NCI-designated Comprehensive Cancer Center that is devoted solely to children. This includes the: Cancer Genetics, Biochemistry & Cell Biology Program; Developmental Biology & Solid Tumor Program; Hematological Malignancies Program; Neurobiology & Brain Tumor Program; and ongoing multi-program efforts utilizing data from the Pediatric Cancer Genome Project towards therapeutics discovery.

St. Jude offers a very competitive package for these positions, including a generous start-up allowance with newly remodeled space and equipment; state-of-the-art facilities for drug discovery; and operational support positions. In addition, appointees have access to a range of institutional core facilities, including analytical chemistry, protein and nucleic acid chemistry, parallel chemistry, gene knockout and transgenic technologies, high throughput and high content screening, pharmacokinetics, structure biology, and multiple advanced xenograft and transgenic animal models of tumor biology.

Those interested in joining this multidisciplinary department and making a difference in the fight against pediatric cancer should arrange to have their CV, a concise future research plan and three letters of recommendation sent by email to R. Kip Guy, Ph.D., Chair Department of Chemical Biology and Therapeutics c/o pam.warrington@stjude.org.

To learn more, visit *www.stjude.org/jobs*

EOE/Minorities/Females/Vet/Disability

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Memorial Sloan Kettering Cancer Center

Faculty positions at the Sloan Kettering Institute Memorial Sloan Kettering Cancer Center

OPPORTUNITIES IN CANCER SCIENCE

- **Genomic Integrity and Editing**
- **Human Cancer Genetics**
- **Epigenetics**
- **Cell Signaling and Regulation**
- **Computational and Systems Biology**
- **Experimental Therapeutics**
- **Stem Cell Biology**
- **Chemical Biology**
- **Tumor Immunology**

JOIN OUR FACULTY

Successful candidates will hold an appointment in one of SKI's Research Programs:

Cancer Biology & Genetics
Chair: Scott Lowe, PhD

Cell Biology
Chair: Alan Hall, PhD

Computational Biology
Chair: Chris Sander, PhD

Developmental Biology
Chair: Kathryn Anderson, PhD

Immunology
Chair: Alexander Rudensky, PhD

Molecular Biology
Chair: Kenneth Marians, PhD

Molecular Pharmacology & Chemistry
Chair: David Scheinberg, MD, PhD

Structural Biology
Chair: Nikola Pavletich, PhD

Research and Training

- 130 research laboratories housed in state-of-the-art buildings
- Core facilities offering cutting-edge scientific services
- Over 800 pre- and post-doctoral trainees
- Appointments in the Gerstner Sloan Kettering Graduate School of Biomedical Sciences and the Weill Cornell Graduate School of Medical Sciences

Visit **www.ski.edu** to learn more.

Apply at: **facultysearch.ski.edu**

MSK is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply and will receive consideration without regard to race, color, gender, gender identity or expression, sexual orientation, national origin, age, religion, creed, disability, veteran status or any other factor which cannot lawfully be used as a basis for an employment decision.

Science Careers online @sciencecareers.org
FACULTY POSITIONS

UNIVERSITY OF NOTRE DAME

FACULTY POSITIONS IN DRUG DISCOVERY

The University of Notre Dame, Notre Dame, IN, is actively seeking candidates for multiple faculty positions with research interests emphasizing modern chemical approaches to the study of disease-related biology and/or the discovery of therapeutic agents. With major funding support from the William K. Warren Foundation, the University of Notre Dame is launching a Center for Drug Discovery and Development. The Center will complement the university's recent major strategic research investments to create and promote a highly collaborative environment for the multidisciplinary biomedical research. The positions range from junior level through tenured senior faculty and endowed chair appointments. The successful candidate will occupy state-of-the-art research space in a new 200,000 square-foot molecular sciences building currently under construction.

Senior and senior-endowed chair candidates should have a distinguished record of accomplishments. Candidates for all levels are expected to establish and maintain a strong, extramurally-funded research program and a commitment to excellence in teaching at the undergraduate and graduate levels also is required. The positions include salary, start-up funding and laboratory space commensurate with level.

Junior candidates should include a cover letter, curriculum vitae, detailed research plan, and statement of teaching interests addressed to Professor Richard Taylor, Chair of the search committee through **https://academicjobsonline.org/ajo/jobs/4346**. In addition, junior candidates must also arrange to have at least three letters of recommendation sent to the search committee via the application website. Senior applicants need only provide the search committee chair a limited set of documents that will be handled in confidence. To request further information please contact Professor Taylor (**taylor.61@nd.edu**). Review of applications will commence immediately and will continue until the positions are filled.

The University of Notre Dame is an Equal Opportunity Employer with a strong institutional commitment to diversity and endeavors to foster a vibrant learning community animated by the Catholic intellectual tradition.

FACULTY POSITIONS AT THE ROCKEFELLER UNIVERSITY

The Rockefeller University seeks exceptional, interactive, and creative scientists to join its faculty. We invite applications from outstanding candidates for tenure-track positions.

The University has a laboratory-based organizational structure that fosters interdisciplinary research. We encourage applications in the following areas:

- **Chemical & Structural Biology**
- **Genetics & Genomics**
- **Immunology, Virology & Microbiology**
- **Medical Sciences, Systems Physiology & Human Genetics**
- **Molecular Cell Biology**
- **Neurosciences & Behavior**
- **Organismal Biology & Evolution**
- **Physical, Mathematical & Computational Biology**
- **Stem Cells, Development, Regeneration & Aging**

Details about specific subjects of research can be found at: **http://www.rockefeller.edu/facultysearch.**

The Rockefeller University provides strong support for the research work of its faculty. The positions offer competitive salary, benefits and start-up funds, renovated laboratory space, access to state-of-the-art core facilities and extensive opportunities for collaboration both within the University and with neighboring institutions.

Applications are being accepted electronically through our **Online Application System** at **http://oas.rockefeller.edu**. Applicants should follow the online application procedure.

The deadline for application submission is October 10, 2014.

If you have questions regarding submitting an application, please contact our Administrator at **facultysearch@rockefeller.edu.**

The Rockefeller University is an Equal Opportunity Employer - Minorities/Women/Disabled/Veterans.

THE MOLECULAR & BEHAVIORAL NEUROSCIENCE INSTITUTE
UNIVERSITY OF MICHIGAN

FACULTY POSITION

The Molecular and Behavioral Neuroscience Institute (MBNI) at the University of Michigan is recruiting an Assistant Professor (tenure track) whose research program focuses on fundamental aspects of nervous system development and function. We are especially interested in candidates applying molecular, cellular and genetic techniques to investigate mechanisms underlying synaptic plasticity, neural circuits, behavior or disease. We seek highly motivated and interactive individuals that will establish an internationally recognized, independently funded research program. Applicants must have a Ph.D. and/or M.D. and a strong record of research accomplishments. For more information on the MBNI see: **http://www.mbni.med.umich.edu/mbni/search.html**.

Interested candidates should send a cover letter, curriculum vitae and a 3-5 page statement of current and future research directions as a single PDF to **MBNI.2015@umich.edu** Candidates should also arrange to have 3 letters of recommendation sent as PDFs to the above email address. Applications and letters of recommendation will be considered up until **November 1, 2014**.

Women and underrepresented minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an equal opportunity employer.

UNIVERSITY OF MICHIGAN

Tenure Track Assistant Professor Biophysics

Biophysics at the University of Michigan has a tenure track Assistant Professorship available, with a university year appointment starting September 2015. An appointment at a higher rank may be considered for candidates with an exceptional record of productivity. We are considering applications in all areas of biophysics, especially those areas focused on problems of biological significance that use and develop modern biophysical methodologies. We are primarily interested in quantitative experimental or theoretical work across all length scales. Highly interdisciplinary work that bridges disciplines is strongly encouraged and joint appointments with other departments are possible. Information about our research areas can be found at http://www.lsa.umich.edu/biophysics/. Candidates are required to have a doctoral degree in biophysics or a related field such as chemistry, biological chemistry, physics etc. The successful candidate is expected to establish an independent research program and to contribute effectively to the Department's undergraduate and graduate teaching programs.

Applicants should submit a curriculum vitae, a brief statement of present and future research plans, a statement of teaching philosophy and experience, at least three letters of recommendation, and evidence of teaching experience, if any. The deadline for applications is **October 1, 2014**. A link to the application can be found at **http://www.lsa.umich.edu/biophysics/**. If you should have any questions, please feel free to contact **Ann Titus** at **atitus@umich.edu** or **(734) 764-1146**.

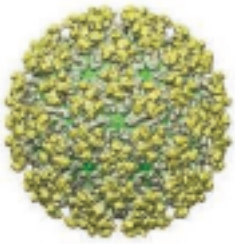
Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

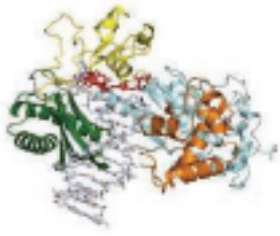
utmb Health

Faculty Positions in Biochemistry and Molecular Biology
The University of Texas Medical Branch

The University of Texas Medical Branch (UTMB Health), Galveston, Texas, seeks outstanding faculty candidates at all ranks for tenure-track/tenured positions in the broad fields of Biochemistry and Molecular Biology under the leadership of a newly recruited Chair in the Department of Biochemistry and Molecular Biology (http://www.bmb.utmb.edu). Investigators working in the areas of DNA and RNA biology are particularly encouraged to apply. The ideal candidate will have extensive experience in elucidating the mechanisms of important biomedical problems at a molecular / atomic level. The candidates should be energized by opportunities to interact with established biomedical researchers in a highly collaborative biomedical research community and to become outstanding mentors to students and postdoctoral fellows. Rich opportunities exist at UTMB for interactions and affiliations with centers of scientific excellence in structural biology and molecular biophysics, biodefense, molecular medicine, cancer biology, infectious diseases, environmental health, aging, and translational sciences (http://www.utmb.edu/centers). In addition to a highly collaborative environment, UTMB offers outstanding core services, including: next-generation sequencing, organic synthesis, mass spectrometry, optical microscopy, flow cytometry, protein expression and purification, molecular biology, solution biophysics, NMR, X-ray crystallography, cryo-electron microscopy, bioinformatics, and computational chemistry (see http://www.utmb.edu/core). Excellent opportunities for scientific interactions also exist through UTMB Health's participation in the Gulf Coast Consortia and the Keck Center for Interdisciplinary Bioscience (http://www.gulfcoastconsortia.org).

A very attractive recruitment package of salary, start-up and newly renovated space will be offered. Interested applicants should submit a curriculum vitae, a summary of research accomplishments and future goals (max 3 pages), and contact information for three references to: **Mariano A. Garcia-Blanco M.D., Ph.D.** at **BMB.Recruiting@utmb.edu**.





UTMB Health strives to provide equal opportunity employment without regard to race, color, national origin, sex, age, religion, disability, sexual orientation, gender identity or expression, genetic information or veteran status. As a VEVRAA Federal Contractor, UTMB Health takes affirmative action to hire and advance women, minorities, protected veterans and individuals with disabilities

FACULTY POSITIONS Science Careers online @sciencecareers.org

HEALTH SCIENCE CENTER TEXAS A&M UNIVERSITY

Tenure-Track Faculty Position Neuroscience and Experimental Therapeutics The Texas A&M Health Science Center College of Medicine

The Department of Neuroscience and Experimental Therapeutics (NExT) invites applications for a tenure-track faculty position at the level of ASSISTANT/ASSOCIATE PROFESSOR. This position offers an outstanding research environment, a newly built research facility with a modern vivarium, and a highly competitive start up package, compensation, and benefits. Successful candidates are expected to develop an innovative, extramurally funded research program and to contribute to graduate and medical student education.

Candidates should have a Ph.D. and/or M.D. or equivalent degree(s), postdoctoral research experience, an excellent publication record and a commitment to graduate and medical education. The ideal candidate's research program will complement existing research strengths in the department, which include molecular, cellular, and behavioral approaches to study of brain development, aging, ocular pharmacology and inflammatory diseases, addiction, circadian biology, neurodegenerative diseases, epilepsy and stroke. For more details see http://medicine.tamhsc.edu/next/index.html). In addition, the neuroscience community at the Texas A&M University consists of 70+ faculty with an active graduate program through the Texas A&M Institute for Neuroscience. Clinical collaborations are possible through the Texas Brain and Spine Institute. Applicants should email (in pdf format) the following (1) cover letter, (2) curriculum vitae with 2-3 page statement of current and future research interests, (3) three selected reprints, and (4) names / addresses of four references willing to provide a letter of recommendation. The application packet should be sent to **NExT-Faculty-Search@medicine.tamhsc.edu**.

The Texas A&M University System is an Affirmative Action/Equal Opportunity Employer dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment. We strongly encourage applications from women, minorities, individuals with disabilities, and covered veterans. Texas A&M University is aware that attracting and retaining exceptional faculty often depends on meeting the needs of two careers and having policies that contribute to work-life balance. For more information, visit http://dof.tamu.edu/content/balancing-work-and-life.

Duke University School of Medicine

Tenure-track or Tenured Faculty Position Department of Molecular Genetics and Microbiology, Duke University Medical Center

Applications are invited for a faculty position in the Department of Molecular Genetics and Microbiology at Duke University Medical Center (http://mgm.duke.edu/). We are seeking individuals that employ genetic and/or genomic approaches to investigate important and fundamental biological questions. While there are no restrictions concerning the research area or the model organism used, individuals working in the following areas are particularly encouraged to apply: (1) the genetic basis for behavior or disease in model systems and/or humans, (2) host and/or pathogen genetic variation that influences infectious disease outcome and progression, (3) genomic and epigenetic mechanisms of disease.

The individual hired is expected to develop a strong, independent research program that will complement existing areas of expertise within the Department. Research strengths within the Department include RNA biology, DNA repair and recombination, microbial pathogenesis (viral, bacterial and fungal), and human/mammalian genetics.

Applications should include a *curriculum vitae*, a description of research accomplishments and plans for future research (please limit to three pages), and reprints of three representative publications. Interested applicants should submit application materials (cover letter, CV, statement of research accomplishments/interests and at least three references) for this job posting on AcademicJobsOnline at **https://academicjobsonline.org/ajo/jobs/4304**. Applications should be received by **November 15, 2014**. Further inquiries may be directed to **Kasey Carroll** at **kasey.carroll@duke.edu**.

Women and minorities are encouraged to apply. Duke University is an Equal Opportunity/Affirmative Action Employer.

AgreenSkills plus — Pathways for inventive researchers

AgreenSkills' Postdoctoral Research Fellowships

Next application deadline: 15 November 2014

AgreenSkills and AgreenSkills+ are international postdoc mobility programmes, accepting applications for fellowships from experienced young and independent researchers in the fields of **life**, **agricultural**, **nutrition**, and **environmental sciences with up to 10 years' experience after their doctorate**. Proposals are accepted, without any guideline topics, for **basic or applied sciences**.

AgreenSkills' programmes are open to incoming researchers who are offered 1-to-3 years fellowships in a research institute in France, within the Agreenium consortium **www.agreenium.org**, while outgoing fellows, already employed by one of these institutes, can spend 6-months-to-2 years mobility period at a research laboratory outside France.

As European Union co-funded mobility programmes, AgreenSkills and AgreenSkills+ strive to offer their fellows **competitive salary and career development packages**, within first-rate research environments. Both programmes greatly focus on the quality of fellowships and their scientific mobility project, the hosting conditions and career prospects.

AgreenSkills and AgreenSkills+ have a continuous call for submissions, with the next selection round deadline on 15 November 2014.

To apply, visit our website: **www.agreenskills.eu**

Co-funded by the European Union — INRA SCIENCE & IMPACT — agreenium Science for food security and sustainable agriculture

University of Missouri SYSTEMS BIOLOGY Faculty Position Assistant or Associate Professor Division of Biological Sciences

The Division of Biological Sciences (**http://biology.missouri.edu**) at the University of Missouri invites applications for an Assistant or Associate Professor in systems biology. We are interested in candidates that integrate empirical and computational approaches to address important biological questions. The campus strengths in life sciences include bioinformatics, behavior, cell and developmental biology, ecology, genetics and genomics, microbiology, neurobiology, and plant biology. The successful candidate will establish a research program that forms linkages with our existing strengths, develops cross-disciplinary collaborations, and attracts federal funding. The successful candidate will join a diverse group of scientists in the Bond Life Sciences Center (http://bondlsc.missouri.edu/), which promotes interdisciplinary research.

We offer a highly competitive salary and start-up package, an active doctoral program with institutional support for students, a highly interactive faculty and outstanding core facilities. Columbia, Missouri, is ranked among the top-ten best college towns in the U.S. *We are committed to ethnic, racial, and gender diversity in our faculty and strongly encourage applications from women and members of groups underrepresented in mathematics and science.*

Review of application materials (cover letter, CV, description of research plans and teaching interests, and contact information for three references, all compiled into a single PDF file) will begin **October 10, 2014**. Application submission instructions can be found at **http://biology.missouri.edu/systemssearch**. Questions should be addressed to **systemssearch@missouri.edu**.

Equal Employment Opportunity: The University of Missouri is an Equal Access, Equal Opportunity, Affirmative Action Employer that is fully committed to achieving a diverse faculty and staff. For more information, call the Associate Vice Chancellor of Human Resource Services/Affirmative Action officer at 573-882-4256. To request ADA accommodations, please call Human Resource Services at 573-882-7976. TTY users, please call through Relay Missouri, 1-800-RELAY (735-2966) or en Español at 1-800-520-7309.

FACULTY POSITIONS Science Careers online @sciencecareers.org

UCSB CHEMISTRY AND BIOCHEMISTRY

FACULTY POSITION

The University of California Santa Barbara announces a search for a tenure-track faculty member for a position beginning Fall 2015 at the Assistant Professor level. Outstanding candidates with research and teaching interests in all sub-areas of inorganic chemistry, including bioinorganic, inorganic materials and organometallic chemistry are invited to apply. In addition to contributing to the research, teaching and service missions of the Department of Chemistry and Biochemistry, we anticipate campus-wide interactions in interdisciplinary programs between departments in the Colleges of Letters and Sciences and Engineering, along with other University-based Centers and Institutes.

Applicants should submit a curriculum vitae, description of their research plans, statement of graduate and undergraduate teaching interests, and arrange to have three letters of recommendation sent on their behalf to **https://recruit.ap.ucsb.edu/**. Review of applications will begin **October 15, 2014** and will continue until the position is filled. A Ph.D. is required at the time of appointment. The Department is especially interested in candidates who can contribute to the diversity and excellence of the academic community through research, teaching and service.

SENIOR FACULTY POSITION

The University of California Santa Barbara seeks to hire a highly creative and productive individual with research and teaching interests in green chemistry or other areas of chemistry or biochemistry relevant to sustainability, to begin as early as July 1, 2015. The successful candidate will be appointed to one of the *Mellichamp Chairs in Sustainability*. Although preference will be given to candidates currently at a senior, tenured professorial or equivalent rank, applications at any rank will be considered. This appointment is one of a cluster of four *Mellichamp Chairs in Sustainability* encompassing green chemistry, sustainable manufacturing, sustainable catalytic processing, and economic analysis of sustainable technologies, made possible by a generous gift from Duncan and Suzanne Mellichamp. This appointment will primarily be in the department of Chemistry and Biochemistry. Other Mellichamp Chair appointments will be made in Engineering and The Bren School of Environmental Science and Management. The four Mellichamp chairholders are expected to work together to foster interdisciplinary research and educational programs in chemical sustainability at UC Santa Barbara. The University is especially interested in candidates who can contribute to the diversity and excellence of the academic community through research, teaching and service.

Applications should be sent to **https://recruit.ap.ucsb.edu/**, and should include a letter briefly outlining career accomplishments and plans, a statement of research and teaching interests, a curriculum vitae, and the names and contact information of 3-5 references. The search will remain active until the positions are filled. However, applicants are encouraged to submit their applications by **October 15, 2014**. Questions regarding this position should be directed to the Chair of the Search Committee at: moskovits@chem.ucsb.edu.

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.

Division of Pulmonary Medicine in the Department of Pediatrics Assistant, Associate or Professor, University Tenure Line, Non-Tenure Line Research, or Medical Center Line Stanford University School of Medicine

The Division of Pulmonary Medicine in the Department of Pediatrics at Stanford University School of Medicine is seeking two faculty members at the Assistant, Associate or Professor rank. Depending upon their qualifications they will be appointed in the University Tenure Line (UTL), the Non-Tenure Line Research (NTLR) or the Medical Center Line (MCL).

The predominant criterion for appointment in the University Tenure Line is a major commitment to research and teaching.

The major criterion for appointment in the NTLR is evidence of high-level performance as a researcher in an area for which a programmatic need exists.

The major criteria for appointment for faculty in the Medical Center Line shall be excellence in the overall mix of clinical care, clinical teaching, scholarly activity that advances clinical medicine, and institutional service appropriate to the programmatic need the individual is expected to fulfill.

Faculty rank and salary will be determined by the qualifications and experience of the successful candidate.

Essential Requirements for candidates: Be an M.D. and/or Ph.D. Candidates must have a demonstrated record of accomplishment in investigator-initiated research.

It is anticipated that the successful faculty candidates will be excellent researchers in a disciple relevant to Pulmonary biology (including but not limited to areas such as inflammation, vascular biology, immunology, developmental biology) committed teachers, outstanding clinicians (MD candidates only), and have the skills to establish an investigator-initiated research program focused primarily upon discoveries that can be applied to human health and disease. The successful candidates will demonstrate the capacity to direct a research program with a focus that is directly relevant to lung biology. All areas of research are of interest: basic, translational, clinical research, epidemiology/statistics, informatics, health services, or health policy.

The appointment will be at the Assistant, Associate or full Professor academic rank with rank being determined by the qualifications and experience of the successful candidates.

Stanford University is an equal opportunity employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women, members of minority groups,protected veterans and individuals with disabilities, as well as from others who would bring additional dimensions to the university's research, teaching and clinical missions.

Interested candidates should send a copy of their curriculum vitae, a brief letter outlining their interests and the names of three references to: **Dr. David Cornfield, Search Committee Chair, dcornfield@stanford.edu**.

THOMAS E. KURTZ CHAIR IN COMPUTATIONAL SCIENCE

Applications are invited for a tenured, full-professor faculty position and the inaugural holder of the Thomas E. Kurtz Chair in Computational Science at Dartmouth College. This is the first of several hires within Dartmouth's newly established William H. Neukom Academic Cluster, which will add three new faculty members in diverse fields, each of whom uses computational techniques in their teaching and research. For the Kurtz Chair, the successful candidate will be an established leader in the field of **Computational and Engineering Principles of Intelligence**, broadly defined as that area of computation associated with the development and application of computational approaches to solve the most difficult computational problems that, by their nature, are either not amenable to modeling from first principles or that have complex, poorly understood interacting components. The holder of the chair will have a primary appointment in one of several possible departments or professional schools, including Biology, Cognitive Science, Computer Science, Mathematics, Neuroscience (housed within Psychological and Brain Sciences), the Thayer School of Engineering, or the Geisel Medical School, with a strong potential for joint or adjunct appointments with other departments or schools. The Kurtz Chair holder will work with other schools, centers and institutes across campus, including the Neukom Institute for Computational Science, in developing Dartmouth's vision of becoming *the* institution in the dissemination and application of computational methods across teaching and research. The holder of the Kurtz Chair will be involved in the process of identifying disciplines for two additional future junior faculty lines, and will serve as part of the search process for those hires.

Dartmouth College is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversity. In that spirit, we encourage applications from a broad spectrum of people, including women, persons of color, persons with disabilities, veterans or any other legally protected group.

Review of applications will begin in **December 2014** with an appointment start date of July 1, 2015 expected. A complete CV, research and teaching statements, and contact information for four references should be sent to: **Search Committee Chair, Thomas E. Kurtz Chair in Computational Science, Office of the Provost, 6004 Parkhurst Hall, Room 102. Hanover, NH 03755-3529**. Applications may also be submitted in PDF format via email to: **Kurtz.Chair.Search@dartmouth.edu**

THE UNIVERSITY of TENNESSEE HEALTH SCIENCE CENTER

Pharmaceutics Faculty Position

The University of Tennessee Health Science Center (UTHSC), department of Pharmaceutical Sciences in the College of Pharmacy, is seeking an outstanding applicant for a twelve-month full-time, tenure-track faculty position at the full, associate, or assistant professor level that is state supported. The successful candidate is expected to have a strong research program in the area of: pharmaceutics, physical pharmacy, drug delivery, gene therapy, nanotechnology, nano-medicine, regenerative medicine, bio-imaging, biosensors, biomedical engineering, or other related discipline with focus on drug discovery and development.

The successful candidate is expected to have a Ph.D. or equivalent degree, the ability to maintain a competitive extramural funding, a commitment to excellence in teaching Pharm.D. and graduate programs, and have excellent oral and written communication skills. Applications will be processed until the position is filled. Applicants must have a commitment to diversity and equal opportunity.

Please submit a cover letter, curriculum vitae, summary of research and teaching interests, and three letters of reference to: **Dr. Duane D. Miller, Chair of Pharmaceutics Faculty Search Committee-Department of Pharmaceutical Sciences. 881 Madison Avenue, Room 435 Memphis, TN 38163**, or by email at **dmiller@uthsc.edu**.

The University of Tennessee does not discriminate on the basis of race, sex, color, religion, national origin, age, disability or veteran status in provision of educational programs and services or employment opportunities and benefits. This policy extends to both employment by and admission to the University. The University does not discriminate on the basis race, sex, or disability in its educational programs and activities pursuant to the requirements of Title VI of the Civil Rights Act of 1964, Title IX of the Educational Amendments of 1972, Section 504 of the Rehabilitation Act of 1973, and the Americans with Disabilities Act (ADA) of 1990. Inquiries and charges of violation concerning Title VI, Title IX, Section 504, ADA or the Age Discrimination in Employment Act (ADEA) or any of the other above referenced policies should be directed to the Office of Equity and Diversity, 62 South Dunlap, Memphis, Tennessee 38163, telephone 901-458-5558 (V/TTY available).

The University of Tennessee is an EEO/AA/Title VI/Title IX/Section 504/ADA/ADEA institution in the provision of its education and employment programs and services.

Ireland seeks World-class Research Talent

Science Foundation Ireland

INSPIRATION • INNOVATION • IRELAND

Ireland is building a reputation as a world leader in scientific research capability, continuously climbing the international citation and innovation rankings to be in the top 20 countries overall for citations, while achieving rankings of 1st, 3rd and 4th in Immunology, Nanoscience and Computer Science research respectively.

Ireland is a small English speaking technologically advanced country (population 4.5 million), home to leading multinational and indigenous companies, with a young growing population (the median population age is 35, the lowest in the EU), ranked 1st in Europe for completion of university education and in the top centile of Eurostat rankings for Innovation Output.

Ireland is committed to funding excellent and impactful research. In 2013 Science Foundation Ireland (SFI) invested, with industry, €300 million ($400 million) in 7 new cutting-edge research centres; funded over 300 new research awards; SFI supported researchers obtained over €125 million ($167 million) of additional research funding from the European Union, Charities and industry. SFI supports over 450 leading researchers who are involved in 2,000 national and international collaborations across 48 countries.

SFI also supports 100 outstanding young researchers at the early stages of their careers.

Ireland has now launched an integrated recruitment programme involving all the Irish Universities and the national science funding agency (SFI) to attract world-class researchers, with exceptional reputations, to assume leadership positions (e.g. Professorial Chairs) in key areas of national importance that include advanced manufacturing; bio-manufacturing; energy; marine; ICT; medical devices; agrifood; climate change; smart cities; ageing; connected health; and digital platforms, content and applications.

The SFI Targeted Research Professorship Programme 2014 will provide competitive research funding from SFI of up to €5 million ($6.7 million) in direct costs, salary and physical accommodation will be provided by the universities.

If you believe that you can inspire and build excellence with impact in a well-resourced research environment, please consult **www.sfi.ie** or email **researchprof@sfi.ie**

NUI Galway
OÉ Gaillimh

University of Limerick
Ollscoil Luimnigh

UCC
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

DCU

NUI Maynooth
Ollscoil na hÉireann Má Nuad

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

UCD Dublin

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FACULTY POSITIONS

CLEMSON UNIVERSITY

The Self Family Foundation Endowed Chair in Human Genetics
Clemson University/Greenwood Genetic Center

Clemson University (CU) is seeking to fill a new endowed professor position. The Self Family Foundation Chair in Human Genetics (Self Chair) is part of a dynamic interdisciplinary initiative to build strength in medical genetics, genomics, epigenetics, and computational biology across South Carolina with national and international partnerships. This position will be located in Greenwood, SC at the Center for Human Genetics (CHG), a research and education building to be built adjacent to the J.C. Self Research Institute and South Carolina Center for the Treatment of Genetic Disorders at the Greenwood Genetic Center (GGC). The GGC is a non-profit institute dedicated to providing clinical and diagnostic genetic services, educational programs, and research in the field of medical genetics.

Individuals with an internationally recognized research program in human genetics or related discipline and a strong history of external funding are invited to apply. Candidates must possess an excellent record of peer-reviewed publications and both teaching and mentoring experience. The Self Chair is expected to maintain a vigorously funded research program and lead the human genetics initiative through recruitment of new faculty hires to be housed at the CHG. Candidates must be eligible for appointment as Full Professor with tenure in the Department of Genetics & Biochemistry at CU.

To apply, submit a cover letter, curriculum vitae, short statement of research interests and future directions, and a list of references in one PDF file emailed directly to **SelfChair@clemson.edu** with Self Chair in the subject heading. To ensure full consideration, please submit all application materials by **October 31, 2014**. Review of applications will begin in November 2014 and continue until the position is filled. The starting date for this position is negotiable.

Clemson University is committed to a policy of equal opportunity for all persons and does not discriminate on the basis of race, color, religion, sex, sexual orientation, gender, pregnancy, national origin, age, disability, veteran's status, genetic information or protected activity (e.g., opposition to prohibited discrimination or participation in any complaint process, etc.) in employment, educational programs and activities, admissions and financial aid. This includes a prohibition against sexual harassment and sexual violence as mandated by Title IX of the Education Amendments of 1972.

UNIVERSITY AT ALBANY
State University of New York

Six Positions at The RNA Institute at the University at Albany

RESEARCH JUNIOR FACULTY: Either a) whole organism, small animal disease model to study RNA function as a target of drug intervention or b) pharmaceutical/clinical organic chemist interested in small molecule RNA intervention in human disease. Full position description and application,
http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=52331

ADVANCED COMPUTATIONAL FACILITY LAB MANAGER: Supervise High Performance Computing (HPC) facility; provide team leadership; conduct and support RNA computational research in structure/function relationships; learn and adapt technologies, provide analysis, integrate, innovate and deliver results supporting human disease research. Full position description and application,
http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=52161

RNA ENVIRONMENTAL BIOCHEMIST JUNIOR/SENIOR FACULTY: RNA epigenetics, environmental and drug insults to RNA with functional consequences. For full position description and application,
http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=43450

LIFE SCIENCE ENTREPRENEURSHIP & TECHNOLOGY COMMERCIALIZATION JUNIOR/SENIOR FACULTY: Research, technology advancement and commercialization, and instruction that elevates and expands UAlbany's program offerings to science and business students in one of the most important and dynamic areas of growth in the U.S. Full position description and application,
http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=43452

SINGLE-MOLECULE BIOPHYSICS POSTDOCTORAL RESEARCHER: Interdiscipli-nary research focusing on the development of single-molecule biophysics. Send cover letter, CV, statement of research interests, and list of at least 3 references as a single PDF document to Dr. Ken Halvorsen, **khalvorsen@albany.edu**.

RNA SEPARATION SCIENCE & MASS SPEC POSTDOCTORAL RESEARCHER: Interdisciplinary research into separation science and small molecule analysis of RNA with strong emphasis on the development of separation science and mass spectrometry-based technologies. To apply, please send detailed curriculum vitae, statement of research experience and interest, and contact info for 3 references including immediate supervisor, and others aware of your research capabilities to **mbasantasanchez@albany.edu**. Full position description and other positions, **http://www.albany.edu/rna/Careers.html**

In applying, candidates must address their ability to work with and instruct a culturally diverse population.

The University at Albany is an EO/AA/IRCA/ADA Employer

Faculty Career Feature
October 3, 2014

Reserve ads by September 16 to guarantee space*

There's only one **Science**

Hiring Faculty? We've got a special feature for your **Faculty ads** this fall! The October 3 feature explores how faculty can best collaborate with professionals in different cultures and countries.

Why you should advertise in this issue of *Science*:

Reach: Your job ad is seen by 570,400 readers around the globe

- The October 3 issue includes a special bonus distribution to 25,000 scientists beyond our regular circulation

To book your ad:
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+86 186 0082 9345

Science Careers
From the journal *Science* AAAS
ScienceCareers.org

*Ads accepted until September 26 if space is still available. Produced by the *Science*/AAAS Custom Publishing Office.

The European Molecular Biology Laboratory is searching for Group Leaders. EMBL offers a highly collaborative, uniquely international culture. It fosters top quality, interdisciplinary research by promoting a vibrant environment consisting of young, independent researchers with access to outstanding graduate students and postdoctoral fellows. EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

EMBL Group Leader Opportunities

DEVELOPMENTAL BIOLOGY UNIT

at EMBL Heidelberg, Germany

GROUP LEADER – DEVELOPMENTAL BIOLOGY

The Developmental Biology Unit studies the development of multicellular organisms, from the cellular to the whole organism level, with a particular interest in the dynamics of developmental processes. Research in the unit is highly interdisciplinary and combines genetic, biochemical, cell biological, genomic/proteomic, comparative and innovative computational modelling and imaging approaches. Current research topics include cell fate specification and polarity, morphogenesis and organogenesis, neurodevelopment, evolution, genome organisation and RNA based control of development.

We are seeking outstanding candidates addressing fundamental principles of organismal development and their mechanistic basis.

Further information about the position can be obtained from the Head of Unit, Anne Ephrussi (**anne.ephrussi@embl.de**).

Interviews are planned for 8, 9 and 10 December 2014.

STRUCTURAL AND COMPUTATIONAL BIOLOGY UNIT

at EMBL Heidelberg, Germany

GROUP LEADER – NMR SPECTROSCOPY

We are particularly interested in candidates that apply NMR spectroscopy techniques to answer fundamental questions in molecular or cellular biology. Interests of the unit include protein-protein interaction networks as well as interactions of proteins and protein networks with small molecule effectors and metabolites.

It is desirable that the successful candidate complements and synergizes with existing activities in the unit. The vision of the unit is to integrate various structural biology approaches (Xray, NMR, electron microscopy, tomography and light microscopy) with computational, chemical and systems biology approaches. Therefore we seek strongly motivated individuals to work in the collaborative and multidisciplinary environment at EMBL. We offer access to an integrated structural biology environment that includes a state-of-the-art NMR facility comprising different NMR spectrometers and high performance computing clusters.

For further information about the position please contact **recruitment@embl.org**.

Interviews are planned for 19, 20 and 21 November 2014.

MOUSE BIOLOGY UNIT

at EMBL Monterotondo, near Rome, Italy

EMBL Monterotondo applies a wide range of modern technologies to diverse problems of whole organism biology. Currently, units address areas of neurobiology, genetics, epigenetics, cancer and developmental biology. The appointed Group Leaders will join the renowned Mouse Biology Unit and head an independent research group.

GROUP LEADER – MOUSE GENETICS AND BIOLOGY

We seek dynamic interactive individuals with an excellent track record and demonstrable experience or interest in the general area of mouse genetics and biology and a desire to join a multidisciplinary environment. We encourage applications from scientists working on diverse questions relating to mammalian organismal biology, which would benefit from the mouse as an experimental system and the use of modern genetic and genomic approaches.

GROUP LEADER – NEUROBIOLOGY

We seek dynamic interactive individuals with an excellent track record and demonstrated experience or interest in the general area of neurobiology and a desire to join a multidisciplinary environment. We encourage applications from scientists working on diverse questions relating to mammalian neuro-biology, which would benefit from the mouse as an experimental system and the use of modern genetic, epigenetic and genomic approaches.

Further information about both positions can be obtained from the Head of the Mouse Biology Unit, Philip Avner (**philip.avner@embl.it**).

Interviews are planned for 26, 27 and 28 November 2014 (Mouse Genetics and Biology) and 15, 16 and 17 December 2014 (Neurobiology).

APPLICATION INSTRUCTIONS

Please apply online through **www.embl.org/jobs** and include a cover letter, CV and a concise description of research interests and future research plans. Please also arrange for 3 letters of recommendation to be emailed to **references@embl.de** at the latest by 19 October 2014.

Further information on Group Leader appointments can be found under **www.embl.org/gl_faq**.

For more information please visit:

www.embl.org

Science Careers online @sciencecareers.org
FACULTY POSITIONS

NYU Langone MEDICAL CENTER

Institute for Systems Genetics

New York, NY

The newly formed Institute for Systems Genetics at NYU Langone Medical Center invites applications for tenure-track faculty positions at the assistant professor level. The Institute, headed by Jef Boeke, Ph.D., will combine the latest technologies in systems, sequence and synthetic biology to tackle fundamental and translational questions in genetics and epigenetics. The Institute's interdisciplinary research programs will center on four general overlapping areas: human genetics, 'omics technologies and their applications, computational sciences, and biological engineering – all practiced in a highly collaborative environment. The Institute will occupy new space in the Alexandria Center for Life Sciences West Tower on the East Side of Midtown. The space is designed to foster highly collaborative and innovative research, and operate side-by-side with new initiatives in metabolism, microbiology, cancer biology, and immunology to complement the vibrant overall biomedical research environment at NYU Langone Medical Center, as well as the growing 'omics community in New York City. State-of-the-art genomics, proteomics, and robotics facilities will be integral components of the Institute, and will leverage outstanding existing core facilities at NYU School of Medicine. Collaborations with the recently founded New York Genome Center and related entities will be encouraged. We seek junior colleagues with hypothesis-driven research programs that exploit genetic, 'omic, engineering and/or computationally intensive methods. Candidates in the following areas are especially relevant:

- **Human Genetics and Genomics**
- **Computational Biology**
- **Functional Genomics**
- **High throughput imaging or phenotyping**
- **Proteomics**
- **Synthetic Biology/Genome Engineering**

Candidates from nontraditional backgrounds such as computer science, engineering, physics, chemistry and mathematics are encouraged to apply. The successful candidate will hold a primary appointment in one of many relevant NYU School of Medicine Departments, and will have opportunities to participate in one or more graduate programs.

For consideration, candidates should send a CV, research plan, and the name, telephone number and email address of four references (consolidated into a single pdf document) by **12/31/14**, to: **ISGNYUjobs2014@gmail.com**. Please have referees send letters of recommendation as pdf documents.

NYU Langone Medical Center is an Equal Opportunity Employer.

VANDERBILT UNIVERSITY

TWO TENURE TRACK FACULTY POSITIONS IN BIOLOGICAL SCIENCES

The Department of Biological Sciences at Vanderbilt University seeks candidates to fill two tenure-track, assistant professor faculty positions. We desire candidates whose research complements existing areas of strength within the department (http://as.vanderbilt.edu/biosci). The scope of the search will reflect the full breadth of the department; we are particularly interested in candidates addressing fundamental questions in the areas of biochemistry or biophysics, organismal, plant, and systems biology. The central criteria for the position are excellence in research and the ability to teach undergraduate and graduate students with a high level of effectiveness. Applicants should send a single PDF containing a letter of application, *curriculum vitae*, statement of current and future research interests, and evidence of teaching effectiveness to **alicia.goostree@vanderbilt.edu**. Applicants should arrange for three letters of recommendation to be sent to the same address. Interested parties should submit their application by **November 1, 2014**, and review of applicants will continue until both positions have been filled.

Vanderbilt University, located in the heart of Nashville, TN, is an Affirmative Action/Equal Opportunity Employer. Women and under-represented minority candidates are especially encouraged to apply.

Leibniz-Institut für Altersforschung
Fritz-Lipmann-Institut e.V. (FLI)

FACULTY POSITIONS (TENURE TRACK) AT THE LEIBNIZ INSTITUTE FOR AGE RESEARCH – FRITZ LIPMANN INSTITUTE, JENA, GERMANY

The FLI is an internationally renowned research institute focused on the biology of aging. We are currently expanding under new leadership and are seeking outstanding enthusiastic individuals to strengthen our research program. We are proud of our highly collaborative and stimulating environment, emphasis on mentoring junior scientists and provide the opportunity to help shape the institute's research focus and fu-ture. We offer an outstanding start-up package, strong scientific core support, a competitive salary and excellent benefits.

The successful candidate will be expected to establish an independent research program using innovative approaches to study mechanisms of aging, preferentially in the areas of stem cell biology, DNA damage, chromatin biology and epigenetics. The candidate will join an interactive and vibrant group of faculty fo-cused on stem cell and telomere biology, stress response and development, regulation of lifespan, organ homeostasis and DNA damage using various model systems.

Applicants must have a Ph.D. and/or M.D. degree and strong record of research achievements. Preference is given to junior faculty candidates.

Qualified candidates are invited to submit an application electronically to <**careers@fli-leibniz.de**>. A curriculum vitae and description of present and future scientific endeavors are required. Please arrange to have three letters of recommendation sent directly from the referees to <**careers@fli-leibniz.de**>.

For more information about the positions please contact Dr. Lenhard Rudolph (vorstand@fli-leibniz.de)

Applications with all required materials will be accepted until October 1st 2014. (code number 1412) Interviews will be conducted from November to December 2014.

The FLI is an Equal Opportunity Employer and we encourage qualified female candidates to apply.

Please see also http://www.fli-leibniz.de/

Systems Developmental Biology Faculty Position

The California Institute of Technology invites applications for a tenure-track assistant professor position in the Division of Biology and Biological Engineering. We are interested in candidates who will approach fundamental questions regarding the cellular and molecular basis of development of multicellular organisms, with emphasis on 'systems' level approaches. Relevant research areas include but are not limited to gene network and circuit design problems in developmental biology, mechanistic studies of morphogenesis, evolution of developmental gene regulatory networks, etc.

Candidates with strong commitments to research and teaching excellence are encouraged to apply. Initial appointments at the assistant professor level are for four years, and they are contingent upon completion of the Ph.D. degree.

Please submit on-line applications at: http://bbe.caltech.edu/Positions, and include a brief cover letter; curriculum vitae; relevant publications, a description of proposed research; and a statement of teaching interests. Applicants should arrange to have four reference letters uploaded. The **application deadline is October 31, 2014**. Applicants must be prepared to **attend a recruiting symposium at Caltech on January 12-13, 2015, where they will present their research and future directions**.

EOE of Minorities/Females/Protected Vets/Disability.

THE OHIO STATE UNIVERSITY COLLEGE OF PHARMACY FACULTY POSITIONS IN PHARMACEUTICS

The College of Pharmacy announces an extraordinary opportunity to build our cancer research program in the area of Pharmaceutics & Pharmaceutical Chemistry. Through support from the College of Pharmacy, the Comprehensive Cancer Center and The Ohio State University, our Division is poised to hire up to 6 positions at the rank of Assistant, Associate or Full Professor. Applicants will be considered with research interests in pharmacokinetics-pharmacodynamics, pharmacometrics, quantitative pharmacology, drug delivery, drug formulation, molecular pharmacology, pharmacogenomics or personalized medicine. Opportunities for leadership positions within the Institution may be available. The successful candidates are expected to establish and/or maintain an externally funded research program and to train graduate students and postdocs. Preference will be given to those with funding in the area of cancer. Laboratory space will be available in the state-of-the-art, Biomedical Research Tower that is directly adjacent to the College of Pharmacy. The Health Sciences campus at The Ohio State University is among the most comprehensive in the world and comprises Colleges and Schools representing all major health science professions. In December 2014, a new 21-level cancer research hospital will be opened providing unparalleled opportunities for clinical and translational research.

APPLICATION INSTRUCTIONS: Applicants should include a statement of research interests and a current curriculum vitae. Review of applications will begin immediately and will continue until all positions are filled. More information is available at h**ttp://go.osu.edu/ceut**. Please send applications to **Thomas D. Schmittgen, Chair Search Committee, College of Pharmacy, Division of Pharmaceutics & Pharmaceutical Chemistry, The Ohio State University, Schmittgen.2@osu.edu**. Final selection of the candidate requires successful completion of a background check.

The Ohio State University is an Affirmative Action/Equal Opportunity Employer.

Genomics Faculty Position at Clemson University

The Department of Biological Sciences at Clemson University invites applications for a full-time tenure-track faculty appointment to begin August 2015 in Eukaryotic Genomics. Clemson University is ranked 21st among national public universities by *U.S. News & World Report* and is located on Lake Hartwell near the Blue Ridge Mountains in beautiful Upstate South Carolina. The Department offers degrees in microbiology, biological sciences, and environmental toxicology.

Applicants must have a PhD, postdoctoral experience, and a strong publication record. The successful applicant is expected to establish a nationally recognized, externally funded research program, and to contribute to the Department's undergraduate and graduate teaching missions. We offer very competitive salaries and start-up packages. We anticipate making the appointment at the Assistant Professor level.

We seek colleagues who are applying genomic tools to address important biological questions ranging from human health and disease to the origin and maintenance of organismal diversity and adaptation.

Applications must include a CV, three reprints, a research plan, and contact information for three references. Review of applications will begin **November 1, 2014** and continue until the position is filled. To insure full consideration, please submit application materials by **October 15, 2014**. Application materials should be sent by e-mail as one PDF file to **biosearch@clemson**.edu. Further information about these positions and the Department are available at: **http://www.clemson.edu/biosci** and **http://findjobs.clemson.edu**.

Clemson University is committed to a policy of equal opportunity for all persons and does not discriminate on the basis of race, color, religion, sex, sexual orientation, gender, pregnancy, national origin, age, disability, veteran's status, genetic information or protected activity (e.g., opposition to prohibited discrimination or participation in any complaint process, etc.) in employment, educational programs and activities, admissions and financial aid. This includes a prohibition against sexual harassment and sexual violence as mandated by Title IX of the Education Amendments of 1972.

EMBL 40 YEARS | 1974–2014 EMBL-EBI

The EBI, part of the European Molecular Biology Laboratory (EMBL), is a world-leading bioinformatics centre providing biological data to the scientific community with expertise in data storage, analysis and representation. EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

Group Leader

at EMBL-EBI in Hinxton, near Cambridge, U.K.

We are seeking an outstanding computational biologist to establish a new research group to complement our current research programme. The EMBL-EBI, located on the Wellcome Trust Genome Campus near Cambridge, U.K., is a global centre for biological data resources and computational biology research. We pursue many different aspects of biology, exploiting and helping to interpret and integrate the biological data held in the databases. Current research topics include sequence analysis, computational genomics, phylogeny, structural biology, regulatory control networks, functional genomics, neurobiology and literature/text mining.

The advertised research position is suitable for outstanding candidates from any area of computational biology, but we are keen to strengthen our research in translational bioinformatics, single cell genomics, systems biology, cellular modelling and literature and data integration. The candidate will join a strong and supportive group of young research group leaders and will interact closely with the large database service teams, benefiting from their technical expertise and scientific knowledge.

Further information about the position can be obtained from the Director of the EBI, Janet Thornton (**thornton@ebi.ac.uk**).

Please apply online through **www.embl.org/jobs** and include a cover letter, CV and a concise description of research interests and future research plans. Please also arrange for 3 letters of recommendation to be emailed to **applications@ebi.ac.uk** at the latest by 19 October 2014.

Interviews are planned at EMBL-EBI in Hinxton near Cambridge (U.K.) for 19 December 2014.

Further details on Group Leader appointments can be found under **www.embl.org/gl_faq**.

www.embl.org
www.ebi.ac.uk

Faculty Positions in Computational Systems Biology At the Boston College Biology Department

The Boston College Biology Department seeks outstanding candidates for full-time, tenure-track faculty positions in the area of Bioinformatics and/or Computational Biology at the rank of Assistant, Associate, or Full Professor depending on qualifications. Boston College provides competitive start-up funds and research space with the expectation that the successful candidate will establish, or bring to the university, a vigorous, externally funded research program.

The research interest of the candidates should be in one of the following areas: population genetics, molecular evolution/phylogenetics, systems biology, or bioinformatics approaches (ex. tool and/or algorithm development for DNA/RNA next generation sequence analyses). The successful candidate will join a vibrant Biology Department, comprised of both computational and experimental groups, that is expanding programs in the area of applying computational approaches to microbial systems biology, viral evolution, host-pathogen interactions, and RNA/protein structural analysis. Special consideration will be given to candidates whose research programs complement the existing focus areas of the department (see http://www.bc.edu/biology for profiles of the Biology Department's current research programs), that have a collaborative track-record, and/or who combine their work with wet-lab experimentation.

Applicants should submit a *curriculum vitae*, a statement of research and teaching plans, and arrange for three letters of references. All application materials should be submitted via Interfolio at **http://interfolio.com/26101**. Review of applications will begin on **October 15, 2014**, and continue until the position is filled.

Boston College is an Affirmative Action/Equal Opportunity Employer and does not discriminate on the basis of race, color, religion, sex, nation origin, disability, protected veteran status, or other legally protected status. To learn more about how BC supports diversity and inclusion throughout the university please visit the Office for Institutional Diversity athttp://www.bc.edu/offices/diversity.

Boston University School of Medicine

Boston University School of Medicine welcomes applications for its departments below at ranks of Instructor, Assistant & Associate Professor, or Professor.

- Anatomy & Neurobiology
- Biochemistry
- Microbiology
- Physiology & Biophysics
- Pharmacology & Experimental Therapeutics
- Pathology
- Genetics (esp. Zebrafish)

Email a cover letter specifying your department of interest with your CV to busmdean@bu.edu.

We are an equal opportunity employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law.

BOSTON UNIVERSITY

www.bumc.bu.edu

BioFrontiers Institute
UNIVERSITY OF COLORADO

Tenure-Track Assistant Professor in Computational Biology at the University of Colorado BioFrontiers Institute

BioFrontiers integrates faculty from nine departments to address significant problems in biology and medicine at the interface of the biological sciences with the physical sciences, mathematics, computer science, and/or engineering (see http://BioFrontiers.colorado.edu/about). While a successful candidate could be rostered in any of our participating departments, this search will focus on candidates seeking to develop an internationally recognized research program in computational biology, with additional emphases on genomics, the microbiome, and/or network science. Successful candidates must demonstrate a strong focus on innovative computation in their research plan.

The tenure-track position is at the Assistant Professor level, although more senior candidates will also be considered. Candidates must have a Ph.D. and a demonstrated commitment to teaching at undergraduate and graduate levels. The successful candidate will hold the Marvin H. Caruthers Endowed Chair for Early Career Faculty for a period of four years, after which the Chair will be recycled to another early career faculty member.

Application materials are accepted electronically at **http://www.jobsatcu.com/postings/86204**. Review of applications will begin on **November 11, 2014** and will continue until the position is filled. The University of Colorado Boulder conducts background checks for all final applicants.

As an Equal Opportunity/Affirmative Action Employer, the University of Colorado is committed to diversity and equality in education and employment and sensitive to the needs of dual-career couples.

Molecular, Cellular, & Developmental Biology Faculty Search

The Department of Molecular, Cellular, and Developmental Biology in the College of Literature, Science and the Arts at the University of Michigan solicits applications for faculty positions at the assistant professor level, but appointment at a more senior level is possible for applicants with suitable experience. The faculty position will be tenured or tenure track with a university year appointment starting September 1, 2015 or January 1, 2016. Successful candidates will be expected to establish a vigorous, extramurally funded research program and to be involved in instruction of both undergraduate and graduate students.

We welcome applications from outstanding biologists in any area of research within the scope of the Department, which includes studies of model organisms (plants, animals, and microbes) with a diversity of approaches (genetics and genomics, biochemistry and structural biology, molecular biology, cell and developmental biology, and physiology). We particularly encourage applications from neuroscientists who use genetic, cellular, molecular and/or *in vivo* imaging approaches. For further information about Department research areas, please visit www.lsa.umich.edu/mcdb.

All applications must be submitted online at **http://labs.mcdb.lsa.umich.edu/search14**. You will be asked to upload the following materials: A cover letter, a curriculum vitae, a brief summary of recent research accomplishments and statement of future research plans, a statement of teaching philosophy and experience, and evidence of teaching excellence for those who have teaching experience. Candidates for appointment as an assistant professor should provide names and contact information for at least three references, as instructed in the on-line application form. To ensure full consideration, all materials should be received by **October 10, 2014**.

Women and underrepresented minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

KOCH INSTITUTE
for Integrative Cancer Research at MIT

Faculty Position

The Koch Institute for Integrative Cancer Research at the Massachusetts Institute of Technology (http://ki.mit.edu) invites applications for a junior or senior faculty appointment. Appointments are expected to be in the MIT Department of Biology, but other departments will be considered if appropriate. The Koch Institute is an NCI-designated Cancer Center, which features research across a wide range of areas in basic cancer research and cancer-oriented engineering. This is an open search with regard to field of study and specific research focus, but cancer relevance will be a consideration. The candidate(s) will be expected to develop and lead an internationally competitive research program as well as participate in undergraduate and graduate teaching. The successful candidate(s) will have laboratory space in the Koch Institute.

Applicants should include curriculum vitae, brief summaries of past accomplishments and a description of future research plans. Letters of recommendation should be sent separately from three individuals able to evaluate the candidate's accomplishments and future potential for both research and teaching.

Please apply online at
https://academicjobsonline.org/ajo/jobs/4427

Complete applications are due
by November 1, 2014.

MIT is an Affirmative Action/Equal Opportunity employer, and we are actively committed to a diverse faculty. Women and members of underrepresented minority groups are strongly encouraged to apply.

Massachusetts Institute of Technology

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL
Joint Department of BIOMEDICAL ENGINEERING
NC STATE UNIVERSITY

THE JOINT DEPARTMENT OF BIOMEDICAL ENGINEERING AT UNC AND NC STATE is seeking five new tenure-track faculty members to complement and expand upon our existing strengths.

All Candidates should have: (1) a demonstrated ability to collaborate with other scientists both internally and externally, (2) and a history of or clear potential for developing extramural research funding, and (3) a Ph.D. in Biomedical Engineering or a field closely related to the research area.

Positions at NC State University:

- **PROFESSOR IN PHARMACOENGINEERING**. Candidate research areas of interest might involve engineering approaches to therapeutic delivery materials, devices, or systems or novel means to assess therapeutic deposition or disease response to treatment. Ideal candidates will have an established independent research program and a demonstrated ability of clinical translation. For inquiries, please contact Fran Ligler at fsligler@ncsu.edu with a reference to the position in the email subject. Position number 00102079.
- **ASSISTANT PROFESSOR IN REGENERATIVE MEDICINE.** Ideal candidates will expand and complement our department's existing research in functional tissue engineering, biomaterials, stem cells, and mechanobiology for engineering, regereration or replacement of living tissues and/or organs. Position number 00061801.

To apply, visit http://jobs.ncsu.edu/ and search for the appropriate position number.

Positions at the University of North Carolina:

- **ASSISTANT PROFESSOR IN BIOMEDICAL IMAGING**, with a focus on hardware design. One area of interest includes RF coil design for Magnetic Resonance Imaging, especially high field imaging; we will also consider other areas in Biomedical Imaging involving hardware development. http://unc.peopleadmin.com/postings/47970
- **ASSISTANT PROFESSOR IN CLINICALLY TRANSLATABLE BIOMEDICAL DEVICE TECHNOLOGIES.** Technologies that integrate with our biomedical microdevice research program are of particular interest. http://unc.peopleadmin.com/postings/47964
- **ASSISTANT PROFESSOR IN REHABILITATION ENGINEERING.** Potential research areas include prosthetics and orthotics, neuromotor rehabilitation, neural technologies, outcomes based musculoskeletal modeling and simulation, and human/animal neuromechanics. http://unc.peopleadmin.com/postings/47978

To apply, please visit the appropriate listed hyperlink.

The University of North Carolina at Chapel Hill is an equal opportunity, affirmative action employer and welcomes all to apply regardless of race, color, gender, national origin, age, religion, genetic information, sexual orientation, gender identity or gender expression. We also encourage protected veterans and individuals with disabilities to apply.

NC State University is an equal opportunity and affirmative action employer. All qualified applicants will receive consideration for employment without regard to race, color, national origin, religion, sex, age, veteran status, or disability. In addition, NC State welcomes all persons without regard to sexual orientation or genetic information. Individuals with disabilities requiring disability-related accommodations in the application and interview process please call (919) 515-3148.

Tenure Track Faculty Positions
The Department of Genetics
Washington University School of Medicine, St. Louis, MO

As part of a major expansion, the Department of Genetics is now recruiting investigators at all academic levels. Individuals with expertise in high-throughput functional genomics, including single-cell analysis; computational and systems biology; development of cellular and animal models of human disease; stem cell biology; genome engineering; epigenomics and analysis of gene-environment interactions; or novel strategies to evaluate the role of genetic variants in disease etiology, are encouraged to apply.

These positions offer superb scientific resources in a collegial department (http://www.genetics.wustl.edu) with traditional strengths in computational biology, genome science, and classical genetics, as well as new activities focused on neurological disorders, cellular bioenergetics, and personalized medicine. The department houses centers that provide access to genomic analysis for research and clinical samples as well as induced pluripotent stem cell and cellular genome engineering technologies. Department faculty members have extensive interactions with clinical and preclinical departments at the University as well as with the Institute for Clinical and Translational Science, Hope Center for Neurological Disorders, Children's Discovery Institute, Intellectual and Developmental Disability Research Center, Siteman Cancer Center, and Genomic and Pathology Services (GPS@WUSTL). In keeping with the interdisciplinary nature of modern Genetics, there is the potential for joint appointments with other departments including, Medicine, Pathology & Immunology, Neurology, OB/GYN, Psychiatry and Computer Science & Engineering.

Applicants should email a single PDF file consisting of a cover letter, curriculum vitae, a one page summary of research accomplishments and a one page description of future research plans, and should arrange for three letters of reference to be sent to Jeffrey Milbrandt, Head of the Department of Genetics at **GeneSearch2014@genetics.wustl.edu**. Completed applications will be reviewed on a rolling basis through **December 8, 2014**. Interviews of qualified candidates will commence immediately and continue until the positions are filled.

Washington University is an Equal Opportunity Affirmative Action Employer.

Washington University in St. Louis

Massachusetts Institute of Technology

Come work with us!

Tenure Track Faculty

The Department of Brain & Cognitive Sciences (BCS) (http://bcs.mit.edu) and **The Picower Institute for Learning & Memory at MIT** (http://picower.mit.edu/) are looking to hire up to three (3) tenure-track faculty at the assistant professor level who work in one or more of the following three (3) areas:

i) *Computational* approaches to intelligence, cognition or neuroscience; an experimental component to the candidate's research would be viewed as a positive but is not necessary. An affiliation with Electrical Engineering and Computer Science, the Computer Science and Artificial Intelligence Laboratory (CSAIL), or other allied departments is possible.

ii) *Molecular & cellular:* The Picower Institute is searching for a candidate studying development, function or plasticity of neuronal circuits at the cellular, circuit, and/or systems levels using a multi-faceted approach combining different methodologies and levels of analysis. Candidates with strong cellular/molecular training who are studying development of brain circuits or using stem cell technologies are particularly encouraged to apply.

iii) *Human cognition and/or cognitive neuroscience* using behavioral methods, especially in the areas of language and/or cognitive development OR using fMRI/neuroscience methods.

Successful applicants are expected to develop and lead independent, internationally competitive research programs and to share in our commitment to excellence in undergraduate and graduate education by teaching courses and mentoring graduate and undergraduate research. PhD must be completed by start day of employment and some postdoctoral training is preferred.

Please submit application materials – cover letter, CV, statement of research and teaching interests and representative reprints – online at https://academicjobsonline.org/ajo/jobs/4202. Please state research area in cover letter. To help direct the application, applicants should indicate which of the three areas listed above is their main research area by answering the mandatory questions included in the application. In addition, please arrange to have three letters of recommendation submitted online. Review of applications will begin on November 1, 2014.

MIT is an affirmative action employer, and we encourage applications from women and underrepresented minorities.

http://web.mit.edu

Colorado State University

Genome Architecture/Function
Biochemistry and Molecular Biology Department

The Department of Biochemistry and Molecular Biology seeks applicants for a tenure-track position at the Assistant or Associate Professor level. We encourage individuals with a strong background in studying DNA-related processes in eukaryotes (e.g. transcription, DNA repair, recombination and replication, cell division, chromosome structure, genomics or epigenetics) to apply. The Department has outstanding research programs in the areas of molecular, cell, and structural biology, with a strong core group studying the molecular mechanisms of cellular functions ranging from transcription to cell division. The position is part of a strategic plan designed to further strengthen and expand these areas of excellence and, therefore, candidates are expected to complement the current strengths in our department. Required credentials include a doctoral degree, post-doctoral experience, and evidence of expertise in the broad areas defined above. Candidates applying for appointment as an Associate Professor must have established an internationally recognized and well-funded research program.

Applicants should submit their application on-line, and upload their curriculum vitae, statements of research and teaching interests at: **http://cns.natsci.colostate.edu/employment/BMB/**, where a full description of the position is available. Applicants at the Assistant level should request three reference letters. Referees will be sent an access code (using the contact information provided by the applicant) for submitting the letters online. Applicants at the Associate level will be asked to supply reference letters should they be selected as a semi-finalist. For full consideration, complete applications addressed to **Dr. Karolin Luger**, as the Chair of the Search Committee, should be received by **November 1, 2014**. However, applications may be considered until the position is filled. Candidates should demonstrate an ability to advance the Department's commitment to diversity and inclusion through research, teaching and outreach with relevant programs, goals and activities.

CSU is an EO/EA/AA Employer. CSU conducts background checks on all final candidates.

NORTHWESTERN UNIVERSITY

The **Department of Molecular Biosciences** seeks outstanding individuals with research interests that complement our existing interdisciplinary strengths. The department is a vibrant and exciting research and training environment on the undergraduate campus of Northwestern University in Evanston, IL. As part of our continuing growth in the Life Sciences, we invite applications for two tenure-track positions at the rank of Assistant Professor. One of these positions may also be filled at the Associate Professor level.

We are particularly interested in the following areas:

- ***Biophysical and biochemical approaches including method development and single molecules to investigate macromolecular and cellular processes.***
- ***Genomic, high throughput and computational approaches including high-content image analysis to address cellular and organismal biology employing diverse model systems including stem cells.***

Applicants should prepare a cover letter, curriculum vitae, research summary and a statement of future research goals, and a statement of teaching experience and interests. For further instructions and to submit an application, please visit Molecular BioSciences homepage at **http://www.molbiosci.northwestern.edu**. Applicants should arrange for at least three letters of recommendation to be submitted on their behalf. Questions should be sent to **molbiosciesearch@northwestern.edu**.

To ensure full consideration, please submit all materials by **November 1, 2014**.

Affirmative Action Employer of all protected classes including veterans and individuals with disabilities. Women and minorities are encouraged to apply. Hiring is contingent upon eligibility to work in the United States.

Faculty Positions in Gene Regulation and Genomics

The University of Texas Southwestern Medical Center in Dallas, the **Cecil H. and Ida Green Center for Reproductive Biology Sciences** and the Division of Basic Reproductive Biology Research in the Department of Obstetrics and Gynecology is recruiting. We invite applications from outstanding candidates for three tenure-track assistant professor positions in signaling, gene regulation, and genome function, especially in the areas of chromatin and transcription, epigenetics, nuclear endpoints of cellular signaling pathways, nuclear receptors, RNA biology, genome organization and evolution, and DNA replication and repair. We are interested in a wide variety of model systems and experimental approaches, including biochemistry, molecular biology, structural biology, animal models, genomics, proteomics, bioinformatics, and computational biology. The Green Center's research programs focus on, but are not limited to, reproduction and development in a broad sense, as well as aspects of endocrinology, stem cells, cancer, metabolism, inflammation, immunity, and neurobiology.

- ***Position 1:* Signaling, chromatin, and gene regulation** – a broad search for candidates using a wide array of experimental approaches to address fundamental questions in nuclear signaling, chromatin, transcription, epigenetics, and RNA biology.

- ***Position 2:* Genomic, bioinformatic, computational, and evolutionary approaches to understanding gene regulation** - a more focused search in areas that will connect to broader genomic initiatives on campus.

- ***Position 3:* Molecular biology of female reproductive systems** - a search for candidates using cell-based or physiological models in combination with molecular or genomic approaches to address fundamental questions concerning female reproductive biology.

The Green Center is an endowed basic science research center at UT Southwestern, which promotes and supports cutting-edge, integrative, and collaborative basic research in reproduction, development, and related areas of biology, as well as strong connections between basic and clinical research. This recruitment is part of a major university and department-supported renovation and rejuvenation of The Green Center over the past four years. Successful candidates will be housed in a newly renovated state-of-the-art research facility and provided a generous start-up package, and are expected to establish scientifically rigorous and externally funded research programs and participate in center, department, and university teaching and training programs. To learn more about The Green Center, visit: **http://www.utsouthwestern.edu/education/medical-shool/departments/green-center/index.html**.

Candidates must have a Ph.D. or M.D. or equivalent in a relevant field of study, postdoctoral or comparable experience, and a demonstrated record of research excellence. Applicants should send a letter of application, curriculum vitae, and a statement of planned research projects as pdf files to **GreenCenter@UTSouthwestern.edu**, indicating the position of interest (1, 2, or 3) in the subject line. Applicants should also arrange for three letters of reference to be sent directly to the above e-mail address. Review of applications will begin on **October 15, 2014** and continue during the 2014 – 2015 academic year or until the positions are filled, although applicants are encouraged to submit their materials as soon as possible.

UT Southwestern is an Affirmative Action/Equal Opportunity Employer. Women, minorities, veterans, and individuals with disabilities are encouraged to apply.

RICE

BIOSCIENCES FACULTY SEARCH
Rice University
http://www.biosciences.rice.edu/
http://www.rice.edu/

Applications are invited for one open-rank faculty position and one assistant professor in broad areas of biological research including biochemistry; cell and developmental biology; neurobiology; plant biology; and systems, synthetic and physical biology. Applicants using experimental and/or theoretical biological approaches that are interdisciplinary with chemistry, physics, engineering, or computational methods are especially encouraged to apply. Candidates must have a Ph.D., postdoctoral training, and outstanding potential in research and teaching. Successful candidates are expected to develop and maintain a vibrant research program supported by extramural funding and participate in the education and mentorship of graduate and undergraduate students. We are especially interested in qualified candidates who can contribute, through their research, teaching, and/or service, to the diversity and excellence of the academic community.

Our application and instructions can be found under the employment section on the left menu on the department website, **http://biosciences.rice.edu**. Review of applications will commence **October15, 2014** and continue until the positions are filled.

Rice University is an Equal Opportunity/ Affirmative Action Employer; women, minority candidates, individuals with disabilities, and veterans are especially encouraged to apply.

STOWERS INSTITUTE
FOR MEDICAL RESEARCH

Faculty Position in the Life Sciences

The Stowers Institute for Medical Research invites innovative young scientists in the Life Sciences to submit applications for a faculty position. We anticipate making an appointment in 2015 at the rank of Assistant Investigator. Research programs of interest include, but are not limited to: computational biology, neuroscience, developmental and cell biology, genomics, stem cell biology, regenerative biology and epigenetics. Our interests encompass a broad gamut of experimental organisms and approaches. The successful candidate will benefit from and complement the Institution's existing strengths in genetics and epigenetics, cell and chromosome biology, stem cells and regenerative biology, developmental biology and evolution, and biochemistry and neuroscience.

The position is fully funded throughout the candidate's appointment. This includes $600,000 per year for full salary support and research funding, in addition to start-up funds and ongoing needs for equipment. The initial appointment is for 6 years and is then subject to renewal every 6 to 7 years. In total, the package for a junior position is more than $3.5 million over the first term and increases significantly after promotion. In addition, investigators may take advantage of exceptional core facilities and technology centers staffed by over 100 scientists. Stowers investigators have multiple opportunities to be involved in the Institute's Graduate School program.

Candidates must have a Ph.D. or equivalent degree and postdoctoral experience demonstrating innovation and excellence in their field. Candidates will be expected to possess a long-term vision of their scientific interests, to establish a vigorous and innovative research program, and to actively contribute to the Institute's mission and collegiality.

Deadline for applications is **October 31, 2014**. Applicants should submit a cover letter, a CV, a research plan and vision statement, and arrange for the submission of three letters of reference through our application page at: **http://www.stowers.org/facultysearch**. Questions should be directed to the Search Committee Chair, **Dr. Alejandro Sánchez Alvarado** (**facultysearch@stowers.org**).

The Stowers Institute for Medical Research is proud to be an Equal Opportunity Employer. All qualified applicants will be afforded equal opportunity regardless of race, creed, color, religion, gender, sexual orientation, pregnancy, national origin, age, disability, military status, or any other status protected by law.

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY FACULTY POSITIONS

The Department of Microbiology and Immunology at the University of Maryland School of Medicine is recruiting new or established investigators with actively funded research programs in host-pathogen interactions. Highly qualified individuals will be considered for tenure-track positions at the rank of Assistant, Associate or full Professor. The Department and the School of Medicine have significant strengths in microbial pathogenesis, vaccine development, HIV biology, genome sciences, inflammation, innate and adaptive immunity, clinical infectious diseases and international medicine. The Department offers excellent laboratory facilities, competitive salary and startup packages, and access to numerous core facilities including state-of-the-art BSL3 and ABSL3 facilities. We are particularly interested in candidates who use multidisciplinary approaches, including genomics, to investigate the interaction of viral pathogens with the host and with a strong desire to interact with other investigators in the institution. Successful candidates are expected to maintain active research programs and participate in department teaching and service opportunities.

Interested applicants are invited to submit the following in a single PDF file to: **microfacsearch@som.umaryland.edu**: (1) detailed curriculum vitae, (2) statement of research interests, goals, and (3) names and contact information for three to five references. Applications should be addressed to the attention of: **Chair, Faculty Search Committee**.

The University of Maryland, Baltimore is an Equal Opportunity/ Affirmative Action Employer. Minorities, women, individuals with disabilities, and protected veterans are encouraged to apply.

Baylor University
Assistant Professor of Biology—Immunology

The Department of Biology invites applications to fill this tenure track position beginning August, 2015. Candidates should have a Ph.D. and expertise in immunology or related fields such as cell or cancer biology. We seek an outstanding scientist who will establish competitive and externally funded research program in an area of science relevant to human health and which contributes to broader departmental goals. Applications will be reviewed beginning **October 15, 2014** and will be accepted until the position is filled. To ensure full consideration, complete applications must be submitted by **October 30, 2014**. For position details and application information please visit: **www.baylor.edu/hr/facultypositions** or from **Myeongwoo_Lee@baylor.edu**.

Baylor University is a private Christian university and a nationally ranked research institution, consistently listed with highest honors among *The Chronicle of Higher Education's* "Great Colleges to Work For." The university provides a vibrant campus community for a diverse student body of over 15,000 students by blending interdisciplinary research with an international reputation for educational excellence and a faculty commitment to teaching and scholarship. Baylor is actively recruiting new faculty with a strong commitment to the classroom and an equally strong commitment to discovering new knowledge as we pursue our bold vision, *Pro Futuris*.

Baylor University is a private not-for-profit university affiliated with the Baptist General Convention of Texas. As an Affirmative Action/ Equal Opportunity Employer, Baylor is committed to compliance with all applicable anti-discrimination laws, including those regarding age, race, color, sex, national origin, marital status, pregnancy status, military service, genetic information, and disability. As a religious educational institution, Baylor is lawfully permitted to consider an applicant's religion as a selection criterion.

UT Southwestern Medical Center

Assistant Professor in Human Genetics

The Center for Human Genetics (McDermott Center for Human Growth & Development) at the University of Texas Southwestern Medical Center (http://www.utsouthwestern.edu/mcdermott-center) invites applications for a tenure-track position of Assistant Professor. We are seeking individuals with innovative experimental research programs in human molecular genetics. Successful applicants will be expected to establish a vigorous independent research program and to teach students at the graduate level.

The individual should hold a graduate degree (MD, PhD or MD/PhD) and have completed a post-doctoral fellowship or be a candidate for the NIH Director's Early Independence Award. The appointment will include a competitive salary, attractive start-up package, excellent laboratory space in a dynamic research environment with access to the Center's state-of-the-art infrastructure for basic and clinical research related to human genetics, including family/patient collection, genetic variation, next-generation sequencing and bioinformatics cores. The faculty member will be jointly appointed to a basic science or clinical department. Applicants should submit their curriculum vitae containing a summary of past accomplishments, a statement of future objectives, and three professional references by email to:

Faculty Search Committee
c/o Susan Hayes, Dept. Administrator
McDermott Center for Human Growth & Development
UT Southwestern Medical Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75390-8591

Susan.Hayes@UTSouthwestern.edu

UT Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women, minorities, veterans and individuals with disabilities are encouraged to apply.

KU THE UNIVERSITY OF KANSAS

Faculty Position
Materials Synthesis/Structure Chemistry

The Department of Chemistry at the University of Kansas, Lawrence, KS, is seeking exceptional candidates for a tenured/tenure-track position in Inorganic or Organometallic Materials Chemistry at the assistant or associate professor level expected to begin as early as August 18, 2015. For appointment at the rank of Associate Professor, candidates must meet the University criteria for tenured Associate Professor.

The University of Kansas is especially interested in hiring faculty members who can contribute to four key campus-wide strategic initiatives: (1) Sustaining the Planet, Powering the World; (2) Promoting Well-Being, Finding Cures; (3) Harnessing Information, Multiplying Knowledge; and (4) Building Communities, Expanding Opportunities. See http://www.provost.ku.edu/planning/themes/ for more information. This specific search is part of an emerging cluster of searches on the topic of "Nanoscale Materials for the Future." The University has state-of-the-art shared instrumentation that supports the characterization of new materials. Effective teaching at both the undergraduate and graduate levels is expected. A statement of teaching philosophy and interests is required. A competitive salary will be offered that is commensurate with experience. A Ph.D. or ABD in chemistry or closely related field is expected by the start date of appointment and postdoctoral experience is desirable.

For a complete announcement and to apply on-line, go to go to **https://employment.ku.edu/academic/1703BR or https://jobs.ku.edu** and click "**Search Faculty Jobs**"; search by key word [Chemistry]. Submit online application cover letter, CV, the names and contact information for three references, a 2-page teaching philosophy statement/expertise/and interests on-line, and a brief summary of 2-3 research proposals (2 pages each). Initial review of applications will begin **November 1, 2014**, and will continue as long as needed to identify a qualified pool.

KU is an EO/AA Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex (including pregnancy), age, national origin, disability, genetic information or protected Veteran status.

Fred Henry Chair of Life Sciences in Cardiovascular Research

San Diego State University (SDSU) is seeking to fill The Fred Henry Chair of Life Sciences, which is an endowed professorship that allows the holder of the Chair to devote full time to research, primarily in the cardiovascular area. The scientist holding this chair will have state-of-the-art laboratory space in the SDSU Donald P. Shiley BioScience Center, a recently constructed facility that houses researchers working at the interfaces among infection, inflammation, and cardiovascular disease. The Shiley BioScience Center is the first multidisciplinary research center focused on the connection among these fields. The holder of the Fred Henry Chair of Life Sciences serves a research leadership role at SDSU, will have a tenured faculty appointment in the Department of Biology and will be a full member of the SDSU Heart Institute.

SDSU Donald P. Shiley BioScience Center

We seek an individual at the rank of professor who has an established research program in areas, such as signal transduction in cardiovascular systems, cardiac physiology and pathology, immune interactions with the heart, microbial infections of the heart, and applications, such as cardioprotection and cardiac regeneration. Additional desired accomplishments and experience include an externally funded, independent research program, a track record of sustained funding by individual research grants, experience with multi-investigator research, equipment, and facilities grants, excellent communication and leadership skills, as well as a demonstrated ability to build, lead, and oversee interdisciplinary, collaborative research teams. The Fred Henry Chair of Life Sciences will participate in graduate training programs at the M.S. and Ph.D. levels, and will be encouraged to develop collaborative relationships with the San Diego biotechnology industry, as well as with translational and clinical centers. Moreover, the candidate will join a highly productive group of well-funded principal investigators in the SDSU Heart Institute who focus on aspects of the cardiovascular system using mouse, Drosophila and cell culture models. Current areas of interest include: signal transduction, stem cells in regenerative medicine, contractile function, amyloidosis and other diseases related to protein misfolding.

Interested individuals should electronically submit supporting documents, including (i) a cover letter, (ii) curriculum vitae that includes education, employment history, publications, research funding record (pending, current and previously completed), awards and honors, presentations, and other related items, (iii) a research statement that describes previous, current and planned research programs, as well as experience developing multi-investigator research collaborations, grants, centers and/or programs, (iv) a teaching/mentoring statement that describes teaching experience, including courses taught and experience mentoring students, post-doctoral fellows, and other researchers, and (v) up to five publications. Applicants should list 3-5 references to be contacted by the search committee at a later date and with the applicant's permission. Supporting documents should be sent to the search committee by e-mail to fredhenrychair@mail.sdsu.edu Review of applications will begin on **November 1, 2014**. The position will remain open until a suitable candidate is identified. A full description of this position can be found at: **http://affiliated.sdsu.edu/ColSci/biology.htm**

University of Massachusetts Medical School

PROGRAM IN SYSTEMS BIOLOGY

Faculty Positions Program in Systems Biology

The newly established Program in Systems Biology invites applications from outstanding candidates for a Tenure-Track Assistant/Associate Professor or tenured Professor position. Rank will be commensurate with ability and experience. The positions will be highly competitive with regard to start-up funds, laboratory space, and salary.

We are seeking innovative, energetic and collaborative individuals who will develop a strong **experimental research program** to tackle important problems in one of the following areas in systems biology: Quantitative Biology, Network Biology, Single Cell Systems Biology, Genome Biology, Evolution and Variation, Systems Biology of Human Disease, Proteomics, Metabolomics, Microbiota Research. Exceptionally strong candidates in other areas will also be considered.

The University of Massachusetts Medical School has built a reputation as a world-class research institution in basic and clinical research. The Medical School attracts more than $250 million in research funding annually, 80 percent of which comes from federal funding sources.

The Program in Systems Biology is housed in the new state-of-the-art Albert Sherman Center. The Program is adjacent to the Program in Bioinformatics and Integrative Biology and the RNA Therapeutics Institute. The Program has high-performance computing facilities, extensive shared equipment, and a full-time systems administrator to support the research activities of its Faculty. Further information on the Program can be found at www.umassmed.edu/psb.

Applicants should submit a cover letter explaining their interest in the Program, a curriculum vitae that includes publications and a succinct research plan to http://www.academicjobsonline.org (Position ID # 4406). To expedite the review process, applicants should invite three individuals who are familiar with their work and potential for success to upload their recommendation letters at the same web address. The deadline for applications is **December 1st, 2014** and interviews will be scheduled for early 2015.

Inquiries, but not application materials, may be directed to Professors A.J. Marian Walhout (**marian.walhout@umassmed.edu**) or Job Dekker (**job.dekker@umassmed.edu**).

UMass Medical School is committed to being an Equal Opportunity and Affirmative Action Employer and recognizes the power of a diverse community. We encourage applications from protected veterans, individuals with disabilities and those with varied experiences, perspectives and backgrounds to consider UMass Medical School as their employer of choice.

Caltech

CALIFORNIA INSTITUTE OF TECHNOLOGY invites applications for tenure-track faculty positions in the Division of Chemistry and Chemical Engineering

Assistant Professor Chemistry

Areas of particular interest include inorganic chemistry, physical chemistry, chemical biology and biochemistry, although applications in any area of chemistry or biochemistry are welcomed. Exceptionally well-qualified applicants at the tenured level may also be considered. Candidates with strong commitments to research and teaching excellence are encouraged to apply. The term of the initial untenured appointment is four years, and the appointment is contingent upon completion of all requirements for a Ph.D. in chemistry, biochemistry, or in a related field.

Interested candidate should apply electronically **https://applications.caltech.edu/job/chemistry**. Candidates unable to apply electronically may submit curriculum vitae, publication list, a description of proposed research, and three letters of recommendation to: **Chair of the Chemistry Search Committee, M/C 164-30, California Institute of Technology, Pasadena, CA 91125**. Applications should be received by **October 7, 2014**.

Assistant Professor Chemical Engineering

Assistant Professor level in the area of chemical engineering. Exceptionally well-qualified applicants at the tenured level may also be considered. Candidates with strong commitments to research and teaching excellence are encouraged to apply. The term of the initial untenured appointment is four years and is contingent upon completion of all requirements for a Ph.D. in chemical engineering or in a related field.

Interested candidate should apply electronically **https://applications.caltech.edu/job/chemeng**. Candidates unable to apply electronically may submit curriculum vitae, publication list, a description of proposed research, and three letters of recommendation to: **Chair of the Chemical Engineering Search Committee, M/C 210-41, California Institute of Technology, Pasadena, CA 91125**. Applications should be received by **December 15, 2014**.

EOE of Minorities/Females/Protected Vets/Disability.

POSITIONS OPEN

EXPERIMENTAL BIOCHEMISTRY

University of the Pacific, Chemistry Department invites applications for a tenure-track, **ASSISTANT PROFESSOR** position in experimental biochemistry. Expectations include teaching chemistry/biochemistry and establishing an externally funded research program. The starting date is fall 2015. Detailed application information can be found at **website: https://pacific.peopleadmin.com/postings/3848**. Application review begins October 31, 2014.

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.*

ENVIRONMENTAL MICROBIOLOGIST

The Department of Biological Sciences at Michigan Technological University invites applications for a tenure-track **ASSISTANT PROFESSOR** position in microbiology, with particular focus in environmental microbiology or microbial ecology. Areas of research interest may include: microbial genomics or evolution, community dynamics, nutrient cycling, environmental toxicology, remediation and restoration, and host-microbe interactions. Job expectations include establishing an externally funded research program complementary to the interests of the department and university and successful teaching at the undergraduate and graduate levels in area of expertise. Applicants must hold a Ph.D. in microbiology or a related field and postdoctoral experience is strongly preferred.

Additional information about this position, the Biological Sciences Department and its programs can be found at **website: http://www.mtu.edu/biological/.**

Applications should be submitted electronically to **website: http://www.jobs.mtu.edu/posting/1898.** For consideration, the application should include: (1) curriculum vitae; (2) research statement (maximum 2 pages); (3) statement of teaching interests and philosophy (maximum two pages), (4) complete contact information for four references. Applications are due at 5 p.m. EST October 13, 2014. Direct inquires to Microbiology Search Committee Chair (**e-mail: microbiosearch@mtu.edu**).

Michigan Tech is an ADVANCE institution, located in the Upper Peninsula of Michigan, one of a limited number of universities in receipt of NSF funds in support of our commitment to increase diversity and the participation and advancement of women in STEM. Michigan Tech. acknowledges the importance of supporting dual career partners in attracting and retaining a quality workforce. Visit **website: http://www.dual.mtu.edu.** *Michigan Technological University is an Equal Opportunity Educational Institution/Equal Opportunity Employer, which includes providing equal opportunity for protected veterans and individuals with disabilities.*

POSITIONS OPEN

ASSISTANT OR ASSOCIATE PROFESSOR
Center for Experimental Therapeutics and Reperfusion Injury
Brigham and Women's Hospital
Harvard Medical School

This Center invites applications for a tenure-track Assistant or Associate Professor position. A Ph.D. and/or M.D. will join this Center's research mission. Successful candidate will have maintained research in either GPCR or metabolomics in inflammation-resolution, with a focus on lipid mediators. Continued research activities and mentoring are integral to this position. Qualified individuals with postdoctoral training and/or faculty experience should submit a cover letter, and curriculum vitae online at **website: https://research.bwhanesthesia.org/research-groups/cetri/serhan-lab/position.** The names of three references and application should be addressed to **Dr. Charles N. Serhan,** Search Committee Chair, no later than November 6, 2014.

Successful candidates will maintain independent research in resolution mechanisms in inflammation and tissue injury.

Harvard Medical School is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITIONS (2) in
Bioanalytical and Computational Chemistry

The Department of Chemistry & Chemical Biology at IUPUI (**website: http://chem.iupui.edu/**) invites applications for tenure-track faculty positions in bioanalytical chemistry and computational chemistry to begin August 1, 2015. The positions will be at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR**, with tenure status to be determined based on prior experience and qualifications. All applicants must hold a Ph.D. in Chemistry or a related discipline along with relevant postdoctoral experience. Candidates must demonstrate the ability to initiate and sustain an externally funded program of research, and be able to teach effectively at the undergraduate and graduate levels. Applicants at the Associate Professor level must have a record of research excellence, a history of external funding, and evidence of effective student mentoring.

For the bioanalytical position, candidates working in all areas of the discipline will be considered, but some preference may be given to applicants working in the areas of separation techniques and/or metabolomics measurements. For the computational chemistry position candidates involved in research at the interface with biology and medicine, or in drug discovery, are particularly encouraged to apply. The successful candidates will be expected to teach a course in the undergraduate chemistry program, and to develop a graduate course in an area of their choice. We especially seek applicants whose research interests can support and expand the department's efforts to complement campus-wide interdisciplinary research programs in the life and health sciences, including ongoing collaborative efforts with the IUPUI School of Science, the IU School of Medicine (on campus), and the Integrated Nanosystems Development Institute (**website: http://indi.iupui.edu**).

Applicants should submit curriculum vitae, statements of research plans and teaching philosophy, and the names and contact information for three references, as a single, merged PDF file to either **e-mail: analytchem@chem.iupui.edu** (bioanalytical position) or **e-mail: pchem@chem.iupui.edu** (computational position). Review of applications will begin in late October 2014 and will continue until the position is filled.

IUPUI is an Equal Employment Opportunity/Affirmative Action Employer with a strong commitment to increasing faculty diversity—we seek to attract a diverse applicant pool for this position, especially including women and members of minority groups.

POSITIONS OPEN

FACULTY POSITION
Biochemistry/Chemical Biology

The Department of Biochemistry & Molecular Biology at the Drexel University College of Medicine invites applications for a tenure-track faculty position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level. We seek interactive individuals who are using biochemical, chemical, and/or biophysical approaches to investigate important molecular problems in biology and medicine. Individuals whose research complements existing strengths in the department are especially encouraged to apply; these strengths include protein structure/function, inhibitor and drug design, mechanistic enzymology, cell signaling, virus-host interactions, and cancer cell metabolism. The Department offers a collegial and stimulating environment with many opportunities for collaboration and state-of-the-art facilities **website: http://www.drexelmed.edu/biochemistry.** Competitive startup funds are available. Successful candidates will have a Ph.D. and/or MD, relevant postdoctoral experience, and a strong record of research accomplishments. Faculty are expected to establish rigorous, independent, and well-funded research programs and to make significant contributions to graduate and medical education.

The Drexel University College of Medicine is a large private medical school located in Center City Philadelphia. Drexel University is ranked among the top 100 universities in the nation, and was named as one of the top "Up-and-Coming" national universities in the 2014 U.S. News College Rankings.

To apply, please submit a single PDF containing curriculum vitae, statement of research interests, statement of teaching philosophy, and names of three references to **e-mail: lucia.boyer@drexelmed.edu**; please include the words "Biochem/ChemBio Search" on the subject line.

MATERIALS CHEMISTRY
Dartmouth College
Hanover, New Hampshire

The Department of Chemistry at Dartmouth College is seeking an outstanding applicant for an open rank (tenured or tenure-track) faculty position in Materials Chemistry starting July 2015. We seek candidates interested in making, measuring, and/or modeling materials, broadly defined including polymers, nanomaterials, surfaces/interfaces, and molecular sensors and machines. Candidates who will help lead, initiate, and participate in collaborative research projects within Chemistry as well as Dartmouth's Thayer School of Engineering and Geisel School of Medicine are particularly encouraged to apply. Teaching responsibilities will include participation in the undergraduate curriculum and advanced/graduate courses in their area of specialty. The department (**website: http://www.dartmouth.edu/~chem/**) is home to 17 tenured and tenure-track faculty with strong Ph.D. and M.S. programs and affiliated with Dartmouth's M.D.-Ph.D. program. Dartmouth College, a member of the Ivy League, is located in Hanover, New Hampshire (on the Vermont border). Dartmouth has a beautiful, historic campus, located in a scenic area on the Connecticut River. Recreational opportunities abound in all four seasons.

Applicants should submit curriculum vitae, a description of their research (funding and future plans), a statement of their teaching interests, and at least three reference letters (tenure-track) or names of three references (senior candidates). All inquiries and applications will be treated confidentially. Application materials should be submitted to **website: http://apply.interfolio.com/25556.** Applications received by October 1 will receive first consideration. *Dartmouth is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversity. In that spirit, we are particularly interested in receiving applications from a broad spectrum of people, including women, persons of color, persons with disabilities, veterans, or any other legally protected group.*

BAYLOR

Assistant or Associate Tenure Track Position in the **Department of Environmental Science** at Baylor University beginning August 2015. Successful candidate will be expected to develop and maintain a nationally competitive research program, while teaching at the undergraduate and graduate level. Immediate needs include environmental health science. Rank and salary are dependent on qualifications and experience. Additional information regarding the Department of Environmental Science and this position announcement is available at www.baylor.edu/environmentalscience.

Baylor University is a private not-for-profit university affiliated with the Baptist General Convention of Texas. As an Affirmative Action/Equal Opportunity employer, Baylor is committed to compliance with all applicable antidiscrimination laws, including those regarding age, race, color, sex, national origin, marital status, pregnancy status, military service, genetic information, and disability. As a religious educational institution, Baylor is lawfully permitted to consider an applicant's religion as a selection criteria. Baylor encourages women, minorities, veterans and individuals with disabilities to apply.

University of Colorado
Anschutz Medical Campus

The University of Colorado School of Medicine on the Anschutz Medical Campus in the Denver metro area is seeking to recruit up to twelve outstanding tenure-track faculty colleagues to expand into innovative areas of research. The departments of Biochemistry and Molecular Genetics, Cellular and Developmental Biology, Immunology and Microbiology, Pharmacology, and Physiology and Biophysics invite applications in the areas of:

- **Structural biology** using single particle cryo-electon microscopy (F01653)
- **Genomics** (F01652)
- **Neuroscience** (F01675, F01656, F01685)
- **Immunology**, the immune/microbiome interface, and microbial pathogenesis (F01444)
- **Cancer biology** (F01708)

Successful applicants will join an exceedingly collaborative and vigorous group of faculty dedicated to excellence in research and teaching. They will enjoy access to state-of-the-art facilities on a new and growing biomedical campus located in one of the country's most livable and desirable metropolitan areas.

Details for applying to each position can be found at jobsatcu.com, by entering the job posting code listed above as a keyword.

The University of Colorado strongly supports the principle of diversity. We encourage applications from women, ethnic minorities, persons with disabilities and all veterans. The University of Colorado is committed to diversity and equality in education and employment.

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Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

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To learn more, visit
aaas.org/plusyou/sciencecareers

AAAS + U = Δ

IOWA STATE UNIVERSITY
College of Liberal Arts and Sciences

Interdisciplinary Hiring Initiative in the College of Liberal Arts and Sciences

Spanning the sciences, social sciences and humanities, the College of Liberal Arts and Sciences constitutes the core of research and teaching at Iowa State University. As a member of the AAU located in the family-friendly city of Ames, ISU offers individuals and families opportunities to build their professional careers and personal lives in a thriving community, centered on one of the leading research universities in the nation. We look for people who are creative, curious and collaborative, and who know that the critical challenges of today and tomorrow cannot be solved by one person or one discipline alone.

To support the growth of collaborative research, we have defined five Signature Themes, which outline broad priority areas for research and scholarship in the college. Guided by these themes and building on existing strengths, the LAS College has launched an interdisciplinary hiring initiative under which it will hire over the next **2-3 years** *at least* **4-6 faculty** in each of the following four areas:

Mesoscale science – To devise new complex materials with mesoscale architectures.
Physical biology of the cell – To chart a quantitative roadmap of the living cell.
Sustainability science – To expand fundamental, multidisciplinary research on sustainable systems across multiple scales.
Bioinformatics and computational biology – To advance and support the need for massive data analysis and interpretation in the biological sciences.

las.iastate.edu/faculty-careers

California State University Northridge

ASSISTANT PROFESSORSHIP
Two Tenure-Track Positions
Department of Biology

California State University, Northridge invites applications for two tenure-track Assistant Professor positions in Genetics and Prokaryotic Microbiologist in the Department of Biology to begin August 19, 2015. Applicants must hold a Ph.D. and have postdoctoral experience. The successful candidate shall develop a vigorous research program involving undergraduate and Master's students, seek extramural research funding, demonstrate teaching excellence and must demonstrate ability to effectively work with a diverse student population.

For more information and application procedure visit **website: http://www.csun.edu/facultyaffairs/openings/sm/.**

Screening application will begin on October 15, 2014.

California State University, Northridge is an Equal Opportunity Employer committed to excellence through diversity.

FACULTY POSITIONS In
Structural Biology

The Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology at Iowa State University in Ames, Iowa has embarked on a transformational expansion of its structural biology research enterprise through a large philanthropic gift from the Roy J. Carver Charitable Trust. This major research initiative in Biomolecular Structure includes long-term investment in new instrumentation, endowed funds for graduate student training, and a series of new faculty hires working at the forefront of any aspect of structural biology.

As part of the faculty positions associated with this initiative, and as part of the interdisciplinary effort across the Iowa State University campus to expand structural biology research, the Department seeks two new tenure-track **ASSISTANT PROFESSORS** to establish vibrant, externally funded research programs of international prominence and participate in graduate and undergraduate teaching.

Applicants should have a Ph.D. or equivalent degree and research accomplishments indicative of the ability to establish an independent research program of national prominence.

To view the entire vacancy **#140747** and apply, create an electronic application at **website: http://www.iastatejobs.com.**

To guarantee consideration the applications must be received by November 15, 2014.

Iowa State University 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, or protected Vets status.

ASSISTANT/ASSOCIATE PROFESSOR

Food for Public Health & Wellness. University of Georgia. Candidate will participate in research, teaching, and service activities in the departments of Food Science & Technology and Environmental Health Science Research areas include nutritional or allergenic behavior of food ingredients, food chemical toxicology & safety, role of food in prevention of chronic diseases and obesity, development of innovative model systems, and human population studies for degenerative diseases & obesity prevention. Successful candidate will have a record of publications, extramural funding, and demonstrated teaching effectiveness. Applications due September 30, 2014. Provide letter of application, curriculum vitae, transcripts, four reference letters, and a one-page summary outlining research & teaching visions electronically to Search Committee Co-Chairs: **Dr. Erin Lipp** (**e-mail: ehsdept@uga.edu**), Department of Env Hlth Sci, UGA, Athens, GA and **Dr. Ron Pegg**, Department of Food Sci & Tech. *The University of Georgia is an Equal Opportunity/Affirmative Action Institution. Women and Minorities are encouraged to apply.*

POSITIONS OPEN

University of Illinois at Chicago, Department of Chemistry invites applications for a tenure-track **ASSISTANT PROFESSOR** position in the general area of experimental physical chemistry effective August 2015 pending budgetary approval. The successful candidate will be expected to carry out a full and vigorous program of innovative research and to contribute to the teaching of graduate and undergraduate students. A Ph.D. required. Please submit an online application, including the names and e-mail addresses of three references and upload curriculum vitae, list of publications, summary of past research, plans for future research to **website: https://jobs.uic.edu/job-board/job-details?jobID=44837** by October 12, 2014. *The University of Illinois at Chicago is an Affirmative Action/Equal Opportunity Employer, dedicated to the goal of building a culturally diverse pluralistic faculty and staff committed to teaching in a multicultural environment. We strongly encourage applications from women, minorities, individuals with disabilities and covered veterans.*

ASSISTANT PROFESSOR OF BIOLOGY
Eukaryotic Genetics

POSITION SUMMARY: Lycoming College invites applications for a tenure-track Assistant Professor position in the Biology Department starting fall 2015. Teaching responsibilities will include Genetics, Molecular Biology, a non-majors course, and one or more upper-level courses in the candidate's areas of interest. The candidate will also be expected to develop a research program involving undergraduate students. Ph.D. required, ABD considered.

TO APPLY: Electronic submission of application materials required. Candidates should submit cover letter describing teaching philosophy and research interests, curriculum vitae; graduate and undergraduate transcripts; and contact information for three individuals asked to submit recommendation letters on the candidate's behalf to **e-mail: jobs@lycoming.edu.** Please reference "Biology" and the candidate's name in the subject line of the e-mail for candidate materials and letters sent by the writers. Review of applications will begin immediately and continue until the position is filled; priority will be given to completed applications received by October 15, 2014.

Lycoming College is a National Liberal Arts College with about 1,400 students located in Williamsport, Pennsylvania **website: http://www.lycoming.edu.** Williamsport is a small city of approximately 30,000 that is nestled in the mountains of north central Pennsylvania. Williamsport is centrally located from New York City, Washington DC, and Philadelphia and offers ample opportunities for cultural entertainment and outdoor recreation.

Post-offer, pre-employment drug screen conducted. Equal Opportunity Employer.

FACULTY POSITION

The Department of Chemistry at the University of Michigan invites applications for a tenure-track position at any rank in any sub-discipline or interdisciplinary area of chemistry (including analytical, chemical biology, education, inorganic, materials, organic and physical) with an anticipated start date of September 1, 2015. This will be a University-year appointment (nine-month academic salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels.

Detailed information regarding the electronic application process and required materials is available online at **website: https://www.chem.lsa.umich.edu/chem/faculty recruit/index.html.**

Review of applications will begin on October 1, 2014.

Information about the Chemistry Department is available at **website: http://www.umich.edu/~michchem.** Questions about the application process may be sent to **e-mail: chemfacrecruit@umich.edu.**

Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

University of Illinois at Chicago, Department of Chemistry invites applications for a tenure-track **ASSISTANT PROFESSOR** position in the area of bioorganic and chemical biology effective August 2015, pending budgetary approval. Areas of greatest interest are cutting-edge research at the intersection of synthetic organic chemistry, biology, and medicine. The successful candidate will be expected to carry out a full and innovative program of experimental research and to teach graduate and undergraduate courses in organic/bioorganic chemistry and chemical biology. A Ph.D. is required. Please submit an online application, including the names and e-mail addresses of three references and upload a curriculum vitae, list of publications, summary of past research, plans for future research to **website: https://jobs.uic.edu/job-board/job-details?jobID=44839** by October 12, 2014. *The University of Illinois at Chicago is an Affirmative Action/Equal Opportunity Employer, dedicated to the goal of building a culturally diverse pluralistic faculty and staff committed to teaching in a multicultural environment. We strongly encourage applications from women, minorities, individuals with disabilities and covered veterans.*

ASSISTANT PROFESSOR POSITION
Molecular or Cellular Systems Biology
University of Tennessee, Knoxville

The Department of Biochemistry and Cellular and Molecular Biology at the University of Tennessee, Knoxville invites applications for a tenure-track faculty position at the rank of Assistant Professor in the area of molecular and cellular systems biology. Systems biology seeks to understand and predict how molecules and cells interact and communicate to give rise to emergent properties and behaviors of biological systems. We seek applicants whose research interests lie in understanding central biological phenomena by analyzing multi-dimensional, or large-scale experimental data within a quantitative framework. Ideal candidates will integrate experimentation with biomolecules, genomes, cells, or organisms with a tailored computational approach, such as bioinformatics, in the case of an omic-centered program, and/or mathematical modeling. The successful candidate should address questions that complement existing strengths in the department (cellular and developmental biology, plant and microbial biology, structural biology, neurobiology; see **website: http://bcmb.utk.edu/** for details). We anticipate that the candidate will also take advantage of scientific interactions with the National Institute for Mathematical and Biological Synthesis on our campus or the nearby Oak Ridge National Laboratory.

A Ph.D. in a relevant field and postdoctoral research experience are required. The successful candidate is expected to establish an innovative, externally funded research program and contribute to the departmental teaching mission at the undergraduate and graduate levels. We are seeking candidates who will actively contribute to the diversity and intercultural goals of the University. The position will start as early as August 1, 2015, and the salary will be competitive. Applications should include a brief cover letter, curriculum vitae with list of publications, a two- to three-page outline of research interests, and a description of teaching interests. Please e-mail the application as a single PDF file to **e-mail: bcmbsysbio@utk.edu**, and arrange for three letters of recommendation to be sent directly to **e-mail: referenceletters@utk.edu.** For informal inquiries, please contact **Albrecht von Arnim,** Ph.D., at **e-mail: vonarnim@utk.edu.** Review of applications will begin October 15, 2014 and will continue until the position is filled.

The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA Institution in the provision of its education and employment programs and services. All qualified applicants will receive equal consideration for employment without regards to race, color, national origin, religion, sex, pregnancy, marital status, sexual orientation, gender identity, age, physical or mental disability, or covered veteran status.

Assistant/Associate Professor of Computational Genomics

Tenure Track-9 month Academic Year Appointment – Department of Molecular Biology and Genetics
College of Agriculture and Life Sciences – Cornell University

Cornell is a community of scholars, known for intellectual rigor and engaged in deep and broad research, teaching tomorrow's thought leaders to think otherwise, care for others, and create and disseminate knowledge with a public purpose.

The Department invites applications for a tenure-track faculty position at the Assistant or Associate Professor level. We seek candidates who are developing innovative computational genomics methods to solve novel problems in genome biology, and are interested in interacting with experimental biologists who generate large-scale genomic data sets. Position will be 70% Research & 30% Teaching.

Areas of specialization might include, but are not limited to:
- Functional associations, quantitative genetic, and/or population genetic analyses of large-scale resequencing and phenotyping data sets of humans and/or model organisms.
- Systems-level integration of diverse genomic data sets.
- Functional predictions and/or evolutionary inferences from comparative genomic data.

Qualifications: Individuals seeking a creative integration of experimental and computational approaches will find Cornell a rich environment in which to work (see **http://mbg.cornell.edu/cals/mbg**). An advanced degree (Ph.D., M.D., or equivalent) is required and postgraduate training is highly desirable.

ANTICIPATED START DATE: July 1, 2015

SALARY AND BENEFITS: Competitive and commensurate with qualifications and experience. An attractive fringe benefit package is included.

APPLICATION PROCEDURE: Electronically submit online to **https://academicjobsonline.org/ajo/jobs/4452** CV, a two to four page research statement, which indicates how your program could synergize with existing Cornell faculty, a one page teaching statement, and pdfs of two of your most significant papers. Application review begins on October 15, 2014. Administrative questions can be addressed by Robin Lampman at **RLL2@cornell.edu** .

The Department of Molecular Biology and Genetics and the College of Agriculture and Life Sciences at Cornell embrace diversity and seek candidates who will help create a climate that attracts students of all races, nationalities, religions, genders, and sexual orientations. We strongly encourage women and members of underrepresented minorities to apply.

Cornell University is an innovative Ivy League university and a great place to work. Our inclusive community of scholars, students and staff impart an uncommon sense of larger purpose and contribute creative ideas to further the university's mission of teaching, discovery and engagement. Located in Ithaca, NY, Cornell's far-flung global presence includes the medical college's campuses on the Upper East Side of Manhattan and in Doha, Qatar, as well as the new CornellNYC Tech campus to be built on Roosevelt Island in the heart of New York City.

Diversity and Inclusion are a part of Cornell University's heritage. We're an employer and educator recognized for valuing AA/EEO, Protected Veterans, and Individuals with Disabilities.

UNIVERSITY OF SOUTH ALABAMA
MITCHELL CANCER INSTITUTE
COMPREHENSIVE ONCOLOGY HEALTHCARE & RESEARCH

Faculty Positions
Molecular and Metabolic Oncology

The Mitchell Cancer Institute at the University of South Alabama is developing a new program in Genome Stability, DNA Repair and Metabolism and looks to recruit faculty at the junior and mid-career stage to develop outstanding research programs that complement existing strengths. Interested applicants who are using novel biochemical and molecular biology tools to address fundamental and translational problems in those areas are encouraged to apply. Candidates with a track record of independent funding and publications in high impact journals will be given the highest consideration.

Successful candidates will be expected to run a vibrant collaborative program supported by external funding. A competitive salary and 6-year research start-up package will be provided.

Positions will be within the Department of Oncologic Sciences and the Mitchell Cancer Institute at the University of South Alabama and are tenure track. To apply, please send your curriculum vitae, a two-page summary of your research plans, and three letters of recommendation to: **Robert W. Sobol, PhD; Molecular and Metabolic Oncology Program Director and Point Clear Charities Professor of Oncologic Sciences, Mitchell Cancer Institute, 1660 Springhill Avenue, Mobile, AL 36604** or by email to **sallen@health.southalabama.edu**. Applications will be reviewed and evaluated on an ongoing basis.

The University of South Alabama is an Equal Opportunity Employer Minorities/Females/Veterans/Disabled.

Keck School of Medicine of USC

Department of Stem Cell Biology and Regenerative Medicine
Assistant Professorship in
Stem Cells and Regenerative Medicine

The Department of Stem Cell Biology and Regenerative Medicine is recruiting up to two candidates whose research focuses on understanding fundamental principles of regenerative processes and developing knowledge-based approaches to organ repair. The Department is housed within the newly created Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research within the Keck School of Medicine of USC. Significant resources are available to support all aspects of stem cell research within the building and adjacent centers. Excellent collaborative opportunities exist across the USC campuses. The Department is particularly interested in researchers employing tissue engineering strategies to the goals of regenerative medicine. In addition to its research mission, all members will play an important role in the educational mission of this newly created Department. Generous start-up packages will be awarded to the successful candidates.

Online applications will be accepted; please apply to **http://jobs.usc.edu:80/postings/30002**. Applications should include a letter of interest, curriculum vitae, brief 2-3 page outline of research past, present and future, and four letters of reference. The applicant is responsible for ensuring that the completed application is received before **October 31st, 2014**.

Women and individuals belonging to minority groups are particularly encouraged to apply. The University of Southern California is an Equal Opportunity Affirmative Action Employer.

POSITIONS OPEN

FACULTY POSITION
Johns Hopkins University
School of Medicine
Institute for Cell Engineering

The Institute for Cell Engineering invites applications from outstanding individuals with creative, rigorous, and integrative research approaches to enhance its cell engineering investigational strengths in immunology, stem cell biology, neurosciences, and vascular biology. For additional information about the institute, visit **website: http://www.hopkinsmedicine.org/institute_cell_engineering/**. Candidates should have an M.D. and/or a Ph.D. degree with appropriate postdoctoral experience and an outstanding publication record. Primary department affiliation will be determined by the applicant s qualifications and by relevance of the applicant's research program to departmental initiatives. The successful candidate will have experience in any aspect of stem cell biology or related field. Special attention will be give to investigators in the areas of vascular biology or organogenesis.

To apply, submit curriculum vitae, three letters of reference, copies of relevant publications and a concise description of research interests and research plans (up to three pages) to **e-mail: icesearch@jhmi.edu** to the attention of **Ted M. Dawson**, Director, Institute for Cell Engineering. Applications will be assessed on an ongoing basis and the deadline for submission is December 15, 2014. The appointment is expected to be made in 2015.

The John Hopkins University School of Medicine is an Affirmative Action/Equal Opportunity Employer that embraces diversity.

FACULTY POSITION in Biological Chemistry

The Department of Chemistry at the University of Michigan invites applications for a tenure-track position at any rank with an anticipated start date of September 1, 2015. Candidates with research interests in the area of biochemistry/chemical biology, broadly defined, and an interest in teaching in the biochemistry curriculum will be given priority. This will be a University-year appointment (nine-month academic salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels.

Detailed information regarding the electronic application process and required materials is available online at **website: https://www.chem.lsa.umich.edu/chem/facultyrecruit/.** Review of applications will begin on October 1, 2014.

Information about the Chemistry Department is available on **website: http://www.umich.edu/~michchem.**

Questions about the application process may be sent to **e-mail: chemfacrecruit@umich.edu.**

Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

DIVISION OF MATERIALS RESEARCH

National Science Foundation, Arlington, Virginia. The Division of Materials Research (DMR) announces a nationwide search for senior-level researchers to serve as **Program Directors** in the Materials Research Science and Engineering Centers Program (MRSEC) and the Condensed Matter and Materials Theory Program.

For additional information and how to apply, please refer to the Materials Research Science and Engineering Centers Program and the Condensed Matter and Materials Theory Program Dear Colleague Letters at **website: http://www.nsf.gov/materials.**

Because NSF has a rotator program, there are often opportunities in diverse areas of materials research. Those interested are welcome to request further information from **e-mail: dmr-recruit@nsf.gov.**

For additional information on NSF's rotational programs, please visit **website: http://www.nsf.gov/about/career_opps/rotators/.**

NSF is an Equal Opportunity Employer committed to employing a highly qualified staff that reflects the diversity of our nation.

ASSISTANT PROFESSORS, BIOLOGY

Canisius College, Buffalo, New York. Two tenure-track position, fall 2015. Will contribute to introductory biology (evolution/ecology and/or physiology) and/or sophomore level biochemistry/cell biology sequences. Microbiology: teach upper-level course and laboratory in general microbiology and one or more upper-level courses and associated labs in area of specialty. Research program studying microbiology from biochemical, cellular, and/or molecular approach involving undergraduates expected. Developmental Biology: teach upper-level course and laboratory in developmental biology and one or more upper-level courses and associated labs in area of specialty. Research program studying developmental biology using molecular techniques involving undergraduates expected. Candidates should complement existing expertise, which includes: animal behavior/behavioral neuroscience, immunochemistry, endocrinology, cellular neurobiology, vertebrate ecology, cancer epigenetics, plant biochemistry, primatology, evolutionary biology (population genetics), evolutionary ecology/entomology, and freshwater biology. Expected to seek external funding to support scholarship. Ph.D. is required/postdoctoral experience preferred. Send letter of application, vitae, transcripts, teaching philosophy, plan of proposed research, info for three references by November 1, 2014, to **Dr. Susan Aronica** at **e-mail: hr.recruiter@canisius.edu** (put MICRO BIO or DEVELOP BIO in subject, PDF preferred) or mail to: **HR, 2001 Main St, Buffalo, NY 14208.** *Affirmative Action/Equal Opportunity Employer.*

FACULTY POSITION – VIROLOGY

The University of Texas Southwestern Medical Center, Department of Microbiology, is seeking a new faculty member in virology at the **ASSISTANT PROFESSOR** (tenure track) level. The appointee will be expected to develop a front-rank, competitive, independent research program on a medically relevant viral pathogen(s). The appointee will contribute to the teaching of medical and graduate students. An attractive startup package, including a competitive salary and generous laboratory space in a new building, is available to conduct research in a dynamic environment. Candidates also will be considered for UT Southwestern's $1.2M Endowed Scholars (startup) Program (**website: http://www.utsouthwestern.edu/education/programs/nondegree-programs/other-programs/endowed-scholars/index.html**). Candidates should have a Ph.D. and/or M.D. degree with at least three-four years of postdoctoral experience and an exceptional publication record. Please send a cover letter, curriculum vitae, contact information for three letters of recommendation, and a brief summary of future research to **e-mail: virologysearchcommittee@utsouthwestern.edu.**

University of Texas Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women, minorities, veterans, and individuals with disabilities are encouraged to apply.

ASSISTANT PROFESSOR IN CHEMISTRY
Utah State University

The Department of Chemistry and Biochemistry at Utah State University invites applications for a tenure-track position at the Assistant Professor level beginning fall 2015. Candidates must have a Ph.D. in chemistry or a related field and postdoctoral or equivalent experience. The position requires the development of an externally funded research program and teaching at the undergraduate and graduate levels. Investigators with interests at the interface of inorganic and organic chemistry are encouraged to apply. Application information can be found online at **website: http://jobs.usu.edu** (**REQ ID 054825**); letters of reference should be submitted to the same site. Evaluation of applications will begin October 24, 2014 and will continue until the position is filled. For further information, please visit **website: http://www.chem.usu.edu.** *Utah State University is an Equal Opportunity/Affirmative Action Employer committed to assembling a diverse faculty. Women and members of minority groups are strongly encouraged to apply.*

EUKARYOTIC CELL BIOLOGIST
Tenure-Track Position

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the level of **ASSISTANT PROFESSOR**. We are seeking a Eukaryotic Cell Biologist with research interests that may include (but are not limited to): vertebrate/molecular physiology, molecular cell biology, immunology, endocrinology, parasitology, neurobiology, hematology, or developmental biology. The candidate will demonstrate ability and enthusiasm for teaching an upper division Cell Physiology/Cell Biology course with an associated lab. Other teaching responsibilities may include majors introductory level courses and/or upper division electives related to the candidate's expertise as well as Master's level graduate courses. In addition to teaching the candidate will be expected to establish a successful research program involving undergraduate and Master's students and to compete for extramural funding, to participate in student advising and departmental governance, and be involved in committee service to the university. A Doctoral degree in an appropriate academic discipline is required; postdoctoral experience and prior teaching experience at the college level is strongly preferred. This position is contingent on the availability of funding. Send cover letter, statements for teaching and research, curriculum vitae, and three letters of reference to:

Eukaryotic Cell Biologist Search Committee
c/o Ms. Debbie Reynolds
Dept. of Biology
California State University San Bernardino
5500 University Pkwy.
San Bernardino CA 92407
E-mail: dreynold@csusb.edu

Deadline: Applicant files will be reviewed beginning on November 1, 2014. Position open until filled.

California State University, San Bernardino is an Affirmative Action/Equal Opportunity Employer. We consider qualified applicants for employment without regard to race, color, religion, national origin, age, gender, gender identity/expression, sexual orientation, genetic information, medical condition, marital status, veteran status, or disability.

THREE OPEN FACULTY POSITIONS
Boston College
Chemistry Department

The Chemistry Department of Boston College invites applications for three tenure-track positions to be effective in the fall of 2015. Applicants will be evaluated based on their potential to establish a prominent and well-funded research program and to excel in teaching at the graduate and undergraduate levels. Successful applicants will join a department of approximately 120 doctoral students, 30 postdoctoral fellows, 200 undergraduate majors, and an internationally recognized faculty.

ASSISTANT PROFESSORS in the areas of Chemical Biology, Organic Chemistry, and Physical/Materials Chemistry: requires a Ph.D. in Chemistry or related areas; postdoctoral experience is desirable but not required. The candidate is expected to have published in top-refereed journals and demonstrated the ability to perform outstanding independent research.

Interested applicants must submit a cover letter (which includes the names of three references), a graphical executive summary of research plans (one page), curriculum vitae, a summary of research plans (eight pages maximum), a statement of teaching philosophy and arrange to have three letters of reference submitted via the online faculty application at: **website: http://apply.interfolio.com/25361** for physical/materials chemistry, **website: http://apply.interfolio.com/25362** for chemical biology, **website: http://apply.interfolio.com/25357** for organic chemistry.

All application materials must be submitted electronically on or prior to 15 October 2014.

Boston College, a university of eight schools and colleges, is an Equal Opportunity Employer and supports Affirmative Action.

KHRI

OTOLARYNGOLOGY-HEAD AND NECK SURGERY
UNIVERSITY OF MICHIGAN HEALTH SYSTEM

Kresge Hearing Research Institute
Department of Otolaryngology-Head and Neck Surgery
University of Michigan Medical School
Assistant or Associate Professor

Applications are being considered for a full-time, tenure-track position in the Kresge Hearing Research Institute and the Department of Otolaryngology-Head and Neck Surgery, University of Michigan School of Medicine. The successful candidate will hold a PhD, MD or MD/PhD degree and will have research interests relevant to auditory and vestibular function broadly defined, including the development, function and pathologies of the auditory and vestibular systems, from peripheral to central. The faculty position is within the vibrant and highly interactive research environment of the Kresge Hearing Research Institute under the leadership of its new director, Gabriel Corfas, PhD. The Institute is affiliated with Department of Otolaryngology-Head and Neck Surgery and resides within a very strong and collegial research community of the University of Michigan. We are seeking exceptional scientists to establish vigorous independent research programs, form productive collaborations and promote translation of their research.

Please submit a current CV, a 2- or 3- page description of research interests and future directions, and three to five reference letters. Review of applications will begin **October 15, 2014** and continue until the position is filled. Materials should be sent to:

Gabriel Corfas, Ph.D.
khri-facultysearch@med.umich.edu

Equal Opportunity/Affirmative Action Employer.

Tufts UNIVERSITY

TENURE TRACK FACULTY POSITION
OPTOGENETICS

The Department of Biology at Tufts University invites applications for a tenure-track Assistant Professor with a research focus using optogenetics or related technologies to study behavior across multiple levels of biological organization. The new professor will be expected to maintain an innovative, externally funded research program involving postdoctoral associates, graduate students, and undergraduates. We are seeking candidates whose research uses model systems that are tractable for genetic, molecular, optical, and computational approaches. A clear commitment to teaching excellence at the undergraduate and graduate levels is essential. Doctoral degree and a record of research productivity required. Postdoctoral experience preferred The appointment will begin in September 2015.

The Biology Department has 21 full-time faculty members spanning molecular, organismal, and population biology. The collaborative and supportive research environment includes highly active groups in genetics, molecular biophysics, neuroscience, animal behavior, and computational modeling. We maintain an active graduate-training program at the master's and doctoral levels. We are strongly committed to quality research and education in basic and applied biology. The department is closely allied with other programs including Biomedical Engineering, Chemistry, Environmental Studies, Psychology, and Computer Science. Individual members of the department also maintain active collaborations with colleagues at the Tufts Schools of Engineering, Medicine, and Veterinary Medicine.

To Apply: Applicants should use the link **https://secure.interfolio.com/apply/26040** to submit a cover letter, curriculum vitae, and separate statements of (1) research interests and plans and (2) teaching experience and philosophy. Submission of one to three selected reprints in PDF format is encouraged. Applicants should also ensure that three confidential letters of reference are submitted to this link. Questions about the position may be directed to Karin Murphy, Assistant to the Chair at **Karin.murphy@tufts.edu**. Review of applications begins **November 30, 2014**, and continues until the position is filled.

Tufts University is an Affirmative Action/Equal Opportunity Employer. We are committed to increasing the diversity of our faculty. Members of underrepresented groups are strongly encouraged to apply.

VANDERBILT UNIVERSITY

FACULTY POSITIONS: Assistant or Associate Professor Level Physician Scientists, Infectious Diseases

The Vanderbilt Division of Infectious Diseases of the Department of Medicine in Nashville, TN seeks candidates for physician-scientist, tenure-track positions (Assistant or Associate Professor). Prerequisites include MD degree or equivalent, excellent clinical and teaching skills, and eligibility for Tennessee medical licensure. Preference will go to candidates with a track record of translational lab research, publications, and grant support. We will consider applicants in any field of Infectious Diseases. Particular areas of interest include: (1) HIV (e.g. continuum of care, personalized HIV care, cure) (2) maternal/fetal health (e.g. infection of pregnancy, preterm birth); (3) tuberculosis (e.g. mechanisms of drug resistance, immunogenetics); and (4) "big data" science (e.g. systems biology, next generation sequencing, pharmacogenomics).

Successful candidates will enter a dynamic environment with many opportunities to collaborate across diverse disciplines. We seek applicants who share our goals to advance discovery through developing and utilizing molecular, genomic, computational and other innovative lab approaches relevant to the etiology, prevention, detection, pathogenesis, and treatment of infectious diseases.

Vanderbilt University is one of the world's top academic institutions with a robust clinical Infectious Disease Program, and a tradition of excellence and national-level funding. There is a strong environment for physician-scientist career development, with opportunities for joint/affiliate appointment in other departments.

Applicants should submit a cover letter and CV to **Ms. Robbie Loupé** (**robbie.loupe@vanderbilt.edu**). Queries should be directed to **David Haas, MD** (**david.w.haas@vanderbilt.edu**).

Tulane University

FACULTY POSITIONS IN DEVELOPMENTAL BIOLOGY AND/OR NEUROSCIENCE

The Department of Cell and Molecular Biology at Tulane University (http://tulane.edu/sse/cell/) anticipates filling two tenure-track Assistant Professor position(s), beginning July 1, 2015. Targeted are individuals whose research interests focus on developmental biology, with an emphasis in mammalian organogenesis, or cellular/molecular neuroscience.

The Department is undergoing a rebuilding process and has targeted neuroscience and developmental biology as areas for rapid growth. Applicants must have a Ph.D., at least 2 years of postdoctoral experience, a strong publication record, and show strong potential for obtaining external funding. The successful applicant will be expected to establish a vigorous, independent research program and to participate in graduate and undergraduate teaching. Opportunities exist for research collaborations with and participation in the Tulane Cancer Center, the Tulane National Primate Research Center, the Center for Bioenvironmental Research, and the Tulane Neuroscience Program.

Applicants should submit their curriculum vitae, a brief statement of research interests and three letters of recommendation by clicking on the following link: **apply.interfolio.com/26241**.

Application deadline is **November 30, 2014**.

Tulane University is an Equal Opportunity/Affirmative Action/ADA Employer and encourages minority and female applicants to apply.

Cleveland Clinic

Director, Orthopaedic and Rheumatologic Research Center

The Departments of Biomedical Engineering, Rheumatology and Orthopaedic Surgery within the Lerner Research Institute and Orthopaedic and Rheumatologic Institute of the Cleveland Clinic are recruiting for a leadership level position as Director of the Orthopaedic and Rheumatologic Research Center (ORRC) in any area of musculoskeletal bioengineering, imaging, immunology, inflammation research.

The Cleveland Clinic is consistently rated as one of the top 5 hospitals in the country, and the Departments of Orthopaedic Surgery and Rheumatology are both ranked within the top 5 in their disciplines by the U.S. News and World Report. The Department of Biomedical Engineering is the largest of eleven highly interactive departments in the Lerner Research Institute, and encourages innovation and translation of its technologies into the clinical arena. The Director of the ORRC will have the exciting opportunity to facilitate and build upon the existing multidisciplinary research and teaching programs that exist within and across these vibrant departments.

We are seeking a full time research and leadership commitment from individuals holding an MD, DO and/or PhD degrees. Recruitment resources and salary will be commensurate with qualifications and scope of responsibilities. Academic title will be at the Associate or Full Professor level in the Cleveland Clinic Lerner College of Medicine, with joint appointment in the Lerner Research Institute and Orthopaedic and Rheumatologic Institute. The successful candidate will have a demonstrated track-record and active peer-reviewed federal funding as Principal Investigator. She/he will be expected to build a center of excellence, internationally recognized for its strong basic and translational research and academic reputation.

A substantial recruitment package is offered, comprising:

1. An endowed chair
2. Generous start-up funding
3. Executive level administrative support
4. Executive office and conference room located within Lerner Research Institute
5. Several additional independent faculty level positions for the Center

Interested candidates should submit a letter of interest along with their CV to:
Nathan Elting, Physician Recruiter: eltingn@ccf.org

Cleveland Clinic is pleased to be an equal employment/affirmative action employer: Women/ Minorities/Veterans/Individuals with Disabilities.

Smoke-free/ drug-free environment.

Assistant Professor Bacteriology/Microbiology

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

The Department of Bacteriology at the University of Wisconsin-Madison invites applications for a faculty position in Bacteriology/Microbiology at the Assistant Professor level.

Degree and area of specialization: Ph.D. in microbiology, biochemistry, genetics, cell and molecular biology, or related field.

Minimum number of years and type of relevant work experience: At least 2 years of postdoctoral experience.

Principal duties: Tenure Track Position in Bacteriology, University of Wisconsin-Madison. Academic (9 month) appointment. The Department of Bacteriology at the University of Wisconsin-Madison (http://www.bact.wisc.edu/) invites applications for a faculty position in bacteriology/ microbiology at the Assistant Professor level. The Department is seeking candidates whose research is at the forefront of any area of microbiology with an emphasis broadly on molecular mechanism in any domain of life. The University and Department provide an excellent environment for the development of an outstanding research program. The position carries a commitment to the three functions of resident instruction, research, and outreach/service, as appropriate to the position and rank. Thus, the successful candidate will be expected to develop a vigorous, extramurally funded, independent research program and to participate in undergraduate and graduate teaching and university service.

Additional Application Procedures: All materials should be sent by email to the Bacteriology Faculty Search Committee (**facultysearch@bact.wisc.edu**) and received by **October 31, 2014** to ensure full consideration. Applications should be sent as a single pdf that includes: a cover letter referencing position vacancy listing #80284, a curriculum vitae documenting research and teaching experience, a research plan, teaching interests, and names and contact information for three individuals who will serve as references. Please also ask three referees to submit letters of reference to the same email address (**facultysearch@bact.wisc.edu**) with the applicant's name in the header.

Job listing can be found here:
http://www.ohr.wisc.edu/WebListing/Unclassified/PVLSummary.aspx?pvl_num=80284

UW-Madison is an Equal Opportunity/Affirmative Action EAmployer. We promote excellence through diversity and encourage all qualified individuals to apply.

POSITIONS OPEN

PLANT ECOLOGIST
Tenure-Track Assistant Professor
Starting in August 2015

Primary responsibilities will be to teach upper-level courses in ecology and in plant biology, contribute to introductory and intermediate courses in the Biology major, participate in the College's First-Year seminar in critical inquiry, and mentor undergraduates in our nationally recognized senior research program. Ability to teach biostatistics is a plus. Ph.D. required; postdoctoral research and/or teaching experience preferred. Upload resume, unofficial undergraduate and graduate transcripts, statement of teaching philosophy, description of research program, and three letters of reference to **website: http://apply.interfolio.com/25908.** Address questions regarding the position to **Rick Lehtinen** (**e-mail: biology@wooster.edu**). Application deadline is October 20, 2014.

Wooster seeks to ensure diversity by its policy of employing persons without regard to age, sex, color, race, creed, religion, national origin, disability, veteran status, sexual orientation, or political affiliation. The College of Wooster is an Equal Opportunity/ Affirmative Action Employer.

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- Career Advice
- Career Forum

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From the journal *Science* AAAS

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www.ScienceCareers.org

UNIVERSITY of VIRGINIA

FACULTY POSITION
Biochemistry & Molecular Genetics

In a major new initiative in Genomics and Bioinformatics, the School of Medicine at the University of Virginia (**www.virginia.edu/bmg**) plans to hire several tenure-track faculty members (open rank). As part of this initiative, the Department of Biochemistry & Molecular Genetics seeks candidates who use genomics or bioinformatics to address contemporary problems in cancer or other diseases, regenerative medicine, or the biology of the mammalian cell nucleus.

Candidates must hold a Ph.D. and/or M.D. in Biochemistry, Bioinformatics, Genomics, Oncology or a related field and must have demonstrated the capacity to develop and/or maintain a high-quality independent research program. Applicants with outstanding credentials from all fields will be considered. Support will include attractive start-up packages, laboratory space, and an exceptionally interactive research environment. Clinically qualified applicants will be considered for a joint appointment in the appropriate clinical department.

To apply, visit **https://jobs.virginia.edu** and search on **posting number 0614778**. Complete a candidate profile online, attach a cover letter, curriculum vitae, and statement of research interest **(two pages or less including references)**.

Three letters of recommendation should be submitted electronically, directly to **hn6u@virginia.edu** with the applicant's name in the subject heading.

Positions will remain open to applications until filled.

For additional information please contact Helen Norfleet-Shiflett, Department of Biochemistry & Molecular Genetics, via telephone **(434) 924-1667** or **hn6u@virginia.edu**.

The University of Virginia is an Equal Opportunity/Affirmative employer. Women, minorities, veterans and persons with disabilities are encouraged to apply.

MICHIGAN STATE UNIVERSITY

Chairperson, Department of Plant Biology

Michigan State University invites applications and nominations for the position of **Chairperson** of the **Department of Plant Biology**. The Chair would lead an internationally recognized department with a long history of university commitment to excellence in the Plant Sciences. The Department has more than 30 faculty members, strong graduate and undergraduate programs, and a supportive administrative and technical staff. It is built on the concept of integrating all sub-disciplines of plant biology, ranging from evolution and ecology to cellular, molecular and genomic biology. Research is conducted in modern research facilities on campus and at field sites across Michigan and throughout the world. The department includes faculty affiliated with the Kellogg Biological Station, the MSU Department of Energy Plant Research Laboratory, and the Great Lakes Bioenergy Research Center.

The Chair is expected to provide leadership and to promote a creative environment that fosters innovation and excellence in research and teaching/educational programs in Plant Biology. Candidates must possess an established record of strong scholarship in a relevant field, proven academic leadership, and strong interpersonal skills. Continuation of an active research program is encouraged and will be supported. Candidates should be qualified to hold the rank of tenured Full Professor. Applicants should submit: a complete curriculum vitae; a statement of interest highlighting specific strengths related to this position, including research and administrative experience and accomplishments; and the names of three references (who will be contacted only with your permission). Every effort will be made to maintain confidentiality until a list of candidates for interviewing is determined. All materials should be assembled into one PDF and uploaded to: **https://jobs.msu.edu** for position **#9727**. Review of applications will begin **October 15, 2014**, and will continue until the position is filled. Questions regarding the position may be sent to the Chair of the search committee, at **PLBChairsearch@plantbiology.msu.edu**.

MSU is an Affirmative-Action, Equal-Opportunity Employer and is committed to achieving excellence through diversity. The University actively encourages applications of women, persons of color, veterans, and persons with disabilities, and we endeavor to facilitate employment assistance to spouses or partners of candidates for faculty and academic staff positions.

CALIFORNIA STATE UNIVERSITY LONG BEACH 1949

Assistant Professors

The Department of Biological Sciences at California State University, Long Beach (CSULB) invites applications for two tenure-track Assistant Professor positions starting August 17, 2015. CSULB has a long tradition of training undergraduates and Masters students for careers in science by involving students in productive research and through a commitment to a scholarly approach to pedagogy. We have a diverse student body and our goal is to be a leader in placing students from groups underrepresented in science into research careers. We have a vibrant research community and excellent research facilities. The successful candidates will be expected to develop an externally funded research program involving undergraduate and MS students and teach at the undergraduate and graduate levels. Applicants must have a PhD and postdoctoral experience.

Plant Evolutionary Biologist

We seek broadly trained applicants investigating plant evolution utilizing both morphological and molecular approaches. For further information, please see the position description at **http://www.csulb.edu/divisions/aa/personnel/jobs/posting/2179/index.html**. Screening of applications will begin **October 10, 2014**. CSULB is an Equal Opportunity Employer.

Aquatic Toxicologist

We seek broadly trained applicants investigating the mechanisms of toxicity and/or the responses to toxic agents in aquatic environments at the molecular or cellular level. For further information, please see the position description at **http://www.csulb.edu/divisions/aa/personnel/jobs/posting/2190/index.html**. Screening of applications will begin **November 3, 2014**.

CSULB is an Equal Opportunity Employer.

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CUNY

Harry S. Truman Fellowship
in National Security Science and Engineering

Sandia National Laboratories

Sandia National Laboratories is one of the country's largest research facilities employing nearly 8,500 people at major facilities in Albuquerque, New Mexico and Livermore, California. Please visit our website at www.sandia.gov.

We are seeking applicants for the President Harry S. Truman Fellowship in National Security Science and Engineering. Candidates for this position are expected to solve a major scientific or engineering problem in their thesis work or have provided a new approach or insight to a major problem, as evidenced by a recognized impact in their field.

Sandia's research focus areas are: bioscience, computing and information science, engineering science, materials science, nanodevices and microsystems, radiation effects and high energy density science, and geoscience.

Candidates must meet the following requirements: U.S. citizenship, the ability to obtain and maintain a DOE "Q" clearance, and a Ph.D. (3.5 undergraduate and 3.7 graduate GPA preferred), awarded within the past three years at the time of application, or completed Ph.D. requirements by commencement of appointment. Candidates must be seeking their first national laboratory appointment (pre postdoc internships included).

The Truman Fellowship is a three-year appointment normally beginning on October 1. The salary is $111,200 plus benefits and additional funding for the chosen proposal. The deadline to apply is November 1st of each year.

For complete application instructions, please visit: http://www.sandia.gov/careers/students_postdocs/fellowships/truman_fellowship.html

Please submit the complete package to: Yolanda Moreno, Sandia National Laboratories, P.O. Box 5800, MS0359, Albuquerque, NM 87185-0359, or email ymoreno@sandia.gov. Please reference Job ID: 646048. All materials must be received by November 1, 2014.

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HUNTSMAN CANCER INSTITUTE
UNIVERSITY OF UTAH

UNIVERSITY OF UTAH HEALTH SCIENCES

University of Utah
Huntsman Cancer Institute
Department of Oncological Sciences

The **Department of Oncological Sciences** and **Huntsman Cancer Institute (HCI)** invite applications for a tenure-track faculty position at the **assistant** or **associate professor** level. We seek an outstanding cancer biologist with a program focused in **genomics/genome sciences**. The ideal candidate develops genome-wide assays and/or analysis approaches that utilize high-throughput sequencing to make novel biological discoveries. Areas of interest include gene regulation, epigenetics, RNA biology, cancer genetics, somatic mutation analysis, or the use of genomics in cancer diagnostics. Requirements include a PhD or MD/PhD, experience analyzing genome-scale datasets, and a track record of scientific excellence. Departmental strengths include transcriptional regulation, epigenetics, human cancer genetics, stem cell biology, mouse and zebrafish models of cancer, cell signaling pathways, apoptosis, DNA repair, cell motility, and cancer metabolism. HCI is an NCI-designated cancer center with state-of-the-art laboratories and shared resources, including core facilities for imaging, genomics, drug screening and in vivo testing, and population studies. We offer a collegial and interactive research environment that fosters the development of junior faculty and robust graduate programs for training PhD and MD/PhD students.

Inquiries may be submitted to Recruitment Director at
hci.recruitment@hci.utah.edu

Please apply by November 10, 2014 at the following link:
http://utah.peopleadmin.com/postings/35110

The University of Utah Health Sciences Center is a patient focused center distinguished by collaboration, excellence, leadership, and Respect. The University of Utah HSC values candidates who are committed to fostering and furthering the culture of compassion, collaboration, innovation, accountability, diversity, integrity, quality, and trust that is integral to the mission of the University of Utah Health Sciences Center.

The University of Utah is an Affirmative Action/Equal Opportunity employer and does not discriminate based upon race, national origin, color, religion, sex, age, sexual orientation, gender identity/expression, status as a person with a disability, genetic information, or Protected Veteran status. Individuals from historically underrepresented group, such as minorities, women, qualified persons with disabilities and protected veterans are encouraged to apply. Veterans' preference is extended to qualified applicants, upon request and consistent with University policy and Utah state law. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities. To inquire about the University's nondiscrimination or affirmative action policies or to request disability accommodation, please contact: Director, Office of Equal Opportunity and Affirmative Action, 201 S. Presidents Circle, Rm 135 (801) 581-8365.

The University of Utah values candidates who have experience working in settings with students from diverse backgrounds, and possess a demonstrated commitment to improving access to higher education for historically underrepresented students.

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Science Careers
From the journal *Science* AAAS
ScienceCareers.org

Weill Cornell Medical College

Faculty Position in Chemistry/Chemical Biology

The **Department of Biochemistry** seeks to hire a tenure track faculty member with research interests in synthetic chemistry and chemical biology. All faculty ranks will be considered, based on the applicant's qualifications. The applicant's laboratory will be in the newly opened Belfer Research Building on a floor that also houses the Tri-Institutional Therapeutics Discovery Institute (**https://www.triitdi.org/**) and other synthetic chemistry laboratories.

The new appointee will have a faculty appointment in the Department of Biochemistry (**http://www.cornellbiochem.org/**). The appointee will teach medical students and participate in the Graduate School of Medical Sciences. The appointee will also participate in the Tri-Institutional Training Program in Chemical Biology (**http://chembio.triiprograms.org/**). The specific area of research is open with a preference for candidates interested in using chemical approaches to analyze disease mechanisms and develop therapeutic interventions.

Interested applicants should forward electronic versions of (1) a cover letter; (2) a Curriculum Vitae; (3) a brief overview of current and future research interests; and (4) the names and contact information of at least three referees. (Confidential inquiries can be considered without initial contact of referees.) Material should be sent to Frederick Maxfield, Chair of the Search Committee, at **BiochemSearch@med.cornell.edu**. Evaluation of applications will begin immediately and continue until the position is filled.

EOE/M/F/D/V

PRINCETON UNIVERSITY

MOLECULAR EVOLUTION
Faculty position

Princeton University seeks candidates for a tenured or tenure track faculty position in the area of Molecular Evolution to join a growing collaborative program across the broad interface of evolution, ecology, molecular biology and genomics. We are interested in scientists whose research focuses on questions that integrate across this interface, with the aim of offering novel conceptual advances. The position will be shared between the Department of Ecology & Evolutionary Biology and the Department of Molecular Biology. We seek colleagues who will enthusiastically contribute to a climate of interdisciplinary collaboration, excellence and diversity. The candidate should share our commitment to a mentoring process that attracts students of all ethnicities, nationalities, and genders.

Applicants should write a vision statement, no longer than two pages, that outlines one or more major unsolved problems in their field and how they plan to address them. In this respect, the vision statement should go beyond a summary of the applicant's prior and current research. Applications, including the vision statement, curriculum vitae, three reprints, and contact information for three references can be submitted online via **http://jobs.princeton.edu**, Req **#1400594**. Screening of applications will begin **October 31, 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.

SAN DIEGO STATE UNIVERSITY
Leadership Starts Here

THE DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY AT SAN DIEGO STATE UNIVERSITY announces a tenure-track position in **BIOCHEMISTRY** at the assistant professor level with the appointment beginning Fall 2015. Preference will be given to applicants with research interests that involve the application of physical experimental techniques to study biochemical mechanisms and that complement existing strengths within the department. The successful candidate is expected to develop a robust research program and teach and mentor undergraduate and graduate (M.S. and Ph.D.) students. External funding and independent scientific productivity are expected. A Ph.D. degree and postdoctoral experience in a relevant field are required. SDSU is a large, diverse, urban university and Hispanic-Serving Institution with a commitment to diversity, equity, and inclusive excellence. Our campus community is diverse in many ways, including race, religion, color, sex, age, disability, marital status, sexual orientation, gender identity and expression, national origin, pregnancy, medical condition, and covered veteran status. We strive to build and sustain a welcoming environment for all. SDSU is seeking applicants with demonstrated experience in and/or commitment to teaching and working effectively with individuals from diverse backgrounds and members of underrepresented groups. The College of Sciences is host to a large number of federally-funded minority training programs (described at http://www.sci.sdsu.edu/casa/).

Applicants should submit a cover letter, curriculum vitae, description of research plans, anticipated startup requirements, copies of up to five relevant publications, a brief description of teaching interests, and arrange to have three letters of recommendation sent to: **http://apply.interfolio.com/25906**. Review of applications will begin **October 24, 2014**, and will continue until the position is filled.

SDSU is a Title IX, Equal Opportunity Employer and does not discriminate against persons on the basis of race, religion, national origin, sexual orientation, gender, gender identity and expression, marital status, age, disability, pregnancy, medical condition, or covered veteran status. The person holding this position is considered a "mandated reporter" under the California Child Abuse and Neglect Reporting Act and is required to comply with the requirements set forth in CSU Executive Order 1083 as a condition of employment. (http://www.chemistry.sdsu.edu/)

Tenure Track Faculty Positions in Neuroscience

Washington University in St. Louis

Department of Anatomy & Neurobiology

The Department of Anatomy & Neurobiology at Washington University School of Medicine is undergoing a major expansion and invites applications for tenure track Assistant Professor positions. We are seeking talented investigators who will develop an innovative research program in any field within the following two broad areas of neuroscience: **(1) Cellular and Molecular Neurobiology** including cellular and signaling mechanisms regulating the assembly and function of neural circuits, synaptic plasticity, and developmental disorders of cognition; **(2) Systems Neuroscience** including studies of neural circuit function in genetically tractable model organisms and the development of innovative neurotechnologies.

Applicants should email a single PDF file that includes a cover letter, CV, summary of research accomplishments and plans, and also request 3 letters of support be emailed to **neurosearch@wustl.edu c/o Azad Bonni, MD, PhD, Chairman, Department of Anatomy & Neurobiology, Washington University School of Medicine, St Louis, MO 63110**. Review of applications including letters of support will begin on **October 15, 2014**.

Washington University is an Equal Opportunity Employer AA/EOE M/F/D/V.

Endowed Four Diamonds Research Chair, Pediatric Hematology/Oncology
Penn State Hershey Children's Hospital
Pennsylvania State University College of Medicine

The Division of Pediatric Hematology/Oncology at the Penn State Hershey Children's Hospital invites applications from outstanding scientists and/or physician scientists for the Dance Marathon Endowed Chair, part of a >$50 M endowment supporting the Four Diamonds Pediatric Cancer Research Program. The mission of the Four Diamonds Pediatric Cancer Research Program is to understand pediatric tumor development at the molecular level and to utilize this knowledge to develop novel diagnostic and therapeutic approaches. Currently, the Four Diamonds Pediatric Cancer Research Program supports basic and translational research studies in leukemia, solid tumors, and autophagy, and clinical trials. The search is open to applicants in many areas of basic and translational research with a clear emphasis on pediatric malignancies, their diagnosis and treatment. Rank is at the Associate to Full Professor level commensurate with the applicant's experience and accomplishments. Successful applicants will have a Ph.D., M.D./Ph.D., or M.D. degree with an established track record in cancer research and demonstrated ability to obtain extramural funding (R01-type award).

The Division of Pediatric Hematology/Oncology has 12 full-time Pediatric Hematologists/Oncologists and 9 Ph.D. faculty. Over 100 new oncology patients are seen annually, and there are programs in pediatric stem cell transplantation, neuro-oncology, experimental therapeutics, survivorship, hemophilia, and sickle cell disease. The division has an accredited Pediatric Heme/Onc fellowship program and is an active member of COG and POETIC. Research is supported by the Penn State Cancer Institute, the Penn State Hershey Institute for Personalized Medicine, an NIH-funded CTSA, and Pediatric Clinical Trials Office. Basic core, translational, and clinical facilities provide an excellent environment for research.

All interested candidates should apply on line to: **https://www.healthcaresource.com/hershey/?fuseaction=search.jobDetails&template=dsp_job_details.cfm&cJobId=10147002**

The Penn State Milton S. Hershey Medical Center is committed to affirmative action, equal opportunity and the diversity of its workforce. Equal Opportunity Employer – Minorities/Women/Protected Veterans/Disabled. All individuals (including current employees) selected for a position will undergo a background check appropriate for the position's responsibilities.

Scientific Director,
Institut Pasteur International Network

The Institut Pasteur (Paris, France) announce an international call for the position of Scientific Director for the Institut Pasteur International Network.

The Scientific Director will contribute to the definition of the scientific strategy of the Institut Pasteur International Network, build international partnerships both within and outside the Network. He/She will oversee the research agenda, the education program and will serve as the principal advisor to the President of the Institut Pasteur and the General Secretary on scientific affairs involving the network. He/She will promote the Institut Pasteur International Network to raise it up as an undisputed leader in the global life sciences field.

Candidates must hold an advanced degree in life sciences (PhD, PharmD, MD), as well as a strong and successful experience in developing and implementing international research programs and partnerships, with special emphasis on resource-limited and emerging countries in research.

Effective knowledge of global institutional and private donors requirements would be a plus.

Native English speaker or fluent English speaker is a criterion of eligibility. Fluency in French is an advantage but by no mean mandatory.

Applicants should send their formal application to the General Secretary of the International Network, **Dr. Marc JOUAN**, by e-mail only at **emploi.international@pasteur.fr**, in a single PDF file:

- Letter of motivation
- Curriculum vitae,
- Titles and works

The application deadline is **November 9, 2014**.

Further information on Institut Pasteur International Network can be found at **http://www.pasteur.fr/en/institut-pasteur-world**

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American Association for the Advancement of Science (AAAS)
Washington, DC

AAAS intends to appoint a new Chief Executive.

The American Association for the Advancement of Science (AAAS) is seeking a visionary chief executive to nurture its exceptional suite of current programs and realize its enormous potential. From Science and its other journals, to its marquee fellowship programs, its extraordinary reach in science diplomacy, and influential voice in policy and education, AAAS exists to advance science for the benefit of all people.

AAAS was founded in 1848 and includes some 261 affiliated societies and academies of science, serving 10 million individuals. Science has the largest paid circulation of any peer-reviewed general science journal in the world, with an estimated total readership of 1 million.

AAAS seeks an outstanding and visionary executive to assume overall responsibility for the leadership and management of the organization. Based in Washington, DC and reporting to the Board of Directors, the CEO will be a highly creditable and exceptional leader with unusual vision, operational excellence, intellectual breadth, and practical experience with the scientific, nonprofit, academic, government and business sectors.

Further information about AAAS' reach and impact can be found at: http://www.aaas.org/

AAAS has retained Russell Reynolds Associates to assist with this appointment.
To apply, please electronically submit a letter-of-interest and CV to AAAS@russellreynolds.com

The closing date for applications is October 1, 2014

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Institut Pasteur

Call for projects 2015
Creation of new research groups at Institut Pasteur

The Institut Pasteur has launched an international call for candidates wishing to establish new independent research groups in the cutting edge interdisciplinary environment of its campus in Paris, France.
The Institut Pasteur is a non-profit private foundation dedicated to fundamental, interdisciplinary research and to the translation of the knowledge to medicine and public health. Topics of interest include microbiology (bacteria, viruses, parasites and fungi) and infectious diseases, immunology, developmental biology and stem cells, neuroscience, genomics, genetics and cancer.
The Institut Pasteur is now initiating a new recruitment campaign, with attractive packages for junior and mid-career scientists. Senior investigator candidates are also welcome to apply.

Successful **junior candidates**[1] will be appointed with a permanent position, and as head of a group of 6 people. These groups will be created for a period of 5 years and may thereafter compete for a full research group.
Successful **mid-career and senior candidates** will be appointed with a permanent position, and as head of a research group of 8 to 15 people. The groups will be created for 10 years (mid-term evaluation at 5 years) with the possibility of renewal.

Highly attractive packages to match the experience of the candidate will be provided, including institutional salaries (Principal investigator, permanent scientists, technician, secretary, post-doctoral fellows), a substantial contribution to running costs and equipment, access to on campus state-of-the-art technology core facilities, as well as support for relocation expenses and administrative issues.

We request a Letter of Intent (LOI) in advance of submitting a full grant application. The template can be downloaded from the Institut Pasteur website: **http://www.pasteur.fr/recherche/cfp2015_loi.doc**.

A pdf copy of the LOI should be electronically submitted to **CFP2015@pasteur.fr** no later than **Friday, November 28, 2014** by **5:00** pm (Central European Time).

Shortlisted applicants will be notified by e-mail by mid-January 2015.
A complete application will be requested and due for submission by the end of February 2015. Applicants will be invited for interview to take place in mid-April 2015. The final ranking will be established by the Pasteur Scientific Council during its June 2015 session.

Contacts:
Practical aspects: CFP2015@pasteur.fr
Scientific aspects: alain.israel@pasteur.fr

[1]Institut Pasteur is an equal opportunity employer. Junior group leaders should be less than 8 years after PhD at the time of their LOI submission. Women are eligible up to 11 years after their PhD if they have one child, and up to 14 years after their PhD if they have two or more children.

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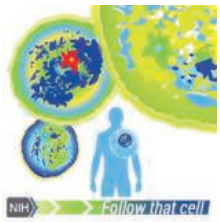
Origins Project Postdoctoral Prize Lectureship

This annual $10,000 international prize will be awarded, beginning in 2015, to an outstanding junior scholar chosen from any field of study relevant to the broad mission of the Origins Project at ASU. Selection will be made among candidates nominated by faculty or senior researchers at their institution, and will be based both on past accomplishment, and future promise, as well as an ability to convey the excitement of their research to a broad audience. The winner will be hosted for one week in the ASU unit closest to their field of study, and will present 3 lectures on their research and one public lecture. In addition to the cash prize, all travel and accommodation expenses will be covered by the Origins Project.

ASU Origins PROJECT
ARIZONA STATE UNIVERSITY

FOR MORE INFORMATION AND INSTRUCTIONS GO TO:
ORIGINS.ASU.EDU/POSTDOC-PRIZE
NOMINATIONS DEADLINE DEC 1, 2014



The **National Institutes of Health** (NIH) announces the **Follow that Cell** challenge. This is a $500,000 prize competition to spur the development of innovative solutions in single cell analysis that will allow us to *Follow that Cell*. Through this competition, the NIH is seeking novel robust methods for analysis of individual cells that can detect and assess changes in cell behavior and function over time. The goal is to develop solutions to follow a single cell in a complex multicellular environment to detect changing cell properties using multiple integrated measures.

If you would like to enter this Challenge and help improve our understanding of cell biology and the cellular basis of disease, provide options for better and more targeted treatments, and become part of a vibrant and engaged community that will stimulate new businesses and support economic growth in biomedical communities, access the registration link through **https://commonfund.nih.gov/Singlecell/index**.

We welcome solutions from individuals, teams and entities from all U.S. sources, including the public sector, private sector, non-profit groups, and those not traditionally supported by NIH. Specific eligibility requirements state federal grantees and federal contractors may not use federal funds to develop Challenge submissions. A recent NIH press release describing this effort and how to register is available online at **http://go.usa.gov/yKcY**

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Arnold and Mabel BECKMAN FOUNDATION

Call for Nominations
2015 Beckman-Argyros Award in Vision Research

The Arnold and Mabel Beckman Foundation is accepting nominations for the 2015 Beckman-Argyros Award in Vision Research. The award was established in 2013 to celebrate the friendship of Dr. Arnold O. Beckman and Ambassador George L. Argyros, and to honor the Ambassador for his 22 years of service as Chairman of the Board of the Arnold and Mabel Beckman Foundation.

The $500,000 Award will recognize and reward one individual who has made significant transformative contributions in vision research.

Nomination Deadline: December 11, 2014

2014 Beckman-Argyros Award Recipient
Krzysztof Palczewski, Ph.D.
Case Western Reserve University

For more information and nomination forms:
www.beckman-foundation.com/beckman-argyros

By **Gale Rhodes**

The career I dreamed of?

All I knew—given the limited range of professional lives I could observe from my hometown of textile mills and surrounding tobacco fields—was that I wanted to teach. When I took organic chemistry, from one of the dullest teachers I ever had, I knew I wanted to teach that and that I could do it better. When I took biochemistry in graduate school, I knew I wanted to teach that, too. In applying for the very few academic jobs on offer in the early '70s, I sold myself as a biochemist and got a small-college teaching job as one. I gradually became one.

I taught happily for 37 years, including leaves for research and writing, in schools where teaching was emphasized and research was, they said, encouraged and supported—sometimes with no more than pats on the back. I watched some great students emerge from academic backwaters into the open seas of original research, while others went off into careers using science in all sorts of unexpected ways. Some wandered off.

My wife and I moved about to meet the demands of family life. I earned tenure three times. For me, it was a game. Rules of tenure vary from place to place, but I could figure out the rules, didn't mind the work, and ended up feeling that I could wake up in the morning and get tenure on the way to the shower. I complied, sometimes cynically, with the demands of deans and chairs that I produce research or scholarship.

Their prodding helped me produce numerous products I feel good about, including a book that's still in print and still helpful, I am told, after more than 20 years; a website from which many people take maiden flights into protein modeling; and a few dozen odds and ends of publications from my grasshopper mind. In the end, that work made me a better, more enthusiastic, more convincing and longer lasting teacher. So, of course, the deans and chairs were right. These products are satisfying, but they are dim background to all the students.

Everywhere I taught, I was the only biochemist on campus. There are many disadvantages to such isolation, but the great advantage was teaching a full-year course all by myself, instead of taking part in committee-built courses like the one I took in graduate school. Seeing biochemistry whole trumped talking to other biochemists every day. But thank goodness for nearby schools and Gordon Research Conferences.

"I could only have dreamed of evading textiles and tobacco with a life of books and chalk."

Many important elements of my career, I could not have foreseen or dreamed of. I wish the same for you. I mean little things like the desktop computer and the Internet, which were not a gleam in any eye when I set out. Nor could I have imagined operating a molecular blackboard (Nicolas Guex's Swiss-PdbViewer) to lead vivid tours of structural elements and active sites, and then sending students off to explore thousands of biomolecular models on their own laptops. In my search for ways to help students learn, new developments meant new ideas.

I don't know how to advise those of you who see so much, read so much, and shudder about what awaits you. I was very naive about the life upon which I embarked. If, somehow, I had known the effort it would take, I might have been daunted. Inexperience, interest, and engagement protected me. I simply did what seemed to be the next thing needed and followed my nose toward what interested me most at the time, shaping the work a bit, if needed, to fit external demands. I found my way mostly by watching and listening, taking my own pulse, and using common sense—leaning, as it were, toward a very general goal and a great love, keeping it fuzzy and doing what I could with what came. Specific expectations might have gotten in my way.

In the details, my career was nothing like what I dreamed. I could only have dreamed of evading textiles and tobacco with a life of books and chalk. (I do miss chalk.) Nevertheless, I had the career I dreamed of. ■

Gale Rhodes is professor emeritus of chemistry at the University of Southern Maine, Portland. For more on life and careers in science, visit www.sciencecareers.org.

ILLUSTRATION: ROBERT NEUBECKER

Downloaded from www.sciencemag.org on September 12, 2014

Published by AAAS