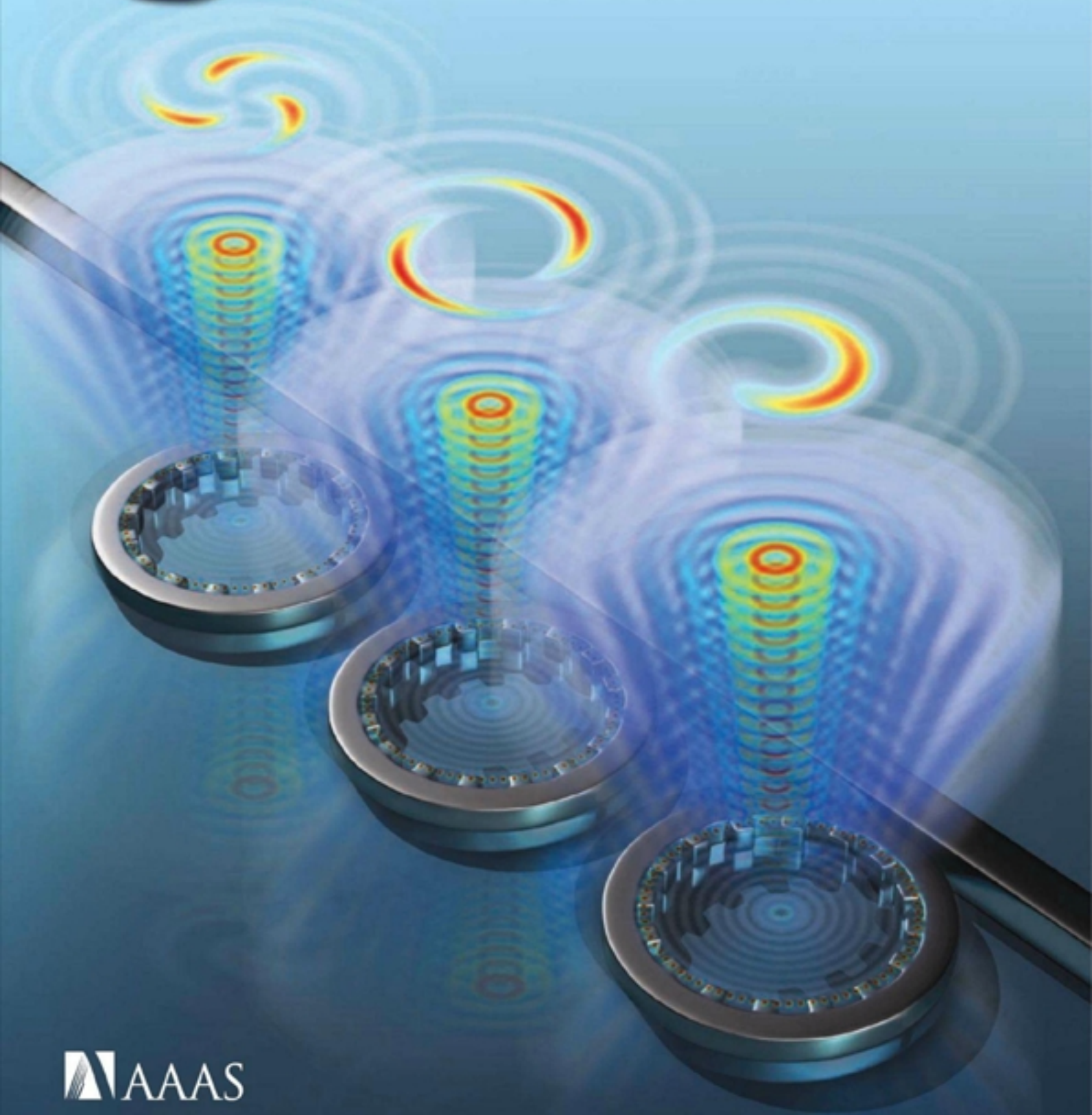


# Science

19 October 2012 | \$10



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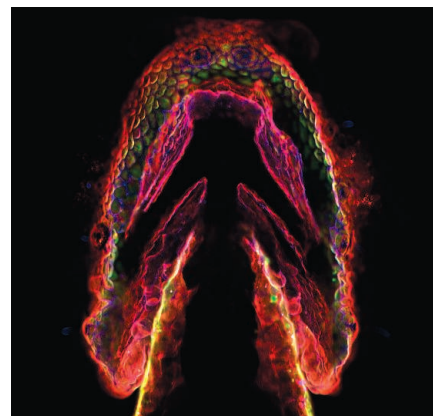
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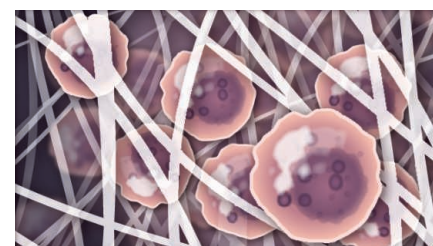
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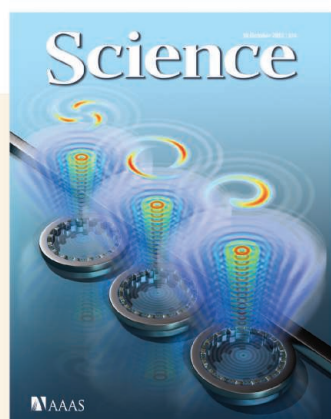
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Optical vortices emitted by an array of silicon ring resonators. The helical phase fronts, indicated by spirals, denote photons with orbital angular momentum. Emission results from the embedded angular gratings that extract the light waves traveling around the ring. The small emitters (diameter: 8 micrometers) can be integrated on a large scale into photonic integrated circuits, enabling new applications in optical communications and sensing, quantum photonics, and lab-on-a-chip. See page 363.

*Image: Yue Zhang (ciel924@126.com), based on data from Xinlun Cai, Jiangbo Zhu, and Siyuan Yu*

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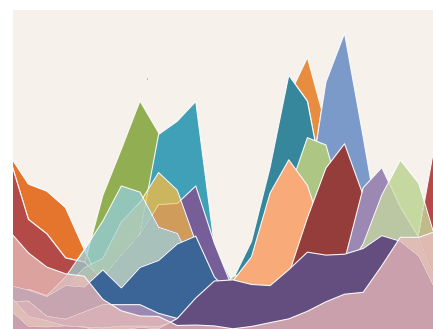
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*M. Čuk and S. T. Stewart*

10.1126/science.1225542

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### Forming a Moon with an Earth-like Composition via a Giant Impact

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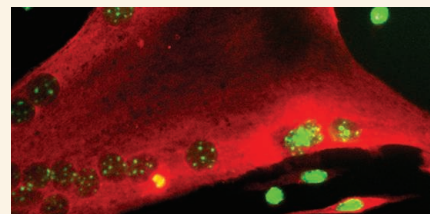
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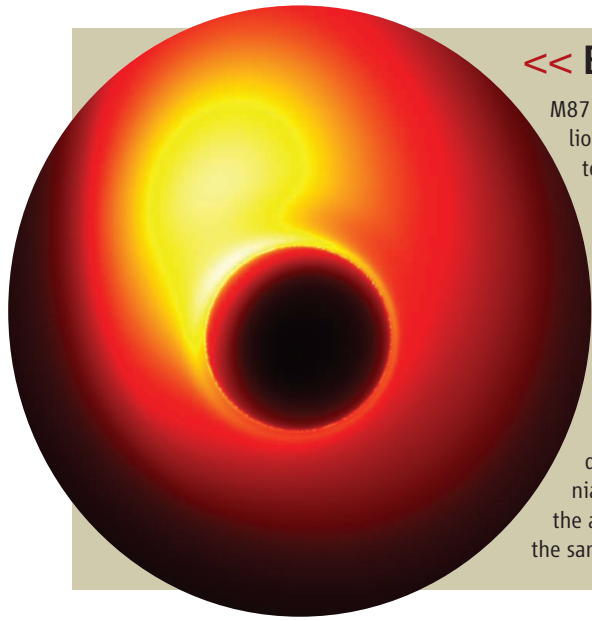
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## << Black Hole Close-Up

M87 is a giant elliptical galaxy about 55 million light-years away. Accretion of matter onto its central massive black hole is thought to power its relativistic jet. To probe structures on scales similar to that of the black hole's event horizon, **Doelman *et al.*** (p. 355, published online 27 September) observed the relativistic jet in M87 at a wavelength of 1.3 mm using the Event Horizon Telescope, a special purpose, very-long-baseline interferometry array consisting of four radio telescopes located in Arizona, California, and Hawaii. The analysis suggests that the accretion disk that powers the jet orbits in the same direction as the spin of the black hole.

## All Change

Research on early warning signals for critical transitions in complex systems such as ecosystems, climate, and global finance systems recently has been gathering pace. At the same time, studies on complex networks are starting to reveal which architecture may cause systems to be vulnerable to systemic collapse. **Scheffer *et al.*** (p. 344) review how previously isolated lines of work can be connected, conclude that many critical transitions (such as escape from the poverty trap) can have positive outcomes, and highlight how the new approaches to sensing fragility can help to detect both risks and opportunities for desired change.

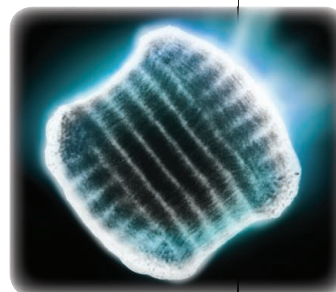
## Transcription Around the Clock

The biological clock that controls daily rhythms in mammalian physiology and behavior is thought to be regulated in large part by transcriptional events (see the Perspective by **Doherty and Kay**). **Koike *et al.*** (p. 349; published online 30 August) produced a comprehensive analysis of these transcriptional events across the entire mouse liver genome over a 24-hour period. Only ~22% of cycling messenger RNA transcripts were driven by de novo transcription, suggesting that posttranscriptional events also play an important regulatory role in the mammalian clock. Biological timing in organisms can also respond to rhythmic cues from the environment. **Morf *et al.*** (p. 379, published online 23 August) explored how one such cue, cycles in ambient temperature, influence circadian timing in mammalian cells. Cold-inducible

RNA-binding protein (CIRP) accumulates when body temperature is low. A systematic search for binding partners of CIRP identified RNA encoding core components of the circadian clock. Loss of CIRP decreased the amplitude of circadian gene expression and cells lacking CIRP adapted more quickly to temperature cycles.

## Beyond Quantum Dots

Semiconducting colloidal nanoparticles—quantum dots—are of interest for their unusual properties. One current challenge is the controlled assembly of colloidal particles into larger structures, such as two-dimensional lattices on a substrate, or three-dimensional superparticles. **Wang *et al.*** (p. 358) present a two-step self-assembly of CdSe/CdS semiconductor nanorods to form mesoscopic colloidal superparticles. The particles show well-defined supercrystalline domains with dimensions ranging from hundreds of nanometers to several microns, and with the particle morphology controlled by the number of constituent rods. Films of the needle-shaped superparticles were able to act as polarizing light-emitting diodes.



## A Twist of Light

The angular momentum of photons can be used to encode and transmit information. **Cai *et al.*** (p. 363) developed a method for gener-

ating and emitting controllable orbital angular momentum states of light from a reconfigurable and scalable silicon photonic chip. Using micro-ring resonators embedded with angular gratings allowed the imprinting of optical angular momentum on the light propagating in the whispering gallery modes of the resonator. The method may enable large-scale integration of optical vortex emitters on complementary metal-oxide-semiconductor-compatible silicon chips.

## Too-Hot Times

Climate warming has been invoked as a factor contributing to widespread extinction events, acting as a trigger or amplifier for more proximal causes, such as marine anoxia. **Sun *et al.*** (p. 366; see the Perspective by **Bottjer**) present evidence that exceptionally high temperatures themselves may have caused some extinctions during the end-Permian. A rapid temperature rise coincided with a general absence of ichthyofauna in equatorial regions, as well as an absence of many species of marine mammals and calcareous algae, consistent with thermal influences on the marine low latitudes. Sea surface temperatures approached 40°C, which suggests that land temperatures likely fluctuated to even higher values that suppressed terrestrial equatorial plant and animal abundance during most of the Early Triassic.

## Dating Carbon

Radiocarbon dating is the best way to determine the age of samples that contain carbon and that are younger than ~50,000 years, the limit of precision for the method. There are several factors that complicate such age determinations, however, some of the most important of which include variability of the  $^{14}\text{C}$  production in the atmosphere (which affects organic samples whose radiocarbon inventories are derived from atmospheric  $\text{CO}_2$ ), surface ocean reservoir effects (which affect marine samples that acquire their radiocarbon signatures from seawater), and variable dead carbon fraction effects (which affect speleothems that derive their carbon from groundwaters). **Bronk Ramsey *et al.*** (p. 370; see the Perspective by **Reimer**) avoid the need to make such assumptions, reporting the  $^{14}\text{C}$  results of sediments from Lake Suigetsu,

*Continued on page 303*

Continued from page 301

Japan. Analysis of terrestrial plant macrofossils in annually layered datable sediments yielded a direct record of atmospheric radiocarbon for the entire measurable interval up to 52.8 thousand years ago.



## African Origins

Humans originated in Africa and then spread across the globe. The high genetic diversity found in sub-Saharan Africa is consistent with this view, but the relationships among and within African populations have been less well explored. **Schlebusch *et al.*** (p. 374, published online 20 September) genotyped 220 individuals from 11 populations representing groups from Southern Africa to determine their relationships and history. The data suggest that modern-day human populations arose from a complex blend of mixing between groups and genetic stratification.

## Made and Modified

The polytheonamides are 48-residue toxins derived from marine sponges that include 18 D-amino acids, as well as many other unusual amino acid modifications. Given the complexity, one might guess that these peptides are the product of nonribosomal, peptide synthetase (NRPS). However, **Freeman *et al.*** (p. 387, published online 13 September) now show that polytheonamides are produced by a bacterial symbiont using a ribosomal pathway. Six candidate enzymes for the 48 posttranslational modifications were identified and three were functionally validated. Such ribosomal systems could be useful in bioengineering.

## Cleave and Leave

Plants produce ethylene gas, which acts as a hormone and is essential for the ripening of fruit, the resistance of plants to pathogens, the adaptation of plants to stress conditions, and stem cell maintenance. Although many components of the ethylene gas signaling pathway have been well studied, little is known about how the ethylene receptors located in the endoplasmic reticulum (ER) membrane can transmit the signal to the nucleus. Studying *Arabidopsis*, **Qiao *et al.*** (p. 390, published online 30 August) found that perception of ethylene gas in the ER promotes signal transduction via cleavage and rapid ER-nucleus translocation of the cytosolic portion of the transmembrane ETHYLENE INSENSITIVE2 protein, which activates ethylene-dependent gene expression and other ethylene response phenotypes in plants.

## A Fine Balance

Intellectual and neurological disabilities can arise from diverse developmental aberrations. **Novarino *et al.*** (p. 394, published online 6 September; see the Perspective by **Beaudet**) have now determined the genetic basis for one such disorder for a small group of patients. Exome sequencing led to identification of mutations in a kinase *BCKDK* (*Branched Chain Ketoacid Dehydrogenase Kinase*) that regulates metabolism of branched-chain amino acids such as valine, leucine, and isoleucine. Mice with homozygous mutations in the *BCKDK* gene showed developmental and neurological abnormalities resembling those in certain mouse autism models. Analysis of transport mechanisms responsible for carrying amino acids across the blood-brain barrier revealed competition between the branched-chain amino acids and large neutral amino acids. Nutritional supplementation with extra branched-chain amino acids in the diet of mice carrying homozygous mutations in the *BCKDK* gene normalized their phenotype.

## Making a Riboswitch

During RNA synthesis by RNA polymerase (RNAP), nascent RNA will start to fold. This cotranscriptional folding can affect the final conformation and the function of the full-length RNA. Using single-molecule spectroscopy and optical tweezers, **Frieda and Block** (p. 397) followed the cotranscriptional folding of the *pbuE* riboswitch from *Bacillus subtilis* directly in real time, observing the formation of the adenine-binding aptamer structure or the alternative transcription terminator hairpin structure. The structures formed within seconds of their sequences clearing the RNAP footprint, confirming that the *pbuE* riboswitch is kinetically controlled.

CREDIT: CARINA SCHLEBUSCH



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## A Threat to National Security

THE UNITED STATES FACES ONE OF THE MOST PERILOUS CONDITIONS THAT I CAN REMEMBER IN my professional life. The greatest threat we face today is not external, but internal. Our inability as a nation to reach a durable consensus on plans that match our national goals with our financial resources is, in my judgment, the greatest National Security risk.

I distinguish here between National Security (capital N and S) and lower-case national security. The latter refers to military capabilities, the size of our army, the effectiveness of our weapons, the quality of military training, the deployment and pace of operations, and so forth. That is lower-case national security, to which I have devoted most of my professional career. Capital-letter National Security concerns the vitality of our economy, the ability to generate new ideas, the consensus in society to carry burdens for national purposes, and conviction in the legitimacy of our political foundation and its institutions.

The budget deficits we now face are a threat to both dimensions of national security. Most immediately, the United States faces the threat of sequester—a mindless process that represents the default of responsibility by U.S. politicians. If the sequester is triggered, it will impose damaging cuts on our defense establishment and programs. It also will impose crippling cuts on domestic spending, to include federal support for the nation's science and engineering research and development system. Serious as these are, the fundamental threat to National Security lies elsewhere: in our inability to find common purpose on an obvious problem. Each political party is proposing to solve the fiscal imbalance only in a manner that satisfies its base constituencies. This will fail. The problem is so vast that it demands a broad-based consensus, and that can come only with compromise.

We have a chart to navigate these waters. President Obama asked Congress to create a national commission—chaired by former Senator Alan Simpson and former White House Chief of Staff Erskine Bowles—to find a solution to this problem. This bipartisan effort produced in 2010 a plan that would set the United States on a healthy path over the next 10 years. At its core is a fundamental compromise: The plan calls for cutting \$3 of spending for every \$1 of new taxes. The cuts will primarily be required in entitlements, especially Social Security and Medicare. The American public will support a plan that affects their lives if it is efficacious and fair. Americans know that we have a serious problem, but they have yet to hear either party present plans that meet the fairness test. Simpson-Bowles gives the nation such a plan. But the United States lacks politicians with the maturity and sense of responsibility to enact it.

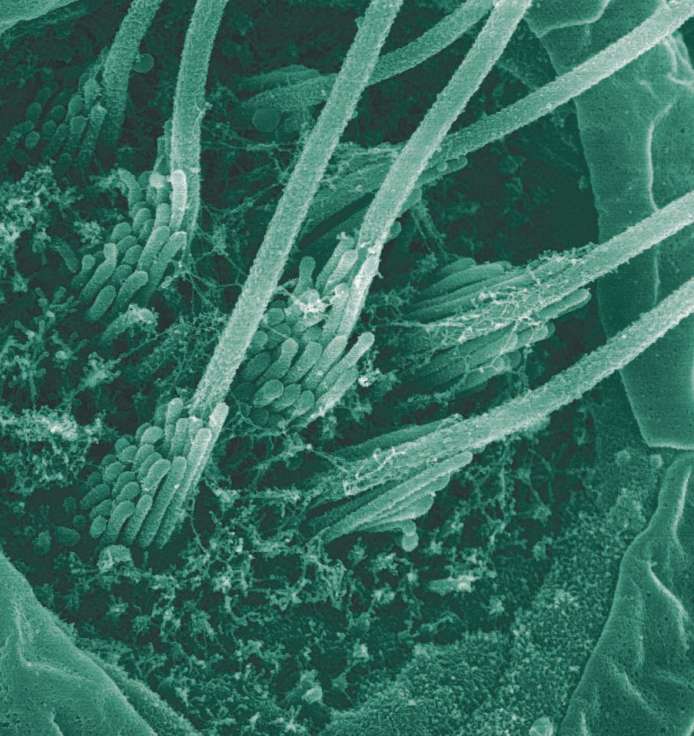
Sadly, Republican and Democratic leaders have landed on identical strategies to win the next election: anger their respective bases so fundamentally that they come out to vote against the other candidate. This may be clever politics, but it is deeply flawed national leadership. Both political parties, through their behavior, are threatening National Security. It goes far beyond the immediate sequester issue. Our fiscal imbalance threatens federal support for the research and development needed to seed future innovation, sovereign creditworthiness, support for education, the refreshment of talent in government service, and much more. This is a gigantic threat to National Security.

Ultimately, the burden rests with the citizens of the United States, because they must solve this problem. Our politicians will continue to disappoint us until we demand that they meet a higher standard. It is the voters who elect national leaders who are responsible for this sad state. Every American needs to demand that the two political parties find a genuine compromise. Our National Security demands it.

— John J. Hamre







## GENETICS

### At the Tip of Hearing

Individuals with the hereditary disorder Usher syndrome suffer from hearing loss. Associated genetic mutations impair function of the inner ear, where sensory cells fail to convert sound waves into electrical signals. Riazuddin *et al.* have determined that mutations in the gene *CIB2* contribute to Usher syndrome and nonsyndromic deafness. *CIB2* encodes calcium and integrin binding protein 2, which is widely expressed in human and mouse tissue. In the mouse inner ear, the protein localizes to the tips of stereocilia of inner ear cells. When deflected by sound waves, ion channels in these hairlike projections open, triggering a mechanoelectrical signaling cascade. *CIB2* interacts with whirlin, a protein that organizes molecular complexes that maintain stereocilia structure and growth. Suppression of *CIB2* expression in zebrafish disrupted responses to acoustic stimuli and caused abnormal balance during movement. Overexpression of *CIB2* in cultured cells decreased the release of calcium from intracellular stores. *CIB2* may help to maintain intracellular calcium homeostasis in inner ear cells by sequestering calcium and influencing the release of stored calcium during mechanoelectrical signal transduction. — LC

*Nat. Genet.* **44**, 10.1038/ng.2426 (2012).

## SOLAR PHYSICS

### When the Sun Erupts

Coronal mass ejections (CMEs) are large-scale clouds of plasma that erupt from the solar atmosphere and travel into interplanetary space. They represent the most energetic events in the solar system and can be harmful to satellites in space, Earth-based power grids, and even to humans if they are in space or on airplanes, particularly on polar routes. CMEs are common, occurring four to five times a day during periods of maximum solar activity, but they weren't detected until the early 1970s when satellites were launched to study the Sun. After four decades of research, however, there is still no complete understanding of the physical mechanisms behind CMEs. Roussev *et al.* describe state-of-the-art computer simulations that couple a detailed magnetic flux emergence simulation extending from the interior of the Sun to its atmosphere with a global model of the solar atmosphere and the solar wind. The results reproduce x-ray observations of CMEs and show how the injection of magnetic flux leads to a catastrophic evolution of the solar atmosphere. To better understand collisions of CMEs as they travel in interplanetary space, Shen *et al.* analyzed observations of two CMEs that erupted from the Sun on 2 November 2008. The two structures collided as though they were solidlike objects and likely experienced a superelastic collision (i.e., a collision in which the linear kinetic energy of the colliding system increases). — MJC

*Nat. Phys.* 10.1038/nphys2427;  
10.1038/nphys2440 (2012).

## ARCHAEOLOGY

### Wild Textile

It's been generally thought that the human use of textiles greatly accelerated with the advent of agriculture. Earlier woven textiles using wild materials are known, and indirect evidence for woven fabrics dates back to glacial times. However, early cultivation of plants such as flax and hemp, and also the domestication of sheep, are thought to have provided more reliable raw materials. Bergfjord *et al.* now show that wild nettle was used widely and valued as

a textile source as recently as 2800 years ago in Denmark. The Bronze Age textile was found wrapped around a body in a burial site; the orientation of the fibers and the presence of calcium oxalate crystals verify that it was made from woven nettle. Using strontium isotopes, the authors further show that the nettle was likely imported to Denmark, probably from Central Europe—an area that had active flax agriculture at the time. Thus, wild plants were still valued for textiles long after extensive cultivation of flax and hemp. — BH

*Sci. Rep.* **2**, 664 (2012).



## APPLIED PHYSICS

### Extended Darkness

Titanium sapphire laser sources rely on a phenomenon termed Kerr lensing to generate ultrashort light pulses. Essentially, the refractive index of the crystal in the laser cavity rises with the intensity of the light passing through it, which induces focusing toward the center where selective amplification sets the mode structure. Kasala and Saravanamuttu have created an environment in which a propagating light beam is instead pushed away from the center. Specifically, they diminish the intensity of a ~0.1-mm central spot in a ~5-mm beam—derived from an incoherent source of incandescent white light—and then direct the beam through a medium containing a photoinitiator and a

*Continued on page 307*

Continued from page 305

polymer precursor. Because the polymerization rate is intensity dependent, and the refractive index of the polymer being formed is high, light migrates away from the center, but not too far away—the confining effect of the polymer keeps the dark core region narrow. As a result, a channel forms, impenetrable by light that can be observed relative to the bright background by optical microscopy. Experiments with smaller ratios of the background beam to the central depression bolstered the authors' posited mechanism. — JSY

*J. Am. Chem. Soc.* **134**, 14195 (2012).

## PLANT SCIENCE

## Salty Roots, Stunted Roots

Too much salt is as bad for plants as it is for us. For plants, salt stress and drought stress go hand in hand. Some of the more rapid responses to salt stress are signaled through actions of the sucrose nonfermenting-related kinase 2 (*snrk2*) gene family. Members of this



gene family fall into three groups, depending on their response to the hormone ABA. Studying *Arabidopsis*, McLoughlin *et al.* analyzed the specific contributions of two members of this gene family to salt stress. *snrk2.4* and *snrk2.10*, which encode proteins that are not particularly responsive to ABA signaling, responded within minutes to salt stress but delivered different functions. SnRK2.10 seemed to primarily sustain the emergence of lateral roots in excessively salty conditions, whereas SnRK2.4 had a more singular effect supporting primary root growth. After the initial, transient response, SnRK2.4 relocalized into punctate subcellular structures, which suggests that mechanical stress triggered through changes in osmotic pressure also signal subcellular relocalization of these rapid-response kinases. — PJH

*Plant J.* 10.1111/j.1365-3113X.2012.05089.x (2012).

## CELL BIOLOGY

## Getting Pulled into a Membrane

When proteins are translocated across the endoplasmic reticulum or bacterial membrane, they

pass through a proteinaceous tunnel, the translocon. However, transmembrane proteins need to slip sideways out of the translocon to become embedded in the lipid bilayer. The recognition of transmembrane protein helices by the translocon is a poorly understood process, and competing models have been proposed. One model is a thermodynamic partitioning model for membrane insertion, in which hydrophobic segments in a nascent polypeptide partition between the translocon channel and the surrounding lipid during their passage through the translocon. By using the bacterial SecM or the mammalian Xbp1 translation arrest peptides as in vivo force sensors, Ismail *et al.* found that a transmembrane helix is subjected to a strong biphasic “pulling force” at the precise moment that it enters the translocon. The pulling force was seen only for peptide segments with hydrophobicity above the threshold for membrane insertion and increased in proportion to the hydrophobicity of the segment. The biphasic force may reflect when the transmembrane segment interacts with and then partitions from the translocon into the membrane. — SMH

*Nat. Struct. Mol. Biol.* 10.1038/nsmb.2376 (2012).

## CELL SIGNALING

## Testing the Signal

In many experiments, cell signaling events are measured in cells given a strong and constant stimulus. Though this has been useful in delineating signaling pathways, cells may often receive signals that are more subtle and dynamic. Tomida *et al.* explored how a common signaling enzyme, the protein kinase known as MAPK-1 (mitogen-activated protein kinase-1), responds to variable input in a sensory neuron of the worm *Caenorhabditis elegans*. They kept living worms in a microfluidic chamber in which they could accurately control exposure of the animals to changes in salt concentration of varied magnitude and duration. They then monitored activity of the enzyme over time within a single neuron by monitoring a synthetic substrate expressed in the cell that gave a fluorescent signal when phosphorylated. Constant stimulation of the neuron did not activate MAPK-1 much. The strongest response came from pulses of stimulation at a moderate frequency—about 20 s of stimulus followed by a rest of the same duration. The authors propose that this pattern of response might make sense in a signaling system that should be inactive in a constant environment, but activated in response to changes, while at the same time filtering out noise. — LBR

*Sci. Signal.* **5**, ra76 (2012).

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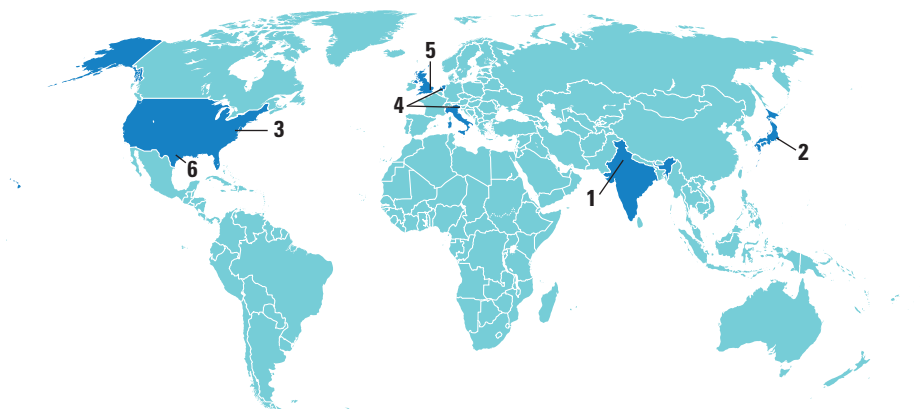
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## AROUND THE WORLD



New Delhi 1

### Advisory Panel Defends GM Research

A report released on 9 October by the Indian prime minister's Scientific Advisory Council (SAC) makes a strong pitch for wider acceptance of genetic engineering and biotechnology. The report from the 32-member SAC panel describes genetic modification as a transformational technology that has benefited agriculture and health. The endorsement differs sharply from the conclusion of a parliamentary panel on agriculture, which gave a thumbs-down to genetically modified (GM) technology this summer.

instill confidence in the GM crop regulatory process. A bill to establish such an authority is before the Parliament.

[http://scim.ag/GMres\\_India](http://scim.ag/GMres_India)

Tokyo 2

### Pioneering Stem Cell Research Called Into Question

A Japanese researcher's claim to have performed a groundbreaking stem cell experiment was quickly called into question by Harvard Medical School in Boston and the University of Tokyo, the institutions that supposedly supported the work. The unusual case of Hisashi Moriguchi blew up last

week when Moriguchi displayed a poster at a New York stem cell conference on 10 October that reportedly trumpeted the transplant of induced pluripotent stem (iPS) cells into heart patients.

Harvard asserted that Moriguchi had been a visiting fellow there for a month in 1999 but had not had any affiliation with the institution since. A spokesperson for the University of Tokyo Hospital confirmed that Moriguchi is on staff as a project researcher, but emphasized that the work apparently reported in New York was not carried out in its labs.

Meanwhile, Moriguchi has listed a secondary affiliation with Harvard on a number of published papers and correspondence over the years. The journals in question are investigating. The University of Tokyo and Tokyo Medical and Dental University have also launched inquiries into the iPS claims, as well as other work described by Moriguchi.

Follow the coverage of this fast-moving story on *ScienceInsider*.

<http://scim.ag/Moriguchi>



A brinjal field.

Opinion is heavily divided on the use of agricultural GM technology in India. Ten years ago, India adopted Bt cotton, which uses modified bacterial genes to control pests. But in 2010, the government blocked similar technology in Bt brinjal, a type of eggplant.

The SAC report also calls for establishing an independent watchdog called the Biotechnology Regulatory Authority of India, housed outside the ministry of science, to



Washington, D.C. 3

### Protections for Whole Genome Data

A report released last week by the U.S. Presidential Commission for the Study of Bioethical Issues makes 12 recommendations for protecting the privacy of patients' whole genome data while allowing it to be used in research.

The 150-page report finds that with prices heading toward \$1000 per genome, whole genome sequencing will soon be widely available. To protect privacy, there should be "clear policies" defining who can access and use whole genome data. Federal and state governments should establish a "consistent floor" of protections that penalize those who sequence someone's DNA without his or her consent. Patients and volunteers in research studies need to be informed about how their data might be used and should know that unexpected results may be discovered.

"This is a proactive and it's a forward-looking report. It's not a response to a crisis. But the commission understands that if this issue is left unaddressed, we could all feel the effects," said the panel's chair, University of Pennsylvania President Amy Gutmann.

<http://scim.ag/genomeprotect>

Amsterdam and Trieste, Italy 4

### A Guide to Responsible Research

The InterAcademy Council (IAC) and IAP—the global network of science academies—have put forward a common set of fundamental values and practices in an effort to promote responsible conduct among researchers around the world.

In a policy report titled *Responsible Conduct in the Global Research Enterprise* released on 17 October, IAC and IAP identified seven qualities—honesty, fairness, objectivity, reliability, skepticism, accountability, and openness—as universal scientific values.

The 62-page document offers researchers guidelines on topics as wide-ranging as social implications of dual-use research and initiating an international collaboration. "You have to agree ahead of time who is on

the paper and who is the first author, because there are cultural differences,” says Ernst-Ludwig Winnacker, co-chair of the report’s international authoring committee and secretary general of the Human Frontier Science Program in Strasbourg, France. The report also offers recommendations to research institutions, funding agencies, scientific journals, and national academies.

Science today is very global, “and, therefore, the mechanisms of how to deal with misconduct should also be global,” Winnacker says.

London 5

## GSK Announces New Data Sharing Policy

GlaxoSmithKline (GSK) last week announced what amounts to a glasnost policy, taking down high walls that until now have



prevented outsiders from closely examining results of individual patients who have taken part in clinical trials run by the pharmaceutical giant. Company CEO Andrew Witty, who made the announcement at an 11 October meeting

at the Wellcome Trust in London, also said GSK will make public details of more than 200 “hits” for possible tuberculosis drugs that their researchers discovered by screening its library of some 2 million compounds. For a long time, Witty said, there was “a mistaken judgment that actually by being more open and more transparent around data somehow that would destroy the fundamental business model.” Michael Merson, head of the Duke Global Health Institute in Durham, North Carolina, says GSK’s new initiatives “provide an excellent example of how pharma can help find solutions to health problems that particularly affect the world’s poorest populations.”

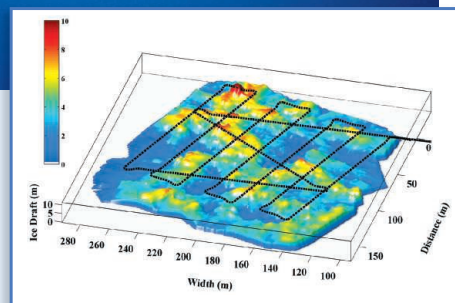
Austin 6

## Texas Cancer Research Agency Loses Peer Reviewers

The \$3 billion Cancer Prevention and Research Institute of Texas (CPRIT) has been shaken by the resignation of its eight-member scientific review council over concerns about the integrity of the agency’s peer review process.

## The View From Beneath

Twenty meters beneath the surface of the Southern Ocean off the coast of East Antarctica, a free-swimming robot peers upward at sea ice floes and assesses their bulk. Most satellite data reveal changes to the area but not to the thickness of the ice, making it difficult to gauge the full impact of global climate change on this frozen environment. Now, this autonomous underwater vehicle (AUV) has produced the first 3D map of the underside of an East Antarctic sea ice floe, revealing an upside-down landscape reminiscent of mountains, lakes, and valleys. Researchers mounted the AUV’s multibeam sonar, normally used to map the seafloor, on top of the vehicle rather than on the bottom, yielding a map of the base of the ice. The AUV’s trek is part of the 7-week Sea Ice Physics and Ecosystem eXperiment II, an international project coordinated by the Australian Antarctic Division and the Antarctic Climate and Ecosystems Cooperative Research Centre.



Approved by Texas voters in 2007, CPRIT has disbursed hundreds of millions of dollars in peer-reviewed research grants and recruited about 50 scientists to Texas. But CPRIT Chief Scientific Officer Alfred Gilman, a Nobel Prize-winning biologist, is stepping down over concerns that the CPRIT board delayed a slate of grants intended mostly for his former institution and approved an \$18 million “incubator” grant without scientific peer review.

Now CPRIT’s scientific review council and many of its more than 100 peer reviewers are following Gilman out the door. “Nothing has changed since last spring” when questions regarding CPRIT’s review process first arose, said Phillip Sharp, who resigned as council chair last week and is a Nobel laureate at the Massachusetts Institute of Technology in Cambridge. CPRIT said in a statement that it is “no surprise” that some reviewers are leaving along with Gilman.

The agency says it has found several candidates to replace the chief scientific officer. <http://scim.ag/Texaspeer>

## NEWSMAKERS

### Biomedical Research Loses Senate Champion

The biomedical research community is mourning the loss of former Pennsylvania Senator **Arlen Specter**, a loyal supporter of the National Institutes of Health (NIH) throughout his 30 years in Congress.

Specter died on 14 October from complications of cancer at age 82.

A moderate Republican who switched to the Democratic Party in 2009, Specter helped lead efforts to double the NIH >>





## Random Sample

## Dance Your Ph.D.: Burlesque Routine Takes Top Honors

Peter Liddicoat, a materials scientist at the University of Sydney in Australia, admits to being shy, “more comfortable hiding behind the computer monitor.” So when his labmates urged him to take part in the “Dance Your Ph.D.” contest, he was reluctant. But he finally caved in to the pressure. “A turning point was my boss’s enthusiastic laughter when encouraging me to do it,” Liddicoat says, “and the realization that this would tackle head-on the ominous question, ‘So what is your Ph.D. about?’ ”

That is no easy task with a Ph.D. titled “Evolution of nanostructural architecture in 7000 series aluminium alloys during strengthening by age-hardening and severe plastic deformation.” But after 6 months of preparation, and the help of dozens of friends, he turned his Ph.D. into

a burlesque. For using juggling, clowning, and a big dance number—representing the crystal lattices that he studies—Liddicoat is the winner of the 2012 Dance Your Ph.D. contest. Visit *Science*’s Web site to see his video for yourself, as well as the other finalists in categories including physics, chemistry, biology, and social science. <http://scim.ag/DancePhD2012>



## &gt;&gt;NEWSMAKERS

budget from 1998 to 2003. He almost single-handedly added \$10 billion in stimulus funding for NIH to the 2009 Recovery Act and sponsored legislation expanding federal funding for research on human embryonic stem cells. Specter also proposed the Cures Acceleration Network, an NIH program aimed at speeding drug development, created in 2010.

Specter’s personal battle with illness fueled his passion for NIH. He had a brain tumor removed, underwent bypass heart surgery, and was treated for Hodgkin’s lymphoma in 2005 and 2008. This past summer, he was diagnosed with non-Hodgkin’s lymphoma.

NIH Director Francis Collins called Specter “a towering champion for biomedical research and the mission of the [NIH]” and said: “I truly miss Arlen’s steady hand and vision for our agency.”

[http://scim.ag/\\_Specter](http://scim.ag/_Specter)

## Climate Change a Priority For World Bank

Dealing with climate change will be one of the World Bank’s priorities, said **Jim Yong Kim**, the first scientist to head the organization, in an 11 October press conference in Tokyo. “Since becoming president of the World Bank,



I have looked deeply into the data on climate change, and I have to say I was surprised that even in the last 6 months to a year, the data has become ever more frightening,” he said. “As a scientist, I feel a moral responsibility to be very clear in communicating the dangers of climate change.”

Kim, a public health specialist with a long track record of involvement in developing countries and the former president of Dartmouth College, took office on 1 July. He added that he wants to go beyond painting a “doomsday picture.” Encouraging companies and countries to understand that developing new technologies and approaches to combat climate change can lead to economic growth is vital, he says.

<http://scim.ag/bankclimate>

## FINDINGS

## Ancient Canals Transported Building Blocks to Angkor Wat

Scientists have long known that the sandstone blocks used to build the famous Angkor Wat temple in the ancient Cambodian city of Angkor came from quarries at the foot of a sacred mountain nearby. But how did the 5 million to 10 million blocks, some weighing more than 1500 kilograms, reach Angkor? Researchers report in a paper in press at the *Journal*

## Science LIVE

Join us on Thursday, 25 October, at 3 p.m. EDT for a live chat on **Neandertal intelligence**. <http://scim.ag/science-live>

of *Archaeological Science* that when they examined Google Earth maps of the area, they saw lines that looked like a transportation network. Field surveys revealed a series of canals, connected by short stretches of road and river, that lead from the quarries straight to Angkor. The roads and canals—some of which still hold water—would’ve carried blocks on their 37-kilometer journey to the budding temple. Researchers don’t know whether the blocks floated down the canals on rafts or via some other method. Scholars had previously assumed ancient builders floated blocks down a canal to the Tonle Sap Lake and then upstream on the Siem Reap River, a 90-kilometer-long route. <http://scim.ag/AngkorCanals>



CREDITS (TOP TO BOTTOM): PETER LIDDICOAT (2); ISTOCKPHOTO.COM; WIKIMEDIA COMMONS

## NOBEL PRIZE IN CHEMISTRY

# Receptor Scientists to Receive Chemistry Nobel

Whether it's feeling that rush at the first sight of dawn, salivating at the smell of an apple pie in the oven, or even fighting a spark of fear at an unknown sound in the forest, our bodies have an impressive ability to sense their surroundings and instantly respond. In all these cases and myriad others, a diverse family of proteins on the surface of cells translates those external stimuli—whether light, odor molecules, or hormones that prompt the fight-or-flight response—to changes inside our cells. Last week, two Americans—Robert Lefkowitz of Duke University in Durham, North Carolina, and Brian Kobilka of Stanford University School of Medicine in Palo Alto, California—won the Nobel Prize in chemistry for tracking down these proteins and revealing the atomic details of how they work.

The award is the seventh chemistry Nobel since 2002 to recognize advances in the biological side of the field. Lefkowitz and Kobilka, in fact, both trained as medical doctors rather than as Ph.D. chemists. This shift of the awards toward biology reflects the versatility of modern chemistry, Bassam Shkhashiri, the president of the American Chemical Society, argued in a blog post last week. "Chemistry permeates all the sciences," Shkhashiri wrote. "Its importance is so all-pervasive that chemistry not only has broken down traditional barriers in science, but even risks losing its own identity."

The selection of Lefkowitz and Kobilka was "a shrewd and wise choice," says Richard Henderson, a structural biologist at the MRC Laboratory of Molecular Biology in Cambridge, U.K. Charles "Chuck" Sanders, a structural biologist at Vanderbilt University in Nashville, agrees. "There have been a lot of people that have contributed to the advances in this field," Sanders says. "But these two stand out. This is an extremely well-

deserved award for them."

Early researchers had no idea that a single family of proteins might govern so many different physiological processes. One hint came at the end of the 19th century, when scientists studying the effects of the hormone adrenaline discovered that it had different effects in various parts of the body. It increased heart rate and blood pressure, but it decreased digestive activity and caused pupils to relax and dilate. Some biologists proposed that proteins called receptors on different cells somehow captured adrenaline molecules and either ferried the hormone into cells or transferred a message inside to

track adrenaline itself and eventually map out nine different subtypes of adrenergic receptors, known as  $\alpha$  and  $\beta$  ARs.

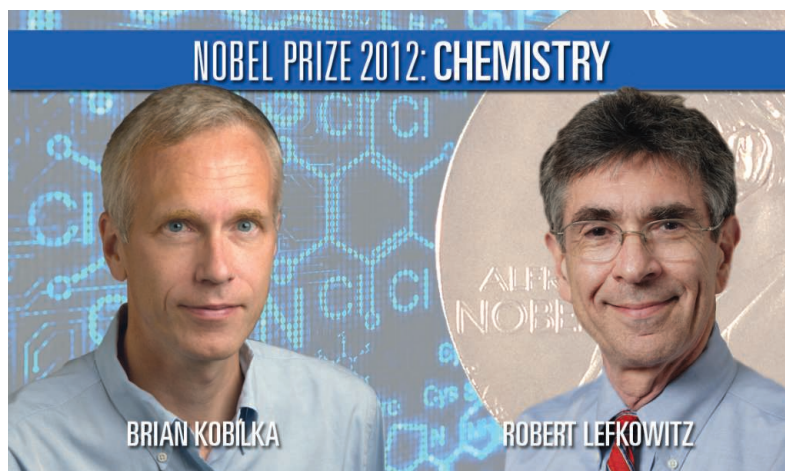
In the 1980s, Lefkowitz set out to identify the gene for the  $\beta$  AR. After a student purified bits of the receptor and teased out a few fragments of its gene, Kobilka, who had joined Lefkowitz's lab, used them to fish out the full gene. The group quickly noticed that the gene showed marked similarities to one for rhodopsin, the light receptor in the retina of the eye. Each receptor had seven rodlike regions that snaked through the cell membrane and coupled to so-called G-proteins inside the cells. By this time, other groups had discovered some 30 other receptors that work with G-proteins, which transmit signals inside cells and were the subject of the 1994 Nobel Prize in physiology or medicine. And in what Lefkowitz later described as a "real eureka moment," he, Kobilka, and the rest of the group concluded that there was likely an entire family of receptors that

look alike and function in the same manner. Today, some 800 GPCRs, also known as seven-transmembrane receptors, have been identified.

After completing his work at Duke, Kobilka took an appointment at Stanford University and set out to capture a three-dimensional picture of the  $\beta$  AR. Using the receptor's gene, the group produced millions of copies of individual GPCRs and coaxed them into crystallizing into a tiny solid shard. They determined the atomic structure of the protein by firing a tight beam of

x-rays at the crystal and charting exactly how the atoms in the crystal caused the x-rays to ricochet off.

But the size and flexibility of GPCRs made it impossible for the group to get a sharp image. Kobilka's team needed ways to stabilize the floppy receptor—primarily the portion of the proteins that protruded outside the cells—as well as the G-protein binding site on the inside. Progress was painfully slow. Kobilka even lost his funding from the Howard Hughes Medical Institute when results sputtered. But by 2007, Kobilka and his colleagues had devised enough tricks for stabilizing the molecule to come up with the first crystal structure of the inac-



**G-whizzes.** Lefkowitz (right) and Kobilka discovered how cell receptors register external stimuli, a discovery with many applications in medicine.

trigger a response. In the 1940s, an American biologist named Raymond Ahlquist inferred that there must be two types of adrenaline receptors: one that causes smooth muscle cells to contract, and another that stimulates the heart.

Lefkowitz picked up the trail in the early 1960s. Then a young cardiology student at the National Institutes of Health in Bethesda, Maryland, Lefkowitz was working with adrenocorticotrophic hormone, which stimulates the production of adrenaline in the adrenal gland. He modified the hormone, tagging it with radioactive iodine, and then tracked its binding to adrenal membrane material. He and others later used the same strategy to



tive GPCR. And in what other experts call a tour de force, in 2011 the group reported in *Nature* that it had obtained a high-resolution structure of the GPCR in which the external domain was being activated by a receptor stimulator at the same time as the internal region was bound to a G-protein. The structures revealed in glorious detail that when a hormone or another trigger molecule binds to the receptor, this causes the external por-

tions of the seven-transmembrane rods to pinch together, forcing the opposite ends of those rods apart. The spreading creates a cleft in the portion of the protein inside the cell, allowing a G-protein to bind, which in turn triggers a cascade of intracellular reactions. "These proteins evolved to be very efficient at detecting small things on the outside of cells and making a big change on the inside of a cell," Kobilka says. "Once that

challenge was overcome in evolution, it was used over and over again."

That has made GPCRs one of the most versatile families of proteins, as well as one of the most medically important. Today, compounds that target GPCRs make up 30% to 50% of all drugs on the market and remain one of the hottest areas of research in medicine and chemistry.

—ROBERT F. SERVICE

## NOBEL MEMORIAL PRIZE IN ECONOMIC SCIENCES

# Economics Nobel Honors Matchmaking Finesse

How do you make a perfect match—between doctors and hospitals, between schools and students, or even between kidneys and patients? Lloyd Shapley and Alvin Roth have won the 2012 Nobel Prize in economics for helping to answer that question. Shapley, 89, is a professor emeritus at the University of California, Los Angeles, and Roth, 60, is a professor at Harvard University and Harvard Business School in Boston.

The Nobel committee awarded the prize to the two economists "for the theory of stable allocations and the practice of market design." Shapley pioneered theoretical concepts to understand and solve the matching problem; Roth clarified those ideas and applied them to engineer algorithms that are now widely used in the real world.

Working in the 1950s and 1960s, Shapley used cooperative game theory to explore matching. Trade between a group of rational actors, Shapley reasoned, would reach a stable state if no individual had anything further to gain by making a new trade. Along with the late economist and mathematician David Gale, Shapley applied this idea of stability to the case of marriages, setting up an example of pairing 10 women and 10 men into opposite-sex couples.

The two came up with a matchmaking method called the "deferred acceptance" algorithm, which could proceed in either of two ways. In one scenario, the men propose to the women, and each woman rejects the men she finds unsuitable while holding on to—but not yet accepting—the proposal she likes best. In the next round, the rejected men propose to their second-best choice,

and the women again retain or choose what they view as the best offer while rejecting the rest. Over several rounds, the pairings become stable.

In the other scenario, the women propose to the men. The result is a different set of pairings, also stable. Although stable matches emerge no matter who does the choosing, Gale and Shapley showed that women end up with a more favorable outcome when they propose to men, and vice versa.

In the 1980s, Roth explored matching between medical interns and hospitals and showed that the algorithm then in use by

of the Toronto, Canada-based company National Matching Services Inc.

Roth went on to apply the Gale-Shapley algorithm to redesign the admission process used by public high schools in New York City. The new process has led to better matching between schools and students, reducing by 90% the number of students assigned to schools they didn't list as preferences.

The Gale-Shapley algorithm has also been applied, with important modifications, to matching kidneys with patients. New versions of the algorithm are also being used to conduct Internet auctions in which search

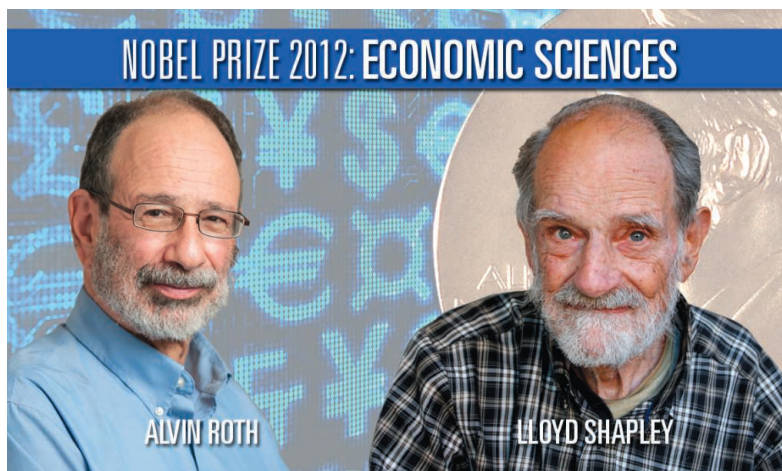
companies sell advertising space. The Nobel committee's announcement of the prize this morning describes the work being honored as "an outstanding example of economic engineering."

Roth's keenness to seek out real-world problems to solve makes him stand out among academic economists, says Peranson, who has been a personal friend of Roth for years and has watched him speak at university symposiums. "The Ph.D. students are very

enamored with Roth because when they hear him talk, they realize, 'Not only am I studying something that's interesting, but it could have real impact.'"

Roth, who is moving to Stanford University next year, is the author of a popular blog, "Market Design" (marketdesigner.blogspot.com). "Blog may be delayed today," he posted on Monday morning, shortly after getting that life-changing phone call from Stockholm. "Count me as surprised."

—YUDHIJIT BHATTACHARJEE



the National Resident Matching Program (NRMP)—a clearinghouse that matched residency applicants to hospitals—was successful because it followed principles similar to those encoded by the Gale-Shapley algorithm. In the mid-1990s, Roth was asked to improve on the NRMP algorithm to make matching more efficient and better at accommodating special needs such as placing dual-doctor couples in the same hospital. Roth delivered an improved algorithm, working with Elliott Peranson, the founder

## GENETICS

# New Company Pushes the Envelope On Pre-Conception Testing

A Princeton University geneticist and a former consultant whose son has a rare metabolic disease have formed a company to predict disease risk in hypothetical children based on the DNA of prospective parents. The company, aptly named GenePeeks, is the brainchild of Lee Silver, a molecular geneticist, and Anne Morriss, who used a sperm donor to conceive her child 6 years ago. Their goal is to help parents improve their chances of having healthy offspring. The company's plans, first outlined at the Consumer Genetics Conference in Boston on 3 October, are creating buzz among geneticists and others, who are questioning

This week, GenePeeks secured \$3 million in funding from venture capital groups.

Initially, the company will focus on at least 100,000 genetic variants linked to rare recessive and other single-locus diseases, according to Morriss and Silver. They hope to expand to sequencing exomes—the DNA that codes for proteins—and eventually entire genomes. In addition, they are keen to work with established couples, whom they expect will turn to in vitro fertilization and embryo testing to avoid a higher-than-comfortable disease risk.

For Silver, the idea dates back a couple of decades. In 1997, he published a popu-

ble “to come up with an estimate of likelihood of disease in the offspring from those two prospective parents.”

GenePeeks is prompting chatter in the genetic testing world. “I’m not against trying to introduce more screening,” says Arthur Caplan, a bioethicist at the University of Pennsylvania. But sequencing part or all of the genomes of sperm donors, and using them to capture risk of genetically complex diseases in offspring, comes with technical and ethical challenges. Caplan and others ask: Will donors be told about what’s uncovered in their DNA? Do we know enough about diseases like type 1 diabetes to predict a child’s risk based on genetics?

Silver is optimistic that the dozens of variants now linked to common childhood diseases can be used to formulate a good risk estimate, but many disagree. “The science really isn’t there to be all that predictive,”

says Lawrence Brody, chief of the Genome Technology Branch at the National Human Genome Research Institute in Bethesda, Maryland. Take diabetes, he says. Variants of human leukocyte antigen genes are known to show up often in children with the disease. But the same quirks pop up in healthy people, too. “We just don’t know enough to try and model risk” for most chronic diseases, says Mildred Cho, a bioethicist at Stanford University School of Medicine in Palo Alto, California.

Silver and Morriss are still sorting out which diseases to include in testing. “The initial approach is to focus on serious, life-changing, life-threatening childhood diseases,” Morriss says. This might include hundreds of rare recessive diseases, “because there’s no controversy” there, Silver says.

One benefit to screening sperm donors, Silver believes, is that individuals with mutations of unknown significance can be avoided if that seems prudent. Currently, classic genetic testing for many conditions, such as cystic fibrosis, doesn’t check for every possible mutation, because many haven’t been clinically validated. GenePeeks will determine what it considers an “unacceptably high” genetic risk and strike those donors from a recipient’s list.

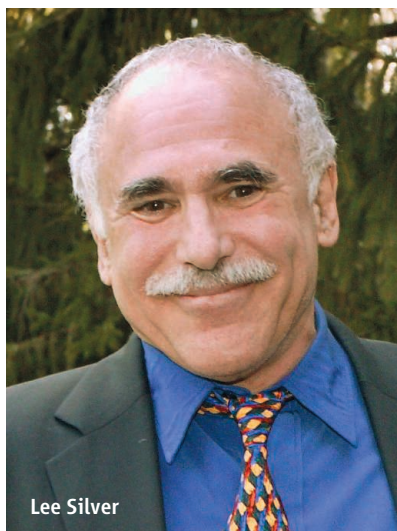
At its heart, the two say, GenePeeks is using Silver’s proprietary computational method, which tracks how genomes tend to recombine,



Anne Morriss

***“We will be identifying risk at the level of the potential child, ... not at the level of donor or client.”***

**—ANNE MORRISS AND LEE SILVER, GENEPEEKS**



Lee Silver

whether the science is up to the challenge and raising possible ethical concerns.

The pair aims to partner with sperm banks, which they say now offer little genetic testing of donors. Morriss, the company’s CEO, believes that expanded testing could help others avoid her family’s fate: Her son was born with MCAD deficiency, a rare recessive disorder in which both parents carry a disease mutation. MCAD deficiency is treatable with dietary modification, and Morriss says her son is thriving. Without treatment, children can suffer heart failure and death. GenePeeks plans to sequence the DNA of sperm donors using saliva or sperm, as well as DNA of potential recipients. They hope to give clients—the women who want to get pregnant—a stripped-down list of sperm donors who are genetically “safer” when matched with their particular genome.

lar science book, *Remaking Eden*, in which he described “virtual children”: embryos selected using preimplantation genetic diagnosis. Silver “entertains even the wildest and most speculative notions because—as he argues persuasively—the future is already here,” wrote *The New York Times* in a review.

Sperm and eventually egg donors are the perfect starting point for the venture, Morriss and Silver say, partly because it’s straightforward to steer clear of risky donors. The pair expects to launch GenePeeks in 6 months or so with a single sperm bank—a partnership, they say, is in the works—focusing on a thousand rare diseases like MCAD deficiency. Over time, they plan to expand to complex childhood diseases such as autism and type 1 diabetes. By examining thousands of “virtual progeny” from each donor-client pairing, Silver says, it’s possi-



to redefine genetic testing. “We will be identifying risk at the level of the potential child, ... not at the level of donor or client,” Morriss and Silver write in an e-mail. But they admit that identifying risk in a potential child might implicate a parent. If someone wants to know more, GenePeeks “will provide raw genotype data to donors or clients” upon request, “and we will encourage them to have it interpreted.” The company plans to consult with its lawyers to determine whether the informed consent already signed by sperm donors is enough to cover the deeper sequencing they plan to do. They also plan to partner with biomedical ethicists, although they decline to name anyone who might participate.

Standards now vary for gene testing of sperm and egg donors across the industry. California Cryobank, a large sperm bank headquartered in Los Angeles, follows guidelines of the American College of Medical Genetics and Genomics, which recommends that anyone planning a pregnancy be tested to see if they carry mutations for cystic fibrosis, spinal muscular atrophy, or eight other diseases in a panel associated with Ashkenazi Jewish ancestry. California Cryobank also performs additional genetic testing in certain circumstances. For example, if a female client knows she carries a mutation for a particular disease and hopes to use a donor, the bank may ask the donor if he is willing to be tested for carrier status for that disease as well.

“We probably coordinate about 100 requests like that a year” and always check back with the donor for additional informed consent, says Pamela Callum, a genetic counselor at California Cryobank. “We never want to turn around and say to a donor, ‘Hey, we tested you for this and we didn’t tell you.’” All donors are offered the chance to learn their genetic testing results.

Callum worries about ever-expanding gene testing panels, especially when the results may be tough to interpret. “It’s really hard to give consent” if the test provider says, “‘We’re going to look at all your DNA, [but] we don’t really know what it means.’”

With genetic technology advancing fast, a company like GenePeeks may be inevitable, some say, to help sperm- and egg-donor operations keep pace. But how far will the company go as its testing strategy develops? “Are they going to test for albinism, risk of deafness, risk of being short?” Caplan wonders. “Once you get into this, you’re quickly sitting face to face with the value question of what counts as a difference that should be classified as a disease, what counts as a difference that should be worth disclosing.”

—JENNIFER COUZIN-FRANKEL

## EVOLUTION

# Gene Duplication’s Role in Evolution Gets Richer, More Complex

In 1970, geneticist Susumu Ohno proposed a simple, yet elegant, idea: New genes arise when a hiccup during cell division produces an extra copy of an existing gene, and that spare copy is free to mutate and take on new functions. This mechanism, he argued, is the single most important factor in evolution. His book, *Evolution by Gene Duplication*, quickly became a classic that’s still cited today, 12 years after his death.

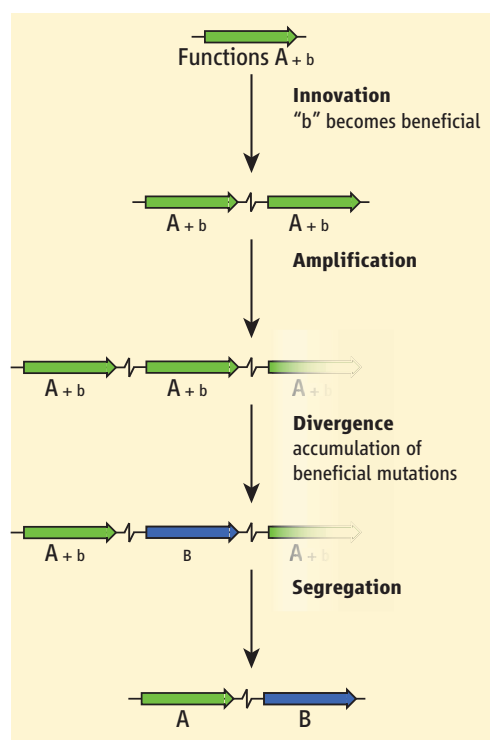
No one is casting aside Ohno’s tome, but some say it’s time for an update.

In 1932, about 20 years before scientists demonstrated the structure of DNA, evolutionary biologist J. B. S. Haldane suggested that gene duplications might be important to evolution. Ohno greatly elaborated on the idea. He proposed that mutations could accumulate in a complete copy of an existing gene because it would not be under natural selection to remain unchanged. In most cases, the extra gene eventually disintegrates, but sometimes the mutations would impart a new function on the copy’s protein product. If that function was beneficial, then natural selection would kick in to preserve that copy and its useful sequence.

In the late 1990s, Michael Lynch, an evolutionary biologist now at Indiana University, Bloomington, proposed that twinned genes would also both stick around if they divided the original gene’s work between them: Each might be expressed to a lesser degree or in different tissues, or code for distinct parts of the original protein. Several studies bore out his “subfunctionalization” theory.

But John Roth, a microbiologist at the University of California, Davis; Dan Andersson, a microbiologist at Uppsala University in Sweden; and their colleagues weren’t completely satisfied with those explanations. Given natural selection’s tendency to purge unnecessary genes, how would the gene copy stick around long enough to take on a new or subfunction? In 2007, this team proposed a different model for evolution by gene duplication, one in which selection prompted the temporary formation of gene copies.

In their scenario, the best candidate for gene duplication is a gene whose single protein product not only performed its primary job but also could carry out a secondary, nonessential function. If new conditions arose that made the secondary function crucial for survival, it would become advantageous for a duplicate gene, even multiple copies of the gene, to arise to meet the need for more of that protein. Once a mutation in one extra copy improved the resulting protein’s efficiency in performing this secondary function, that duplicate’s perpetuity would be guaranteed, and other extra copies



**Expanding the genome.** Selection on double-duty genes helps prompt gene duplication.

An experimental evolution study in bacteria, presented on page 384, shows that at least some genes take another route to giving an organism new functions. And other recent work has established that partial copies of genes—rather than complete duplications of genes or genomes, the focus of Ohno’s work—regularly become useful. All told, a growing body of research demonstrates that Ohno’s ideas were a little too simple. “In the past decade, we’ve realized there’s additional complexity” to evolution by gene duplication, says Richard Meisel, an evolutionary geneticist at Cornell University.

would disappear, they suggested. The original gene would continue carrying out the primary function.

Now Andersson, his postdoctoral fellow Joakim Näsval, Roth, and colleagues have documented this process in bacteria. Näsval first screened mutant *Salmonella* for one that could still make a little tryptophan even though the researchers had disabled the gene for an enzyme, called TrpF, normally needed for the amino acid's synthesis. Tests showed that this ability arose in *Salmonella* because its version of the gene, *HisA*, for making the amino acid histidine, encodes a protein that could also craft tryptophan from its precursors.

Näsval put this dual-function gene and a gene for yellow fluorescent protein into *Salmonella* bacteria lacking typical *HisA* and *TrpF* genes and grew them on media lacking both those amino acids—an environment that should select for microbes able to make both substances on their own. The intensity of the yellow fluorescent protein was a quick indicator of the increase in gene copy numbers.

At first, the bacteria grew slowly, tak-



ing 5 hours to double their population. But in several hundred generations, that doubling time plummeted to about 2 hours. Over the course of a year—and 3000 bacterial generations—Näsval periodically examined the genome of the microbes. He found that the single introduced copy of the dual-function *HisA* gene became amplified into multiple copies. And in some strains, one copy mutated to become much more efficient at making tryptophan and another excelled in making histidine, evidence of the evolutionary process Roth, Andersson, and their colleagues had proposed.

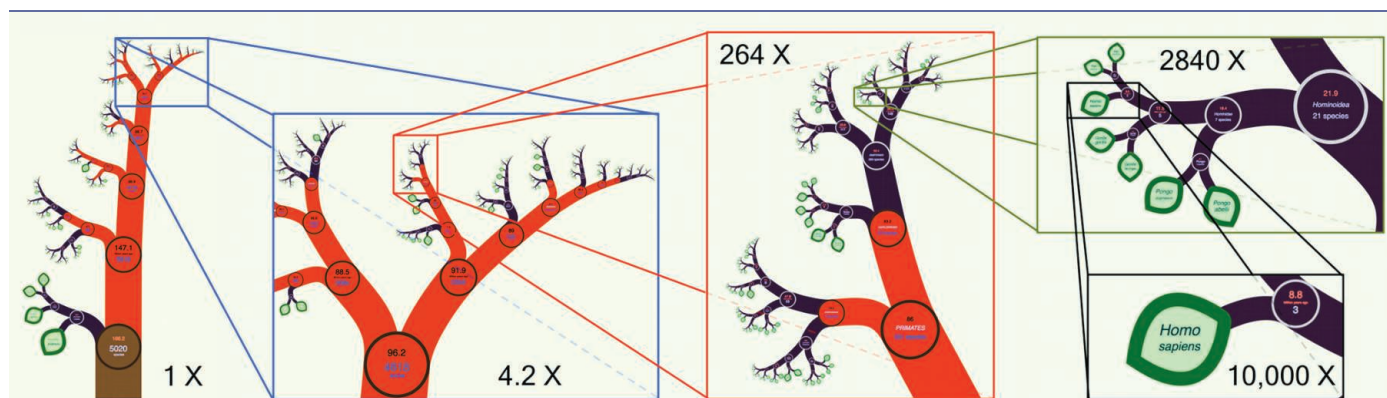
“Ohno will go down as a very important historical figure, but Andersson has the new model for how genes duplicate,” says Antony Dean, a microbial evolutionary biochemist at the University of Minnesota, Twin Cities. “His theory is square one.”

Other researchers have previously explored the idea that multifunctionality in

genes can promote duplication, Meisel says, but this “is a nice, elegant experimental system, and the model seems to be supported.” He cautions, however, that this process might be limited to bacteria and viruses. But Dean and Austin Hughes, an evolutionary biologist at the University of South Carolina, Columbia, suspect it's more common. No matter what, Näsval's experiment will encourage more experimental tests of gene-duplication scenarios, Hughes says.

In a review in the September *International Journal of Evolutionary Biology*, evolutionary geneticist Vaishali Katju of the University of New Mexico, Albuquerque, drew attention to another simplification by Ohno. She documents numerous cases in which partial duplications of a gene, some of which acquire additional DNA, perhaps during the copying process, have become key precursors to novel genes. The work “enriches Ohno's theory by showing that two gene copies need not be identical at birth,” says Jianzhi Zhang, an evolutionary geneticist at the University of Michigan, Ann Arbor. “Ohno would be pleased with these additions and modifications.”

—ELIZABETH PENNISI



## BIOINFORMATICS

### New Way to Look at Life

Ever since Charles Darwin, biologists have been building trees to show how organisms are related to one another. Now, computational biologist James Rosindell of Imperial College London and his colleagues have come up with a tree that outdoes all trees. Called OneZoom, the approach works like Google Maps: A user can drill down the tree's trunks, branches, and tips to view ever-finer details, and the structure can incorporate an infinite amount of information. (See [www.onezoom.org](http://www.onezoom.org).)

The concept is based on fractal geometry, in which patterns repeat themselves at different scales. Rosindell's open-source program embeds biological data—text, graphs, etc.—he and Luke Harmon of the University of Idaho in Moscow reported this week in *PLoS Biology*. “It seems to be a very elegant solution to a problem that's been widely recognized in evolutionary biology: how to have a tree of life that's visually appealing

and accurate,” says Richard Ree, an evolutionary biologist at The Field Museum in Chicago, Illinois.

Researchers can use the program to view their own phylogenetic data and can add information to the nodes, such as references, links to other Web sites, and even whether a species is endangered. OneZoom is for computers only, however. The simplest way to represent the bacteria, if printed out, would be 2000 kilometers long, Rosindell says. Over the next year, a large-scale project sponsored by the National Science Foundation called the Open Tree of Life plans to produce one tree encompassing 1.8 million named species. “Until I saw [OneZoom], I was apprehensive about how we were going to do this,” Ree says. But he thinks OneZoom may offer a solution.

—ELIZABETH PENNISI





# Reinventing the Pill: Male Birth Control

Researchers tinkering with cancer drugs and related molecules have found reversible ways of altering sperm; now they need to secure money and regulatory support for drug testing

IN THE LATE 1950s NEAR SALEM, OREGON, scientists started testing a birth control drug called WIN 18,446 in male prisoners. Men took responsibility for most birth control then, so a male contraceptive seemed a natural fit for American society. WIN 18,446 worked well, too: The prisoners felt fine and seemed quite healthy, except that their sperm was suddenly stunted and feeble. Unfortunately, when clinical trials shifted to the general population, men started getting sick—vomiting, sweating, headaches, blurry vision. They seemed poisoned. After some digging, scientists pinned down the culprit: alcohol. Because prisoners couldn't drink, no one had realized at first that WIN 18,446 did not mix well with liquor. The drug was abandoned, and 60 years later, no one has gotten any closer to the "male pill."

WIN 18,446 is a perfect example of why creating the male pill is so hard. It did exactly what it was supposed to do—stopped sperm production in everyone who took it—and it was reversible. Sperm levels returned to normal after men stopped taking it. Yet it failed anyway as a drug because of an arguably minor side effect.

Male contraceptives are held to high standards partly because the calculus for male and female birth control is different. For example, taking the female pill increases a woman's chances of develop-

ing blood clots. But because pregnancy increases the chances of blood clots by 10 times more, the pill's side effects seem worth the risk. With men, there's no counterbalancing risk of pregnancy, so the tolerance for side effects drops to zero. That's especially true because "you're dealing with healthy people, not people with an illness, and you'd have to use [the pill] for long, long periods," says Diana Blithe, a program director at the U.S. National Institute of Child Health and Human Development (NICHD) in Bethesda, Maryland, which funds research into male contraception.

Drug companies have all but abandoned the male contraceptive field in the past decade. After acquiring smaller companies, for instance, both Bayer and Merck shut down those programs. (Bayer refused to say why, and a Merck spokesperson said only, "It is not a priority area.") In addition to the medical challenges like the high safety standard, Blithe points out one other obstacle for companies: The U.S. Food and Drug Administration (FDA) has no guidelines about what levels of safety and efficacy the male pill would need to have to win approval. FDA declined to comment about whether it planned to develop guidelines or whether it has even discussed doing so.

Over the past half-century, contraception has become largely a female issue—

**Zeroing in.** James Bradner (left), Jun Qi, and others are working on contraceptives that, unlike old compounds, do not target male hormones.

and drugs for women work relatively well, making a male pill seem less urgent. But intrauterine devices require a medical provider to insert, can have serious complications, and cost up to \$1000. And not all women can tolerate the far more popular female pill, says Lawrence Finer, director of domestic research at the Guttmacher Institute in New York City, which specializes in reproductive issues. Because half of all U.S. pregnancies today are unplanned, he says, there's clearly a need "to increase our contraceptive repertoire."

With so little private support for a male pill, Blithe's program focuses as much on getting products to market as on basic biology. She will host a closed meeting in November in Houston, Texas, for about 30 top biologists to swap ideas about new genetic and biochemical leads for male contraceptives. The most promising leads involve disrupting the maturation of sperm in the testes, and although funds are tight, clinical trials for a few approaches could begin within the next few years. These researchers hope they can outsmart nature, but they know that producing an effective, safe, cheap, targeted, well-tolerated, bioavailable, easy-to-manufacture, side-effect-free, and (whew) completely reversible male pill won't be easy.

## Different targets

Plugs. Hormones. Special underwear. Auto-immune attacks. The "dry orgasm." There's no shortage of approaches to male contraception (see diagram, p. 319). But all share the same goal: slowing down the relentless proliferation of sperm in men and holding sperm counts to about 1 million per milliliter of ejaculate.

## Online

sciencemag.org

Podcast interview  
with Sam Kean  
([http://scim.ag/pod\\_6105](http://scim.ag/pod_6105)).

Even the reduced concentration "may sound like a lot," says John Amory, a doctor and reproductive biologist at the University of Washington, Seattle, "but that's a pretty good, effective contraceptive" that will reduce fertility by 99%.

Until recently, scientists modeled most male pills on the female pill and attempted to disrupt male hormones—testosterone above all. The biochemistry is convoluted, but artificially raising testosterone levels suppresses other hormones necessary to make mature sperm. This works wonderfully—sperm levels plummet—but it's a sledge-

hammer approach that affects tissues throughout the body, causing widespread side effects. There's no good way to administer testosterone, either. Oral testosterone breaks down so quickly in the body that men must take several pills a day to keep levels high, and alternatives like testosterone gels or injections are a hassle. Hormonal contraception doesn't work anyway in 10% to 20% of men, Amory says.

This strategy suffered its latest blow in April 2011, when a high-profile study led by the U.S. nonprofit group CONRAD and co-sponsored by the World Health Organization was called off early. The study had monitored more than 200 couples taking various hormones for more than a year, but side effects such as acne, weight gain, and mood changes convinced scientists that the therapy would fail in the marketplace.

Most current research into a male pill has shifted away from widely circulating hormones toward molecules specific to the testes. One promising approach being explored by Amory and others involves disrupting retinoic acid, one of a group of molecules known collectively as vitamin A. Like hormones, vitamin A plays a role in many different tissues, so scientists can't just shut down the pathway. But sperm are so sensitive to retinoic acid that reducing its levels even a little could prove effective.

Amory's research sprang from WIN 18,446, the failed prisoner drug. Because WIN 18,446 sickened people who drank, Amory suspected that it disrupted the breakdown of alcohols. Wine, beer, liquor, and other booze contain ethanol, which the body metabolizes into acetaldehyde. Because acetaldehyde is poisonous, an enzyme called ALDH converts it to acetic acid (vinegar, essentially). In a paper in the *Journal of Andrology* first published online in August 2010, Amory's team proved that WIN

attaches to ALDH and gums up the process, allowing acetaldehyde to accumulate.

Amory, though, saw the bright side. The testes also convert an alcohol (retinol, another form of vitamin A) into retinoic acid. During spermatogenesis, retinoic acid binds to the RAR protein, creating a complex that turns on genes necessary to convert precursor cells into mature sperm. The testes use a unique ALDH called ALDH1a2 in this conversion. So Amory's team tried to tweak WIN 18,446's molecular structure to make it lock onto ALDH1a2 only, and not onto the ALDH that prevents alcohol poisoning.

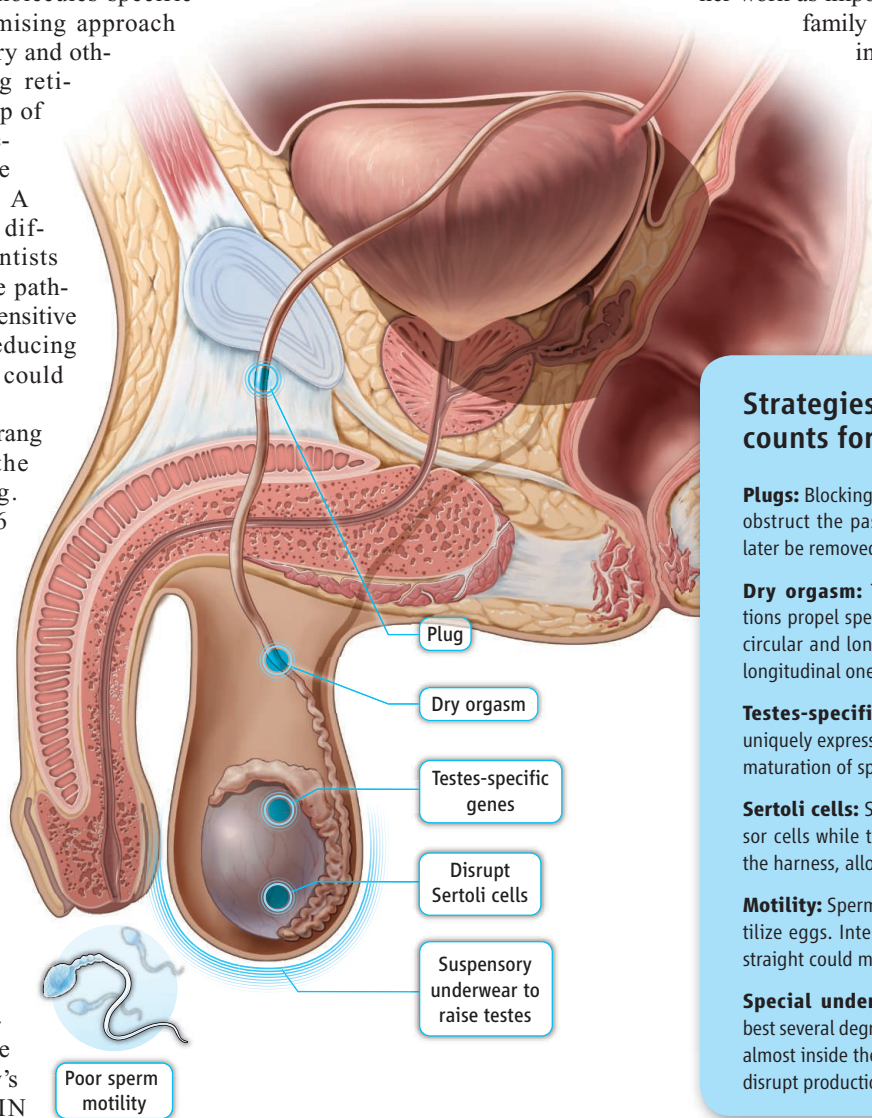
It didn't work. "We tweaked the hell out of WIN 18,446," he laughs. "Our poor organic chemist made 100 versions" in 2009 and 2010, but every one proved a dead end. Still, intrigued by the specificity of ALDH1a2, his team screened 60,000 other molecules and found seven more

that gummed up ALDH1a2. They're now tweaking those seven to make them testes-specific. Amory expects four or five of them to fall short in future tests—some might prove toxic or might not cross the blood-testis barrier, a tissue firewall that separates the testes from general blood circulation. But within 5 years, he hopes to approach FDA about clinical trials.

Debra Wolgemuth, a reproductive biologist at Columbia University Medical Center, is also targeting retinoic acid's role in sperm maturation. Through chemical screening, Wolgemuth's team identified a compound that prevents the binding of retinoic acid to RAR, so the downstream genes never get activated. Here, too, the effects are systemic: Retinoic acid binds to similar RAR proteins in other tissues, so Wolgemuth is working with chemists to tweak the drug and make it testes-specific.

Like many in her field, Wolgemuth sees her work as important not just for domestic family planning but also for keeping Earth's overall population at sustainable levels.

By some projections, Earth's population could top 10 billion by midcentury. She also says the research could help control animal populations around the world, an



### Strategies to lower sperm counts for birth control

**Plugs:** Blocking the vas deferens with plugs could obstruct the passage of sperm. The plugs could later be removed surgically or dissolved.

**Dry orgasm:** Two types of muscular contractions propel sperm forward in the vas deferens—circular and longitudinal. Certain drugs can halt longitudinal ones, pinching the tube shut.

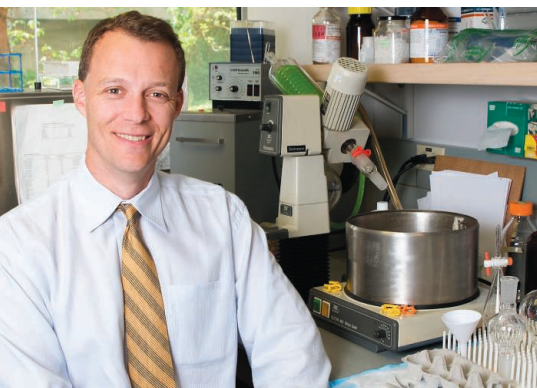
**Testes-specific genes:** Shutting down genes uniquely expressed in the testes could prevent the maturation of sperm.

**Sertoli cells:** Sertoli cells harness sperm precursor cells while they mature. Certain drugs break the harness, allowing sperm to escape early.

**Motility:** Sperm cells travel long distances to fertilize eggs. Interfering with their ability to swim straight could make the journey impossible.

**Special underwear:** Sperm production works best several degrees below 37°C. Raising the testes almost inside the body with tight underwear could disrupt production, as could external heat.





**New approaches.** Research teams led by John Amory (top) and Joseph Tash (bottom, front row) target testes-specific genes and molecules.

important application. She jokes that testing birth control in rodents gave her another idea as well. “We could use it for rats in the New York City subway,” she suggests.

Beyond retinoic acid pathways, there are hundreds of genes expressed only in the testes, and disturbing any one of them might disrupt the machinery of sperm production.

One potential monkey wrench came from James Bradner, a chemical biologist at the Dana-Farber Cancer Institute in Boston. While screening potential cancer drugs a few years ago, Bradner came across JQ1, which inhibits cancer cell division. It does so by interfering with bromodomain (BRD) proteins, which turn on a master regulatory gene that promotes cancer cell proliferation. As part of the screening process, Bradner explores how drugs behave in various tissues, and he found that JQ1 also inhibited a testes-specific BRD called BRDT. Not long before, Wolgemuth’s team had showed that knocking out BRDT made mice infertile, and research led by Douglas Carrell of the University of Utah in Salt Lake City showed that natural BRDT variations in men led to infertility. Bradner also knew from experience that many cancer drugs halt the proliferation of sperm, too. All signs suggested that JQ1 had potential as a contraceptive.

Because Bradner lacked experience in the field, he called Martin Matzuk, a reproductive biologist at Baylor College of Medicine in Houston. Within 3 weeks of trying JQ1 in mice, Matzuk knew he had something special: With BRDT shut down, spermatogenesis all but stopped about halfway through the maturation process, and the sperm that did squeak through couldn’t swim. Bradner and Matzuk reported these results in *Cell* in mid-August. Importantly, they also provided evidence that JQ1 didn’t affect hormone levels and that it was reversible, because 4 weeks after mice went off it, they could father as many offspring as controls.

Tissue targeting remains a challenge: To satisfy the demand for zero side effects, Bradner suspects the team will need to tweak JQ1 and make it perhaps 100-fold more specific for BRDT than other BRDs. As a hedge, they’re also screening for other BRDT inhibitors. JQ1 may go into clinical trials next year as a cancer therapy, though, so Bradner and Matzuk may get early feedback on its contraceptive potential.

Another compound inching toward human trials is H2-gamendazole, which is also a derivative of an old cancer drug. A team led by biologist Joseph Tash at the University of Kansas Medical Center in Kansas City determined that it disrupts Sertoli cells. In addition to providing support for sperm precursors, Sertoli cells bind the precursors in place with a sort of harness, keeping them in the testes until they mature. H2-gamendazole unravels the harness, and sperm drift off into the semen stream before they’re capable of fertilizing eggs.

Like other compounds, H2-gamendazole acts within weeks and is reversible. Its big advantage is that its target, the Sertoli cells, lie on the blood side of the blood-testis barrier. So unlike with other potential drugs, “one doesn’t have to worry about that tight firewall for compounds getting across,” Tash says.

Having completed many safety tests in rodents, Tash had hoped to start talking to FDA last year about clinical trials. But various bureaucratic delays hampered him. He had to focus on renewing his NICHD grant and also needed help from his university to hire consultants before approaching FDA. But for Tash, such delays are nothing new. He has pursued a male contraceptive since his student days in the late 1960s, when a stint in a Chicago hospital showed him how heavily the contraceptive burden fell on women. He laughs now, saying he never imagined back then that creating a male pill

would take so long: “The naiveté of youth had not met the reality of funding and of collaborative research.”

### Valley of death

Tash laments that his team has now entered the drug developer’s “valley of death,” the gulf between cheap early testing and exponentially more costly human trials.

The difficulty of bringing new drugs, especially contraceptives, to market influences how Blithe’s program awards money, she says: “Applications are peer-reviewed with product development in mind. You might hear ‘It’s too applied,’ or ‘That’s something a pharmaceutical company should do’ in normal grant reviews, but not here.” Grants also include money to hire contractors for tasks like toxicology work.

As for partnering with drug companies, most scientists say that companies urge them to keep them updated on any leads. But that’s different from a company committing money. Blithe suspects that some companies view contraceptives as a zero-sum game—that every dollar spent on male contraceptives would mean one less dollar spent on female contraceptives. But she believes many couples will actually double up on pills. Amory does, too. “When I mention what I do at parties, people say men aren’t interested in contraception,” he says. “But men were the ones responsible for most contraception before the 1960s” and still take responsibility for 30% of contraception today, he says, despite limited options. “Men are interested,” he insists.

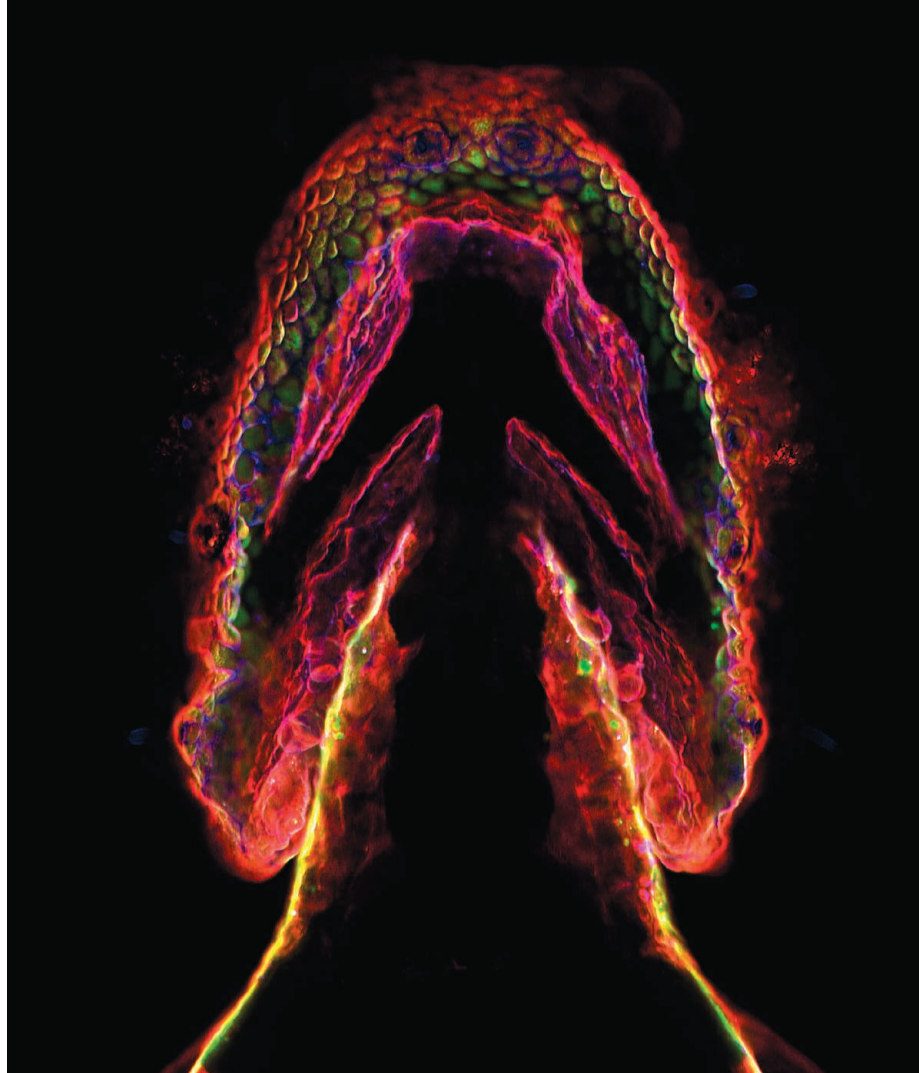
The NICHD meeting in November is closed to the public in part because discussing results openly could interfere with the ability to patent compounds. At the same time, both Blithe and Amory suspect that drug companies may hang back because of fears about litigation. Birth defects and infertility are common in the general population, and, statistically speaking, some couples who use the male pill will experience one or the other for reasons having nothing to do with the drug. But bad luck wouldn’t necessarily stop people from suing, Amory points out.

Still, the prize could be worth the risk. “Female oral contraception is one of the most important medicines ever developed,” Bradner says. The male counterpart could be in the same league worldwide, and the identification of testes-specific genes and enzymes makes scientists guardedly optimistic that the time for a safe, effective, targeted, and so on, male pill might be nigh. Blithe says: “To say it’s a year off or 5 years off isn’t accurate. [But] I suspect that if one gets out there, a lot more will follow.”

—SAM KEAN

CREDITS (TOP TO BOTTOM): CLARE MCLEAN/UNIVERSITY OF WASHINGTON; MEDICINE; ELISSA MONROE/UNIVERSITY OF KANSAS MEDICAL CENTER

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**Development aglow.** Recently produced glycans (red) light up the jaw of this zebrafish embryo, while older glycans (green) have migrated inside cells.

## CELL BIOLOGY

# Looking for a Sugar Rush

The sugar molecules that stud cell surfaces and coat many proteins play critical roles, but they are poorly understood and researchers want new tools to study them

If a cell biologist wants to investigate the details of, say, breast cancer, she can follow a well-trodden path. After comparing the DNA of people with and without the disease to identify genetic sequences associated with the cancer, she may scan genome databases to pinpoint a gene of interest. With the DNA sequence in hand, she can determine the amino acid structure of the protein made by the gene, isolate the molecule to study its biochemical activity in test tubes, and even tag it with a fluorescent compound and watch where it goes inside cells to learn clues about its function. She can delete the gene from mice to learn further clues, and even synthesize drugs to enhance or block its activity. And on and on.

If only life were that easy for researchers studying our cell's sugars.

Chains of these sugar molecules, called glycans, polysaccharides, or sometimes carbohydrates, are a primary class of biomolecules, arguably as important as the nucleic acids DNA and RNA, proteins, and lipids. They decorate the outer surface of nearly all cells, directing communication and interactions not only between our cells but with bacteria and viruses as well. They also coat many proteins and help orchestrate the way those molecules fold into their three-dimensional shapes and bind to their targets. Scientists have never had the tools to synthesize and alter glycans in the same systematic way they've been able to with DNA and proteins. That makes glycans one of the least understood classes of molecules in biology.

Now, glycoscience researchers say the field should make a big push to forge the suite of tools that they need to study their

quarry. Even without these tools, "the pace of progress in glycoscience has been really good" in recent years, says Laura Kiessling, a chemist at the University of Wisconsin, Madison. With an appreciation for the role of sugars rising rapidly among researchers and an influx of scientific talent into the field, she adds, "the time is ripe." That was also the conclusion of a report released in August by the National Research Council (NRC) of the U.S. National Academies, which argued that science funding agencies in the United States should focus their efforts to build tools to facilitate glycoscience research over the next decade, much as the U.S. Department of Energy and the National Institutes of Health have devoted considerable money to advancing DNA sequencing and analysis technologies.

If scientists can master the molecular language of sugars, that could have a major impact on research areas including the development of novel vaccines and the creation of more energy-efficient biofuels. Glycoscience researchers are already pursuing such goals. But David Walt, the chemist at Tufts University in Medford, Massachusetts, who chaired the NRC study, says that without the same kinds of tools available to researchers who study DNA and proteins, such projects are typically heroic scientific pursuits that can only be pursued by specialty labs. "We want glycoscience to be democratized broadly within science and not [be] just a specialized discipline," Walt says.

## Sweeter vaccines

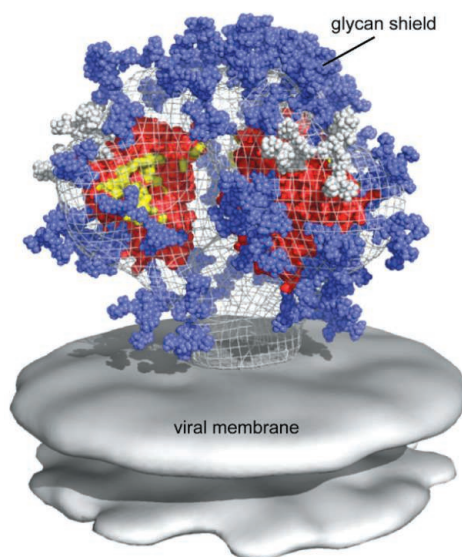
Glycans themselves certainly aren't relegated to a specialized domain. They are universal in living organisms and play a role in virtually all major human diseases. The molecules are the primary component of plant cell walls, where they are synthesized using the carbon dioxide that plants take in during respiration as raw material; as a result, plants serve as one of the biggest reservoirs for sopping up excess atmospheric carbon. Plant-based glycans can also provide a non-fossil source of fuel and novel materials. "People have recognized that glycoscience is important," Walt says. "But by and large, people have viewed it as very complex."

That complexity comes from the fact that glycans are put together in a manner very different from that of more familiar families of biomolecules. In nucleic acids and proteins, the individual chemical units—nucleotide



bases in the case of DNA and RNA, and amino acids in the case of proteins—are all linked by the same chemical bond. Once you master the making and breaking of that bond, you can synthesize virtually any nucleic acid or protein you want.

Not so with glycans, which are constructed from more than a dozen individual sugar units connected with different bonds by a diverse family of enzymes. That diversity generates five main families of glycans and a wide variety of branched and chainlike structures. Moreover, unlike proteins that are synthesized according to their genetic



**Targeting HIV.** Antibodies capable of inactivating the AIDS virus bind to bits of its glycan shield (blue) and the underlying coat protein.

blueprint, the structure of individual glycans is controlled only in part by the genes for those enzymes that link sugars together. Just as important, the architecture of a particular sugar chain also depends on the concentration of different enzymes in the cellular broth. This interplay between genetics and the local environment means that the glycans associated with a particular protein will vary according to the environment in which the protein was produced.

That variability has been a barrier to studying the cell's sugars. For example, biologists have typically probed the structure of a protein—and gained clues to its function—by crystallizing it and using x-rays to map the location of amino acids in the protein. However, because most proteins are decorated with glycans, and the glycans on separate copies of the same protein often differ, it's a tough challenge to make coherent crystals. So structural biologists routinely strip away the glycans. But that means they have

been getting an incomplete picture at best. Researchers are now getting better at keeping those components intact, in part by studying versions of proteins from insects, which typically have more uniform glycans.

The importance of this has been illustrated by work on the glycoproteins of the AIDS virus. Last year, Ian Wilson, a structural biologist at the Scripps Research Institute in San Diego, California, and colleagues from 11 institutions reported that they engineered insect cells to express the crucial outer portion of the HIV envelope protein gp120, which they used to obtain x-ray crystal structures of two anti-HIV antibodies that neutralize up to 72% of all HIV strains (*Science*, 25 November 2011, p. 1097). These structures preserved the glycans that coated gp120 and revealed that the best antibody binds to two bits of highly conserved glycans, while also piercing the sugar shield to bind to the underlying protein. “Now that we’re finding [binding] hotspots means we can use that to design some smaller molecules that can be used as an immune agent” and possibly a vaccine, Wilson says.

Another area of progress involves synthesizing glycans—a skill that may also have biomedical implications. Scientists can easily make almost any small protein and assemble increasingly long strands of RNA or DNA, but they are only slowly gaining the ability to make complex glycans. At the biannual American Chemical Society (ACS) meeting in August in Philadelphia, Pennsylvania, for example, Samuel Danishefsky, a synthetic organic chemist at the Memorial Sloan-Kettering Cancer Center in New York City and Columbia University, described how his team has developed a strategy to synthesize sugars associated with tumor cells in order to spark the immune system to fight cancer.

Researchers have known for years that many cancer cells undergo significant changes to their cell surface glycans. Some have tried to use those changes to identify molecular markers for various cancers to serve as diagnostics and as molecules that stimulate an immune response.

Getting the body's immune system to pay attention to a sugar is a challenge, however. Cell surface glycans, even when altered in cancer cells, are usually not seen as “foreign” by the immune system. So an early cancer vaccine approach was to synthesize a fragment of a common cancer cell surface glycan and link it to an immune-stimulating compound, such as a peptide or nanoparticle, as well as adding additives called adjuvants that can also spark an immune response to

the sugars. One candidate anticancer vaccine following this strategy targets a sugar known as globoH, and the vaccine is now in phase II and III clinical trials against breast cancer and phase I clinical trial against prostate cancer.

Early results have been promising, but different cells in a single tumor often express different cell surface glycans that serve as the “antigen” targets of antibodies. “It’s not likely cancer vaccines will get all the cells, since each vaccine goes against one antigen,” Danishefsky says. That’s why he and his group came up with the idea of synthesizing five known tumor-specific sugars, linking them together, and then attaching them to an immune-stimulating protein. At the ACS meeting, Danishefsky reported that early animal results look promising and that the vaccine has entered initial clinical trials against ovarian cancer.

But that’s only the beginning, Danishefsky says. His group has also begun to tackle complex sugars on prostate-specific membrane antigen (PSMA), a glycoprotein expressed on prostate cancer cells (and different from the well-known prostate-specific antigen, PSA). At the ACS meeting, Danishefsky reported that one of his postdoctoral assistants, Maciej Walczak, recently synthesized two different complex PSMA glycans, one made up of 14 sugars linked together, the other with 17 sugars. A major challenge for the project was that each pair of adjacent sugars in the molecule can bind in one of two different orientations. So Walczak had to control the binding direction at each point. He also broke the problem apart, initially synthesizing multiple pieces of the molecule and then linking them together into the final structures. Those compounds are now being readied as cancer vaccines for testing in animals.

Geert-Jan Boons of the University of Georgia in Athens is taking a more streamlined approach. Although he acknowledges that linking tumor-bound sugars to proteins can induce an immune response, Boons argues that in many cases the strongest response is against the carrier proteins and not the sugars. So he and his colleagues have focused their efforts on linking their sugar targets to a small peptide, known as MUC1, that triggers a strong immune reaction, but is also small enough that antibodies bind both to it as well as to its associated sugars, much as Wilson’s broadly neutralizing antibodies bind both to the HIV coat protein and the sugars on top. At the ACS meeting, Boons reported that his team’s slimmed-down vaccine using sugars from breast cancer cells

reduced tumor sizes by 80% in mouse models of breast cancer. Peng George Wang, a glycochemist at Georgia State University in Atlanta, says that both the slimmed-down and hefty vaccine approaches look promising for now, increasing optimism that at least one will pan out in the clinic.

### Fueling up on sugar

Even though scientists have identified many of the enzymes that assemble sugars in vivo, they are still sorting out exactly how they work and learning how to manipulate the synthesis of glycans in living animals or plants. Eventually, they hope to be able to control the way organisms produce glycans, in much the same way biologists have long used genetic engineering to alter the production of proteins. For example, at the ACS meeting, Markus Pauly, a chemist at the University of California (UC), Berkeley, reported that his group has been making strides in reengineering the way plants make their sugars, which may enable the creation of next-generation biofuels.

Today's primary biofuel, ethanol, is made by microbes that ferment the sugars in corn kernels and sugar cane. Both of those crops compete with food crops for agricultural land, however. Researchers have worked for decades to get those sugars instead from agricultural wastes, such as wood chips and corn stovers. But plant cell walls typically contain lignin and a form of sugars called hemicellulose that microbes that make ethanol can't handle.

Hoping to reengineer plants to produce less hemicelluloses and lignin, Pauly and his colleagues have started with the well-studied mustard *Arabidopsis thaliana*. Like most plants, this one makes several different sugar polymers, each of which contains a sugar backbone with extra side chain sugars dangling off. Pauly's group blocked the genes for the enzymes in the mustard plant that tack on those side chains and, not surprisingly, found that those plants grew poorly, if at all. They then introduced a gene from a tomato plant for an enzyme that tacks on a sugar called arabinose, which *Arabidopsis* doesn't normally use, to growing sugar backbones. The mustard plants grew normally. "It doesn't matter what sugar is [in the side chain] as long as there is a sugar," Pauly says. It's too

*"There is recognition that it is important that we apply the same kind of tools and innovation to glycoscience as areas of molecular biology that have already been revolutionized."*

—DAVID WALT,  
TUFTS UNIVERSITY

early to tell if this will make it possible to design plants with more sugars that microbes can ferment, Pauly says, but he adds that they're beginning such tests now.

Even as some research teams grow more adept at altering the glycans of living organisms, others are finding new ways to image sugars in and around cells—a key technology to showing the impact of any manipulation. In 2006, for example, Carolyn Bertozzi,

The group went on to use that technique to track how glycans change in zebrafish during embryonic development (*Science*, 2 May 2008, p. 664). And more recently, they've begun using the technique to track how bacteria use a combination of sugars and peptides, called peptidoglycans, to build their cell walls. Because peptidoglycans are a primary target of antibiotics, the new approach could lead to novel antibiotics against a host of infectious diseases. "We think that this will be a really nice method to monitor what happens when you block peptidoglycan synthesis," Bertozzi says.

### The road ahead

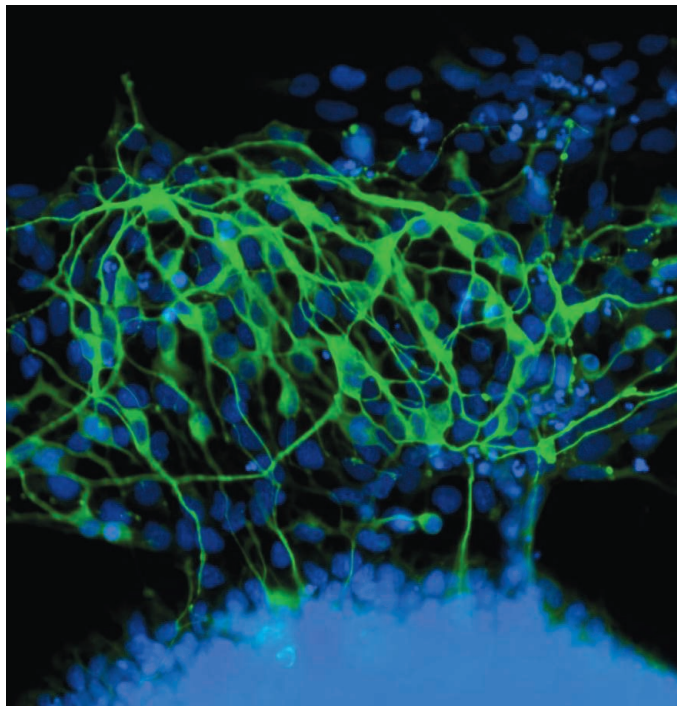
With strides being made across all these areas of glycoscience, Walt and others say the field now needs to focus on making it easier for other groups to reach the cutting edge. "There is recognition that it is important that we apply the same kind of tools and innovation to glycoscience as areas of molecular biology that have already been revolutionized," Walt says. "I think it's more difficult but completely tractable."

NRC outlined some specific recommendations to bring about this glycorevolution. It called on federal agencies to fund technology development programs to create techniques to better image, sequence, and synthesize glycans. As work like that from Danishefsky and Bertozzi shows, the cupboard isn't bare. But, Walt notes, individual advances are typically only applicable to a subset of glycans. He and the other authors of the report also suggest creating a centralized database of mammalian, plant, and microbial glycans, similar to the Protein Data Bank for proteins and GenBank for genes.

In this era of tight budgets, Walt says he doesn't know where the money will come from to

build the needed foundation for glycoscience. But perhaps new money isn't needed: He hopes that the NRC report will help science funding agencies focus their efforts on particular projects and coordinate their work. If so, Walt suggests, in a decade or so, scientists may be ready to launch a human glycome project and pull back the curtain on the role of sugars in biology just as they have done with genes.

—ROBERT F. SERVICE



**Seeing stem cells.** Fluorescent blue compounds attach to glycans on pluripotent stem cells, as they differentiate to form nerve cells (green).

a chemist at UC Berkeley, and her colleagues developed a two-step technique to image glycans in living organisms. They created sugar building blocks containing a chemical group called an azide that's not found in organisms. They then created fluorescent probes containing other compounds that seek out and bind solely to azides. That way, when the modified sugars are incorporated in glycans, Bertozzi's group could image where those glycans ended up.



## NUCLEAR PHYSICS

# Primordial Matter Comes Into Focus in Many Tiny Big Bangs

A standard model is emerging of the quark-gluon plasma produced in collisions of heavy nuclei. But will a key atom smasher run long enough to flesh it out?

**WASHINGTON, D.C.**—It's a scientific sea change that rolled in without a decisive measurement or cry of "Eureka!" Twelve years ago, physicists first used an atom smasher to reproduce an exotic form of nuclear matter known as the quark-gluon plasma, which filled the universe microseconds after the big bang. But only now, after myriad subtle measurements, is a clear understanding of the stuff emerging, as physicists reported here at a recent meeting.\* A standard model of the quark-gluon plasma is finally coming into focus, they say, and another decade or so of experiments should nail that model down.

Each puff of quark-gluon plasma is like a tiny big bang. Studying the stuff won't reveal a lot about how the universe grew up; eddies in the plasma could not leave their traces in the distribution of galaxies. But probing the plasma enables physicists to glimpse the infant cosmos and to explore nuclear matter at its most extreme.

\*Quark Matter 2012 International Conference, 13–18 August.

Physicists say the study of the plasma is evolving much like earlier work on the big bang's afterglow, the cosmic microwave background (CMB). Scientists stumbled on the CMB in 1965 and in 1992 detected tiny variations in its temperature across the sky. But only in 2003 did they measure those variations well enough to deduce the exact composition and age of the universe. "Our field is on the same journey from 'Wow!' effects to numbers and, finally, to textbook physics," says Urs Wiedemann, a theorist at the European particle physics laboratory, CERN, near Geneva, Switzerland.

But will physicists get to complete that journey? Only two atom smashers can make the quark-gluon plasma: the Large Hadron Collider (LHC) at CERN and the Relativistic Heavy Ion Collider (RHIC) at Brookhaven National Laboratory in Upton, New York. And RHIC is the only machine dedicated to such work and can do things that the LHC cannot. However, the U.S. Department of Energy (DOE), which owns Brookhaven, faces a budget crunch that may force it to choose between running RHIC and supporting other nuclear physics facilities (*Science*, 27 January, p. 392). Physicists studying the quark-gluon plasma say they could lose their main machine just as years of effort are about to pay off.

## Nuclear fondue

To create a quark-gluon plasma, physicists must literally melt atomic nuclei. A nucleus consists of protons and neutrons, each composed of three particles called quarks bound by a haze of others called gluons that convey

the strong nuclear force. Quarks attract one another so strongly that one cannot be isolated. Knock a quark out of a proton, and on its way out it will rip quarks and antiquarks out of the vacuum to produce a "jet" of quark-filled particles.

But slam two heavy nuclei together with enough energy, and the protons and neutrons can melt to form a soup of unbound quarks and gluons that is the plasma. More quarks, antiquarks, and gluons spring from the superheated vacuum. For a few trillionths of a trillionth of a second, the trillion-degree plasma is the hottest stuff in the universe. Then it cools to form thousands of ordinary particles, which physicists can detect.

Probing the plasma is a subtle art. Physicists can study how it flows by scrutinizing the almond-shaped puffs of plasma formed when nuclei collide off-center, as most do. In 2001, physicists working with STAR—one of two detectors currently fed by RHIC—found that the ensuing sprays of particles carried more momentum out of the sides of the cloud than through its ends. Such "elliptic flow" shows that, unlike ordinary matter, the plasma flows like a liquid with almost no viscosity.

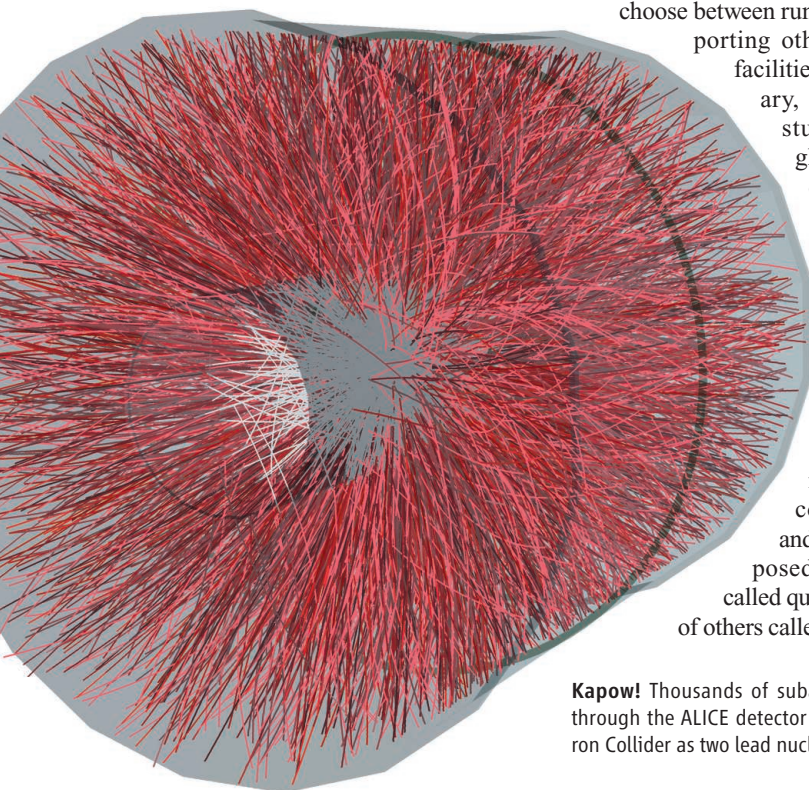
Researchers can also study how the sticky plasma snuffs out jets within it. Measurements from STAR and RHIC's other detector, PHENIX, found that plasma-producing collisions of gold nuclei created fewer jets than collisions of protons did (*Science*, 20 June 2003, p. 1861).

The plasma should also weaken the bond between quarks and antiquarks, and physicists can measure how that effect reduces the production of certain particles in a collision. For example, a particle called the  $J/\psi$  contains a charm quark—a massive and unstable cousin of the up quarks and down quarks in protons and neutrons—and an anticharm quark. In 2007, the PHENIX team found a suppression of  $J/\psi$ s in collisions of gold nuclei relative to collisions of protons.

## The emerging model

Thanks to such measurements, a clearer picture is emerging of how the quark-gluon plasma flashes into and out of existence, physicists say. As the nuclei speed toward each other at near-light speed, the weird effects of relativity take over, explains Raju Venugopalan, a theorist at Brookhaven. The so-called Lorentz contraction flattens the nuclei like pancakes. Time dilation also slows their inner workings, so that the haze of gluons in each nucleus resembles a disk of glass called a color glass condensate.

When the two nuclei collide, the quarks in the protons and neutrons pass through each



**Kapow!** Thousands of subatomic particles streak through the ALICE detector at Europe's Large Hadron Collider as two lead nuclei collide head-on.

other. But the disks of gluons clap together to create a hot nonequilibrium state known as a “glasma.” That stuff quickly equilibrates to make the plasma, which expands and flows before cooling into particles. Basic questions remain: Can plain old “quantum field theory” describe the plasma, or do the quarks and gluons interact so strongly that theorists must use exotic methods borrowed from string theory? However, “for the picture as a whole, there are no big conceptual alternatives,” CERN’s Wiedemann says.

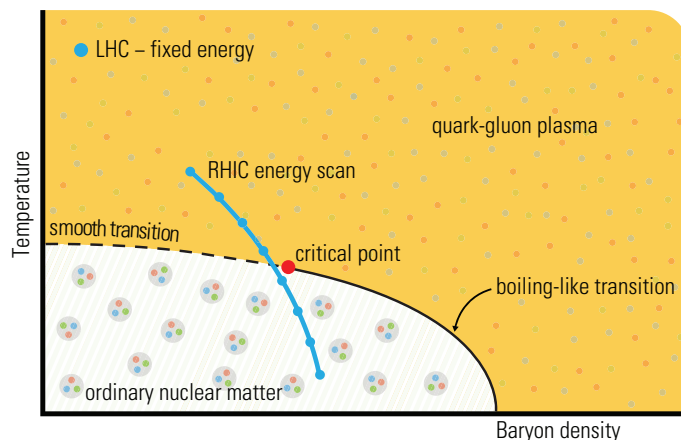
Physicists are now trying to test that model. Some of the most telling data for that work comes from CERN’s LHC, which normally blasts protons together to try to create new particles, such as the recently discovered Higgs boson (*Science*, 13 July, p. 141). But the LHC also smashed lead nuclei for a month in 2010 and again in 2011 at an energy 14 times as high as RHIC’s maximum. The LHC feeds a huge detector called ALICE that is dedicated to quark-gluon plasma work, as well as others known as ATLAS and CMS that study the plasma when not hunting new particles.

Those state-of-the-art detectors can probe the plasma as never before. For example, physicists had assumed that nuclei colliding exactly head-on would produce dull round whiffs of plasma. Instead, researchers at the LHC found that in an “ultracentral” collision, they could detect flow patterns caused by quantum-mechanical fluctuations in the shapes of the nuclei. They could describe each collision’s unique shape as a sum of an oval, a triangle, a square, and other shapes.

Now, Björn Schenke, a theorist at Brookhaven, has fit the average distributions of those shapes measured by ATLAS. To do that, he has to include the distribution of gluons within a proton or neutron as measured in electron-proton collisions. The analysis supports the general scheme and shows that the plasma’s viscosity is within a factor of 2 of a conjectured fundamental lower limit, Schenke reported. “That guy really made a splash,” says Ulrich Heinz, a theorist at Ohio State University, Columbus. “For me, this is like a lot of pieces of the puzzle starting to come together.”

CMS researchers have achieved a similar feat of precision. Within the plasma, they can spot a particle known as the  $\Upsilon(1s)$ , or “upsilon-one-s,” that contains a superheavy bottom quark and an antibottom quark. They can also identify two slightly heavier “excited”

versions of the particle known as the  $\Upsilon(2s)$  and  $\Upsilon(3s)$ . The quark-gluon plasma suppresses the formation of  $\Upsilon(3s)$  more than the formation of the  $\Upsilon(2s)$ , which it suppresses more than that of the  $\Upsilon(1s)$ , reported Camelia Mironov of the École Polytechnique in Palaiseau, France. That’s just what should happen if the quark-gluon plasma gets between the massive quark and antiquark and “screens” them from each other, as the  $\Upsilon(3s)$  is more



**Drilling down.** U.S. researchers have lowered their atom smasher’s energy in search of a transition from nuclear matter to quark-gluon plasma.

loosely bound than the  $\Upsilon(2s)$ , and the  $\Upsilon(1s)$  more loosely bound still. Screening probes other properties of the plasma, theorists say.

### Surprises still

Given such results, some researchers say the LHC has eclipsed RHIC. “It’s undeniably a higher precision tool,” says Georg Wolschin, a theorist at Heidelberg University in Germany. Still, RHIC is more flexible than the LHC. It can collide various nuclei, including unlike ones such as copper and gold, and it can step down in energy.

RHIC’S ability to dial down the energy gives it a shot at mapping out the conceptual border between ordinary nuclei and quark-gluon plasma. At the LHC’s energy and RHIC’s highest energy, the transition from nuclei to plasma and back appears to be a smooth “crossover.” But under the right conditions the transition may become abrupt, like the boiling of water. That change would mark a “critical point” on a graph of the properties of nuclear matter with temperature on one axis and the initial density of protons and neutrons on the other (see diagram).

In 2010 and 2011, RHIC researchers set out to find that critical point. They reduced RHIC’s energy to as little as 8% of its maximum of 200 giga-electron volts (GeV) per proton or neutron, to lower the temperature and, counterintuitively, increase the proton

and neutron density. Between 27 and 19 GeV, subtle changes in the number of protons relative to antiprotons hint that the critical point may be near, reported Lokesh Kumar of Kent State University in Ohio.

Others view the energy-scan data differently. If RHIC turns down its energy until it stops producing quark-gluon plasma, the elliptic flow ought to change dramatically, notes Miklos Gyulassy, a theorist at Columbia

University. But STAR researchers see no such sudden change. That finding could overturn the theoretical apple cart, Gyulassy says. “They haven’t found the critical point, but they’ve found a systematic sameness that’s shocking,” he says. The result suggests that even at the lowest energy, RHIC still produces the plasma, says Jürgen Schukraft, an ALICE team member from CERN. “For 20 years, our biggest problem was to find the quark-gluon plasma,” he says. “Now our biggest problem is not to find it but to make it go away.”

DOE officials may soon have to decide if such problems are worth pursuing. In addition to

RHIC, DOE also runs an electron accelerator at Thomas Jefferson National Accelerator Facility in Newport News, Virginia, to probe the structures of the proton, the neutron, and nuclei. And researchers at Michigan State University in East Lansing plan to build a \$615 million accelerator to generate exotic nuclei. But the three projects won’t all fit into DOE’s \$550 million annual budget for nuclear physics, which is unlikely to increase soon.

RHIC researchers argue that it would make no sense for them to stop now. “You don’t build a Ferrari to drive it 10,000 miles and then give it away,” Ohio State’s Heinz says. “To make this emerging picture really stick together, another 10-year program is still needed.” LHC researchers agree that RHIC should keep going. “The physics that we do at the LHC, we do very well,” Schukraft says. “But we can’t do the same physics as at RHIC.” Besides, the LHC will smash nuclei for only 6 months total in the next 10 years. RHIC can smash them for 6 months each year.

Whether such arguments hold sway should soon become clearer. DOE has asked a panel of experts to evaluate its options and report back in January, says Timothy Hallman, DOE’s associate director for nuclear physics. Physicists may then have a better idea of just how long RHIC will keep banging away.

—ADRIAN CHO



## LETTERS

edited by Jennifer Sills

## Violence: Finding Peace

IN THE NEWS STORY "THE BATTLE OVER VIOLENCE" (SPECIAL SECTION on Human Conflict, A. Lawler, 18 May, p. 829), Steven Pinker asserts that peace anthropologists, whom he calls "anthropologists for peace," inaccurately portray small groups as more peaceful than they are (1). His accusation is unfounded. The term "peace anthropologist" refers to ethnographers who try to understand how people maintain peace. Some of us are pacifists; most are not. The field did not begin by looking for peaceable peoples. The first five modern investigators to study east Semai, for example, did not look for peaceability, but found it anyway (2–6). When ethnographers say that certain types of foragers and a few swiddeners led relatively peaceful lives, the statement is empirical, not ideological. Many ethnographic surveys of peaceable peoples are available [e.g., (7, 8)].

Paleoanthropologists agree that the earliest human ancestors adapted to lifestyles that stressed nonviolence, tendencies that manifested anatomically, such as in the shrinkage of male canines, which anthropoids typically use to fight over females—the main cause of violence in small acephalous human groupings (9). Computer simulation suggests that small egalitarian groups (characteristic of human ancestors and peaceable peoples today) survive if they avoid

violence better than if they practice it (10). Peace anthropologists are not pushing an agenda. They are merely gathering data and drawing the best conclusions they can.

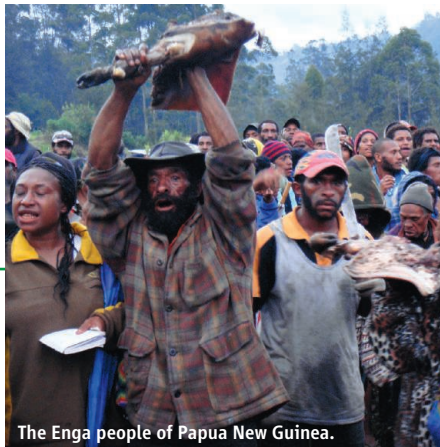
GEOFFREY BENJAMIN,<sup>1</sup> ROBERT K. DENTAN,<sup>2\*</sup> CHARLES MACDONALD,<sup>3</sup>  
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The Enga people of Papua New Guinea.

## Violence: Clarified

TWO RECENT NEWS STORIES DISCUSS THE high average rates of violence in nonstate societies summarized in my book *The Better Angels of Our Nature: Why Violence Has Declined* (1). Both require clarification.

"The battle over violence" (2) quotes an archaeologist who claims that I selected "a few populations...biased toward supporting [my] argument." This is untrue. *Better Angels* reports all the published estimates of per capita rates of violent death in the archaeological and anthropological literature I could find, including those from societies that have been claimed to be nonviolent. Figure 2-3, for example, reports war death estimates from 27 hunter-gatherer and hunter-horticulturalist societies (most data collected between 1825 and 1982), which range from 0 to 1450 war deaths per 100,000

people per year (per capita rates based on the local tribe or village population), with an average of 524 (1). By comparison, the highest estimate of worldwide deaths from war and genocide in the 20th century is 60, and the rate today is far less than 1 (1).

"Turning from war to peace in Papua New Guinea" (E. Culotta, *News & Analysis*, 28 September, p. 1593) notes that I praise Wiessner and Pupu's recent study on peacemaking among the Enga (3), "even though it contradicts one of [my] key conclusions." The study does not contradict my findings. Modern guns did not bring high rates of violence to New Guinea, nor have the Enga's

impressive peacemaking efforts brought the rate down to those of modern Western societies. Even before the gun-fueled rise in Enga violence starting around 1990, the average annual war death rate in published estimates from 11 New Guinean societies is 498 per 100,000 (4–6). During the worst period among the Enga, the authors' estimate is around 100, and after the Enga's peacemaking efforts, it was 32. Compare that to the worldwide average rate of violent deaths today of around 6 to 8 (mostly homicides); the figure for the worst year for American crime, 10; and the range for most Western democracies, 1 to 3 (1). The Enga at their most peaceable still have a rate of violent death that is 3 to 30 times as high as that of much of the modern West.

Although *Better Angels* documents the pacifying effects of successful states, it did



## Readers' Poll Results

### Paying for Tissue

In the 14 September issue of *Science*, R. D. Truog *et al.* opposed the idea of paying patients for discarded tissue that may prove valuable, such as the cells from Henrietta Lacks that became known as the profitable HeLa cell line. In the 14 September issue, S. D. Kominers and G. S. Becker outlined why they think patients should be paid. We asked you to weigh in by answering this question:

When medical procedures result in tissue that would otherwise be discarded, should researchers be required to pay patients for its use?

Nearly 1000 of you responded, from dozens of countries. Here are your results.

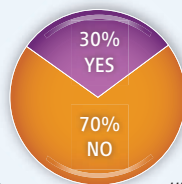
#### A selection of your thoughts:

*I believe a patient should only be compensated when and if the actual tissue becomes commercialized. If the tissue is used for research purposes only and is passed between labs without profit, there should be no patient compensation....*

—reader Amanda Wagner

*The idea that we have to be paid for what is of no use to us seems to overextend the concept of property. Property, in a legal sense, exists because it serves certain purposes. For example, legal protection of property provides incentives for ... its maintenance and improvement.... One might argue that the compensation [of tissue donors] is for the risk of harm from someone using information about the donor to harm the donor, but protection of property rights seems to be an extremely crude way to prevent such harm....*

—reader Jonathan Baron



*Definitely not. This would make it hard for small research institutions to conduct their studies.... [S]cientists don't make much money yet have a crucial role in the development of various aspects of our civilization, and this would just depress the process and place unnecessary pressure on them.*

—reader Samir Younan

*...It would be a really good thing if patients could be compensated when otherwise-to-be-discarded tissue was found to have scientific or commercial value.... The distinction between research and commercial use is becoming increasingly fuzzy as much good research is done in, or in collaboration with, commercial entities. The prohibition of commercial use for many biorepository samples is hampering research now, and promises to hamper it further until the pendulum finally begins to swing the other way again....*

—reader Pat Garrett

*I think the question [should be] "should patients have the option to charge researchers for the use of their tissues that would have otherwise been discarded." I believe the answer to [that] question should be, "yes." ... [W]e pay for commercially developed cells every day. Why should the tissues of humans be worth any less?*

—reader Felicia

*When we recycle our trash at the curb, we receive a little kick-back on our trash costs. A decrement in our cost of medical services—certainly equal to the apportioned cost of tissue disposal—would psychologically have the same effect. We want a sense of control, courtesy, and justice, not an incalculable and probably unrealizable market value....*

—reader Heidi Draffin

not, of course, claim that any state, no matter how weak or inept, magically reduces violence, nor that state control is the only means by which violence may be reduced. Indeed, the book devoted several pages to Wiessner's studies of peacemaking among the Enga, an excellent example of a "civilizing offensive" that has reduced violence at several points in history.

Facts about violence in nonstate peoples

have often been politicized because people worried that high estimates would imply that efforts at reducing violence are futile [e.g., (7–10)]. The value of Wiessner and Pupu's meticulous study is to show why this fear is misguided.

Regarding Benjamin *et al.*'s Letter, it is encouraging that "anthropologists of peace" now see their discipline as empirical rather than ideological, a welcome change from

the days when many anthropologists signed manifestos on which their position on violence was "correct," and censured, shut down, or spread libelous rumors about colleagues who disagreed (7–10).

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### TECHNICAL COMMENT ABSTRACTS

#### Comment on "Restoring Voluntary Control of Locomotion After Paralyzing Spinal Cord Injury"

Urszula Ślawińska, Serge Rossignol, David J. Bennett, Brian J. Schmidt, Alain Frigon, Karim Fouad, Larry M. Jordan

Van den Brand *et al.* (Reports, 1 June 2012, p. 1182) claim to have restored voluntary control of locomotion after paralyzing spinal cord injury. They have not considered recent findings that their upright posture paradigm contributes to locomotor capability after such injuries. We propose that postural adjustments that activate the locomotor central pattern generator in the upright posture, rather than direct voluntary control of locomotion, account for their results.

Full text at <http://dx.doi.org/10.1126/science.1226082>

#### Response to Comment on "Restoring Voluntary Control of Locomotion After Paralyzing Spinal Cord Injury"

Grégoire Courtine, Janine Heutschi, Rubia van den Brand

Ślawińska *et al.* questioned the involvement of supraspinal centers in restoring locomotion after multisystem neuroprosthetic training in rats with paralyzing spinal cord injury. Here, we clarify misconceptions and present additional results illustrating the robust influence of brain input on electrochemically enabled spinal circuitries. We reassert that our intervention reestablished supraspinal control over hindlimb locomotion in paralyzed rats.

Full text at <http://dx.doi.org/10.1126/science.1226274>

### Letters to the Editor

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# Comment on “Restoring Voluntary Control of Locomotion After Paralyzing Spinal Cord Injury”

Urszula Sławińska,<sup>1</sup> Serge Rossignol,<sup>2</sup> David J. Bennett,<sup>3</sup> Brian J. Schmidt,<sup>4</sup> Alain Frigon,<sup>5</sup> Karim Fouad,<sup>6</sup> Larry M. Jordan<sup>4\*</sup>

Van den Brand *et al.* (Reports, 1 June 2012, p. 1182) claim to have restored voluntary control of locomotion after paralyzing spinal cord injury. They have not considered recent findings that their upright posture paradigm contributes to locomotor capability after such injuries. We propose that postural adjustments that activate the locomotor central pattern generator in the upright posture, rather than direct voluntary control of locomotion, account for their results.

Van den Brand *et al.* claimed to have restored voluntary control of hindlimb locomotion after paralyzing spinal cord injury using an electrochemical neuroprosthesis combined with overground locomotor training in adult rats (*1*). They used epidural electrical stimulation together with systemically applied drugs and bipedal locomotor training in a robotic postural interface to force rats to walk toward a food reward. Given the impact of such claims for paraplegic patients around the world, it is important to critically examine the evidence provided. In a recent publication, Sławińska and others (*2*) clearly showed that the upright bipedal posture alone provides sensory feedback that promotes coordinated hindlimb locomotion in the absence of training, drug application, or supraspinal influence. We propose that the bipedal training procedure used by van den Brand *et al.* (*1*) initiated locomotion due to forward shifts in posture, thus engaging powerful feedback from load receptors of the hindlimbs that potently facilitates locomotor activity (*2, 3*). The videos [supplementary materials for (*1*)] show that the rats move their forelimbs, head, and trunk, thus shifting the center of mass forward. The net effect is an increased loading of the hindlimbs that per se could lead to spinal stepping, especially in the upright posture. Injections of potent excitatory drugs combined with lumbosacral electrical stimulation, as used in their paradigm, likely increases the responsiveness of the spinal locomotor cen-

tral pattern generator (CPG) to load-bearing inputs. As their figure 4 shows, the onset of overground locomotion is accompanied by an increase in the ground reaction force (GRF), thus facilitating locomotor activity without having to engage direct voluntary control of the locomotor CPG. We propose that the training paradigm resulted in a strategy that shifted the center of mass forward so that the afferent feedback associated with the upright posture, together with the resultant hip extension, engaged the spinal CPG for locomotion and propelled the animal toward the food reward. This interpretation does not require that the brain regain “supraspinal control over the electrochemically enabled lumbosacral circuits,” as the authors propose [supplementary materials for (*1*)].

If the training is effective in restoring voluntary locomotion, and this is not because the animals are in the upright posture as we propose, then the rats should display marked recovery of overground quadrupedal locomotion, the normal position for progression in rodents. However, a description of the animals in a quadrupedal locomotion task was not included in this paper. There are reports (*4–6*) that recovery of quadrupedal locomotion occurs spontaneously in adult rats and mice with staggered hemisection injuries similar to that used by van den Brand *et al.* The concept that propriospinal neurons can relay a locomotor message is derived from experiments in Schmidt’s laboratory, where it was demonstrated that neonatal rats subjected to simultaneous staggered hemisections can recover locomotion (*7–9*). They have recently demonstrated (*6*) spontaneous recovery of hindlimb locomotion in adult rats subjected to simultaneous staggered hemisections, such as those described in the van den Brand paper (*1*). Barrière *et al.* (*10, 11*) showed that a previous thoracic hemisection facilitates recovery of hindlimb locomotion after a subsequent total transection in cats, and they have confirmed this observation in adult rats (*12*). Therefore, it seems

likely that spontaneous recovery of propriospinal activity plays a role that was not revealed by the analysis used in the van den Brand *et al.* study. The *N*-methyl-D-aspartate (NMDA) lesions (*1*) of the thoracic region between the two hemisections may be effective in stopping locomotion by removing spontaneous propriospinal plasticity rather than by blocking newly developed voluntary control of the locomotor system.

Forelimb movement observed in these experiments likely contributed substantially to propriospinal signals that help drive locomotor activity in the hindlimbs (*13*). Videos in (*1*) suggest that the rats learned to flail their forelimbs as part of the strategy to attain forward progression to the food reward. Thus, the results with NMDA injections into the thoracic relay site can be explained as an interruption of the relay of propriospinal messages for control of locomotion from the forelimbs.

The results with muscimol injected into the motor cortex (*1*) are also consistent with our suggestion that the animals developed a new strategy to facilitate postural adjustments required to initiate locomotion, rather than establishing new connections with the lumbosacral locomotor centers. Indeed, after injecting muscimol, the forward shift of the trunk does not occur [movie 3 in (*1*)]. Moreover, van den Brand used obstacle avoidance and stair climbing to support their claim of voluntary control of hindlimb locomotion. It must be pointed out that stairs and obstacles do not actually constitute “two conditions requiring voluntarily mediated gait tuning” as stated in the van den Brand *et al.* paper. The review by Drew *et al.* (*14*) cited in support of this claim addresses only visually guided movement, but visual guidance does not appear to be involved in the cases reported here. It is well known that spinal-transected animals can negotiate obstacles after contact, as is the case in the videos accompanying this paper. Thus, the conclusion that these tasks require voluntary control is not warranted, and the data provided do not establish voluntary control as the basis for the rats’ success with these tasks.

Recordings of cortical activity provided in (*1*) are more supportive of a role for the cortex in controlling postural adjustments than hindlimb locomotor activity, because the neuronal activity recorded from the cortex is associated with an increase in GRF that occurs at the onset of hindlimb locomotion and not with the rhythmic activity during locomotion (their figure 4). It would be of interest for the authors to attempt to correlate the recorded cortical activity with the GRF changes or other signals from the hindlimbs to examine this possibility. They show that postural adjustments occur in the overground-trained group (PC2 in their figure S5), which “exhibited enhanced lateral body movements that alternatively loaded the left and right limbs during locomotion.” This observation is consistent with our interpretation that the development of

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new strategies to maximize the impact of the sensory feedback associated with the upright posture accounts for the recovery of hindlimb locomotion.

Thus, the conclusion is inescapable that the data presented by van den Brand *et al.* do not provide convincing evidence for “restoring voluntary control of locomotion after paralyzing spinal cord injury.” Furthermore, the interpretation of experiments in which bipedal locomotion is used to monitor and/or train the recovery of locomotion after injury must include consideration of the direct physiological and biomechanical consequences of placing rats in an upright posture.

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# Response to Comment on “Restoring Voluntary Control of Locomotion After Paralyzing Spinal Cord Injury”

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Slawińska *et al.* questioned the involvement of supraspinal centers in restoring locomotion after multisystem neuroprosthetic training in rats with paralyzing spinal cord injury. Here, we clarify misconceptions and present additional results illustrating the robust influence of brain input on electrochemically enabled spinal circuitries. We reassert that our intervention reestablished supraspinal control over hindlimb locomotion in paralyzed rats.

A century ago, Sherrington reported the unexpected ability of isolated spinal circuits to generate rhythmic leg movements in response to sensory input (1). Since this seminal observation, spinal locomotion has been described in various species and conditions (2). We exploited this concept to design an electrochemical neuroprosthesis (3) and training procedures (4, 5) that restored full weight-bearing locomotion in rats with a complete spinal cord injury (SCI). Slawińska *et al.*, as well as many others (6, 7), reported comparable observations in mice (8), rats (9), and cats (10) over the past three decades.

These hindlimb movements, however, remain involuntary. In spinal animals, quadrupedal hindlimb locomotion occurs in the complete absence of supraspinal input and is controlled via sensory ensembles (5, 11, 12). For example, the intact forelimbs initiate locomotion and sustain propulsion during quadrupedal gait, which generates sensory feedback in the hindlimbs due to biomechanical coupling. Like the rear person in a pantomime horse, lumbosacral circuitries can recognize these task-specific sensory states and use this information to coordinate complex movements. In a recent study (11), we demonstrated that, as early as 10 days after a paralyzing SCI, quadrupedally positioned rats produced weight-bearing locomotion, climbed a staircase, and steered in a curve during electrochemically enabled motor states. We did not report these results in our *Science* paper (13) because lumbosacral circuits control these seemingly complex quadrupedal movements without contribution from supraspinal centers. Therefore, they presented no novelty in the framework of this study. Indeed, rats with the same SCI were incapable of initiating bipedal walking [figure 1C and movie S1 in (13)]. They also failed to sustain robotically ini-

tiated locomotion [figure S6 in (13)]. Moreover, when the robot moved nontrained rats toward a staircase, the isolated spinal circuits failed to adjust gait movements, which led to a dramatic collapse against the staircase. Together, these results demonstrate that the absence of supraspinal input led to the inability to initiate, sustain, and modulate bipedal hindlimb locomotion overground. They also reveal that quadrupedal locomotor testing yields inconclusive results and that bipedal walking is a more appropriate paradigm to uncover the ability of supraspinal centers to functionally access lumbosacral circuits.

In (13), we described a type of motor control that is radically different from automated spinal stepping: that is, the recovery of supraspinal control over a range of locomotor behaviors (13). Rats with paralyzing lesions could initiate bipedal locomotion from a resting position on their own will, walk for extended periods of time, pass obstacles, and climb a staircase. To accomplish these tasks, the rats implemented feedforward strategies because they significantly increased their step height before negotiating the staircase [figure S6 in (13)]. In the majority of trials, the hindpaw did not come in contact with the staircase, which excludes the contribution of proprioceptive feedback for triggering these task-specific adjustments (see movie S1, staircase). Thus, active training under highly functional states promoted extensive and ubiquitous remodeling of axonal projections, which allowed the brain to regain adaptive control over electrochemically enabled lumbosacral circuits (13). This plasticity of descending systems did not occur in rats trained on a treadmill. Although these animals showed full weight-bearing stepping on a treadmill and coordinated quadrupedal overground locomotion (11), they failed to produce voluntary bipedal locomotion despite head, shoulder, trunk, and arm movements during their unsuccessful attempts to walk.

Undoubtedly, the biomechanical design of the rodent locomotor system and the remarkable ability of lumbosacral circuits to use sensory input as a source of control for locomotion extensively contributed to the produced movements (5, 13).

In fact, the same type of neural operations most likely underlies the production of locomotion in healthy terrestrial mammals, including humans. Whether this neural process evidences voluntary or involuntary motor control is a problem of definition: What is voluntary motor control? Everyone will agree that trained rats initiated locomotion voluntarily, because they triggered their walk from a resting position at the view and/or smell of the reward. They thus have developed strategies to initiate walking and, in the case of climbing a staircase, even to anticipate the need for increased step height and enhanced locomotor output to resist gravity. We deployed a series of complex additional experiments to demonstrate that neural inputs arising from supraspinal regions were required for the expression of locomotion overground and that cortical networks were critically involved in generating these movements. All these experiments emphasized the necessity of intraspinal and cortical circuitries for the production of bipedal hindlimb locomotion in trained rats.

To further document the supraspinal modulation of hindlimb locomotion in trained rats with paralyzing SCI, we performed two complementary experiments, which are illustrated in movie S1. In the first sequence, a paralyzed rat shows repetitive whole-limb extension, and even jumps, to reach a reward while continuously walking on the treadmill under electrochemical stimulations. When the reward is withdrawn, the rat willingly blocks hindlimb movements. How could these voluntary actions be triggered? Due to the constraining trunk support system, increase in weight-bearing input via tilting of the trunk cannot explain this modulation. Slawińska *et al.* (14) postulate that arm movements could elicit these hindlimb movements. Although forelimb movements do contribute to facilitating gait in rats, just like arm movements facilitate leg movements in humans (15), it is difficult to conceive that these inputs would be sufficient to elicit forces substantially larger than the body weight in order to jump. Moreover, Slawińska *et al.* recognized that forelimb movements were insufficient to initiate hindlimb locomotion after inactivation of the motor cortex. The second sequence shows that trained rats display continuous and coordinated hindlimb swimming despite the lack of useful weight-bearing input to trigger movements in water.

In the absence of experiments that would prove otherwise, we conclude that this series of multifaceted evidence demonstrates that the production of overground locomotor movements in trained rats resulted from powerful interactions between electrochemically enabled spinal circuits and supraspinally originating motor commands.

Finally, it is worth remembering the pioneer recovery of supraspinally mediated movements after several months of electrically enabled training in a paraplegic man (16). Despite three years of chronic paralysis, this individual regained supraspinal control over joint-specific leg movements during stimulation. These results are consistent

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with our conclusions and suggest that the plasticity of spared neuronal systems observed in rats may also occur in humans with severe SCI. We hope that our work will inspire new thinking to restore motor function for affected individuals in the future.

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#### Supplementary Materials

[www.sciencemag.org/cgi/content/full/338/6105/328-c/DC1](http://www.sciencemag.org/cgi/content/full/338/6105/328-c/DC1)  
Movie S1

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variety just as we are to be intrigued by the similarities among them. And we are led to wonder: What is the appeal of the tree metaphor? What general representational utility makes this form so persistent, even as the ideas it represents change?

In sum, Pietsch provides a comprehensive visual document of an idea, which I recommend to anyone interested in the history of science and scientific representations. It collects diagrams that, although now (or perhaps soon to become) obscure, helped shape the development of one of the most important ideas in modern science. With the concept of evolution now often iconified to the point of misrepresentation, *Trees of Life* reminds us that both the idea and its representations were—and are—fluid, debated, and reconstructed.

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## GEOGRAPHY

# Why We Are What and Where We Are

John P. Hart

I love big cities. Not only is there always something interesting to do, but where else can you experience so much human diversity in such relatively compact areas? As you walk down the street, your ears pick up a multitude of languages and accents, your eyes capture a huge range of human physical traits, and your nose catches the smells of cooking from scores of ethnic restaurants

and food carts. All of this reflects the modern world, in which rapid travel allows a mixing of people from around the globe. But how did the patterns of human variation that contribute to the modern cityscape arise? This is the subject of a relatively new field of human biogeography and of Alexander Harcourt's book of the same name.

In the spring of 1974, a group of prominent anthropologists, biogeographers, and population geneticists met at the Smithsonian Institution to discuss the potential of using biogeographical theory and methods to analyze geographical patterns of human variation (1). Participants shared a sense of great promise for the development of an interdisciplinary field of human biogeography. A wide range of investigations have since contributed to the fledgling discipline, even if the researchers were not specifically concerned with or even aware of their contributions to the field. By summarizing a vast, disparate literature from a variety of scientific disciplines (the bibliography comprises 861 publications) as well as offering new analyses and insights, Harcourt (an anthropologist at the University of California, Davis) captures the continued potential of human biogeography as well as its challenges.

Concerned with geographic patterning within and between species, biogeography itself is an old discipline, which Harcourt traces back to 18th-century European explorations and Alfred Russel Wallace's foundational work (2). Since then, we have developed good understandings of the biogeography of many species.

For example, we know that maize (*Zea mays* ssp. *mays*) evolved from a teosinte (*Z. mays* ssp. *parviglumis*) native to central Mexico and has spread around the globe over the past 9000 years as the result of its interactions with humans. It is highly variable both genetically and phenotypically across its range. Its thousands of varieties differ in, for example, plant size, number of days to maturation, inflorescence shape, and kernel shape, size, and color. We know that the historical patterning in variation was the result of founder effects, drift, isolation, hybridization, and natural and artificial selection in varying environmental settings—that is, it reflects the history of genetic variation within and among maize populations.

We are also a globally dispersed species. Having evolved in Africa, *Homo sapiens* dispersed within and from our home continent beginning some 55,000 years ago (to

use Harcourt's compromise figure). Over 50,000 years later, we reached the farthest reaches of the globe, islands in the Pacific Ocean. As we spread out and encountered widely ranging and changing environments, we became highly variable both physically and behaviorally. As in other species, this variation is patterned.

Can the patterns of physical and behavioral variation in humans be explained with the same theories and methods used to explain geographical patterning in other species? That theme of the 1974 meeting remains a primary question for a discipline of human biogeography. Early in his narrative,

Harcourt notes that there is more genetic diversity in a single subspecies or population of chimpanzees than in the global human population. As a result, he states that “much of the biogeography that [he discusses] has to do not with

genes determining human behavior, or even influencing it, but with humans' reactions to the environment.” In other words, whereas some of the patterning in human variation is the result of genetics, much more of it (unlike in most species) is not.

Perhaps what is most interesting about the book and the field is that at this juncture, human biogeography is most successful in explaining patterns in physical traits, whether they be adaptations to environmental pressures or to human niche construction. It seems less adept at explaining variation in cultural patterning. Although Harcourt recognizes that equating languages with cultures is questionable, he bases most of his analyses on that concept. The issue of appropriate units of analysis was raised at the 1974 meeting and has been ongoing in anthropology and other sciences that contribute to human biogeography. Harcourt offers ample justification for his analytical methods. Given that the selection of analytic units should be situational, readers are left to decide how meaningful his results are.

Regardless of any such issues, *Human Biogeography* is a remarkable achievement. The book firmly establishes its subject as an important discipline bridging the social and biological sciences. By bringing together such a vast corpus of analytical results, it will undoubtedly attract more attention to the field.

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### Human Biogeography

by Alexander H. Harcourt

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## RESEARCH FUNDING

# Toward Variable Funding for International Science

Jakob Edler

Science and innovation are a considerable part of the answer to global challenges. However, the demands placed on public science (1) are not well supported by mechanisms for international collaboration. We need to rethink how we organize funding for scientific cooperation in regional and global contexts. Fully integrated global structures to offer collaboration opportunities might be a desirable vision but, unfortunately, a long-term one. Instead, schemes should allow a combination of national funds by ministries and agencies that are flexible enough for the requirements of different knowledge areas and societal challenges. The European Research Area (ERA)-NET scheme offers some generic lessons.

There are well-known benefits from international collaboration in science (2), e.g., papers jointly authored by international authors tend to be more highly cited (3, 4) and “on average, research collaboration pays off” (5). There are limits to productivity gains when broadening international collaboration, especially if it becomes a goal in itself, a response to funding requirements, or is used as an indicator of excellence (5). Thus, the mission is not maximizing international collaboration but optimizing the opportunity structure for appropriate collaboration to achieve scientific and societal goals.

A range of funding opportunities for international research collaboration has been developed. Intergovernmental large-scale infrastructure organizations and laboratories [e.g., the European Organization for Nuclear Research (CERN), the European Molecular Biology Laboratory, and the ITER project (originally International Thermonuclear Experimental Reactor)] are well established. Several problem-driven initiatives focus on international research, often as part of larger, mission-oriented international organizations [e.g., (6, 7)]. However, those organizations fall short of funding collaborative research on a large and sufficient scale (6, 7). There is a very small number of truly global, integrated research programs,

where national funders or ministries invest in a single “common pot” of money to be allocated to the best proposals wherever they come from, e.g., the excellent Human Frontier Science Program (8). Some national ministries, research councils, or foundations are opening up programs to international researchers (9), but participation of external researchers is still very low, as the funds are limited. There is a growing number of bilateral or multilateral cooperation agreements, as well as more integrated, joint programs of funding organizations (10). The G8 countries (minus Italy) have initiated a Research Councils Initiative on Multilateral Research Funding at an intercontinental level.

Despite these efforts, the scale and scope of funding for international collaboration remains insufficient. National governments have increasingly ambitious internationalization strategies, which are not systematically implemented. The lack of funding opportunities for international collaboration is a key obstacle for better international scientific activity [e.g., (11)]. Although recent assessments found some good practice for selected areas (12), no overall appraisal of supporting structures for international collaboration of public science is available. Setting up full-scale, well-functioning global organizations is an important long-term goal. But, given the scale of the challenges we face and the lost opportunities and societal costs stemming from poor conditions for international collaboration, more creative and quicker solutions must be developed.

## The European Approach—a Learning Lab

The ERA-NET scheme supports funding arrangements between ministries and funding organizations from a variable group of countries. This complements the central, integrated Framework Programme (FP) administered by the European Commission (EC), which mainly funds transnational research collaboration. The ERA-NETs bring together funds and capabilities in areas in which participating agencies and ministries from different countries perceive joint strategic interest. They are cofunded

European ERA-NETs offer lessons for flexible international funding to better meet the needs of science and society.

by the EC to cover the costs of setting up and managing the network and—in limited cases (ERA-NET Plus)—to support joint calls for proposals. The scheme foresees a range of activities, building up to joint funding: (i) systematic exchange of information and practices on existing programs and activities, (ii) identification and analysis of common strategic issues; (iii) planning and development of joint activities between national and regional programs; and (iv) implementation of joint transnational activities, including joint calls and programs. The ERA-NETs provide a pathway from mutual learning to joint policy and funding action, which is used in flexible ways by different networks (13).

Some ERA-NETs have been issue- and problem-driven, others have been organized around the requirements of specific science knowledge areas. The majority of those networks whose European Union (EU) cofunding ran out have continued all or some of their activities (13). Despite some persistent implementation challenges, the ERA-NET scheme has largely been assessed as highly successful (14). Not only has it created new opportunities for research collaboration beyond the FP and answered existing demand in the research community (14), it has also catalyzed a positive, dynamic approach in Europe toward more joint funding of international collaboration (10). The main advantage for researchers has been the opportunity to collaborate internationally in areas for which funds for collaboration have not been readily available. Compared with supranational funds for collaboration, researchers have lower transaction and learning costs, as they apply through their national agency, but benefit from quality control through international peer review (15).

The ERA-NET scheme was not designed for global, extra-European cooperation, but it enables participation of non-EU partner agencies and ministries. Extra-European participation has been limited, but the idea of reaching beyond European partners was a learning process between ERA-NETs and has been spreading slowly. The networks with extra-European partners are more het-



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erogeneous, and the challenge has been to include all participants from the beginning as “equal partners” (16), to clearly define research priorities and funding modalities, and to involve those partners with autonomy to commit resources (16). However, the low extra-European participation of agencies and ministries signifies that, especially in broad, heterogeneous networks, there is a need for some seed funding. Further, the more heterogeneous the networks are, the more they need to be designed as dynamic learning tools, taking advantage of the multistep approach outlined above.

### Ways Forward, Lessons Learned

It is important to enable and construct international approaches both top down—driven by social challenges and political intent—and bottom up—driven by the needs of scientists and the initiative of funders. The strength of the first wave of ERA-NETs in FP6 was the bottom-up mobilization of ministries and funding agencies (14). In FP7, the design of new ERA-NETs is confined to politically predefined areas, limiting variety and spontaneity of new combinations, but allowing a politically set focus and bundling with other political initiatives.

Flexibility is needed and feasible. The geometry of agency and ministry participations and scope and design of activities must align with the different requirements of the targeted knowledge field or problem area (17). In some areas, exchange of personnel and training might be a preferred activity; in others, it might be a fully fledged transnational program to enable large-scale joint research (17). For others, innovative modes of joint action, such as prizes or crowd-sourcing, may be more appropriate.

Appropriate models must be chosen. Political pressure often tends toward “juste retour,” where national money spent in transnational schemes is expected to come back to support national researchers and organizations. Transnational funding will only work if those who are politically accountable for the allocation of funds are fully committed to the transnational engagement, where benefits for all may outweigh potential funding asymmetry. A variety of different funding modalities can be applied, corresponding to the political will to share budgets, ranging from integrated joint funding—“common pots” that ignore the juste retour problem—to so-called “virtual common pots,” whereby national participants only pay for those from their own country.

No matter which models are chosen, the ERA-NET experience offers a set of gov-

ernance lessons: (i) commitment of all parties and a strategic alignment of nationally rooted goals with transnational activities; (ii) explicit expectation management as to the common or complementary goals and joint principles; (iii) alignment of the funding mode of the chosen goals with national regulations (including adjustment of national rules if needed); and (iv) clear decision-making structures and a dissemination framework. International schemes can develop independently, without support from regional or international organizations (10); this emerging, still-limited trend needs strengthening. To overcome the challenge of different funding mechanisms and rules that often limit the scope of joint action, a gradual, multistep approach as demonstrated by ERA-NETs is required, from mutual learning and trust building through to joint funding. However, even if ministries and funding agencies perceive a potential benefit from cooperation, the additional costs and risks involved for the organization often limit cooperation. The main advantages of the EU-supported schemes lie in the moderating—and limited—cofinancing role of the EC and in well-institutionalized discourse and decision-making routines within the EU.

To catalyze the idea of flexible international funding models beyond Europe (17), one way forward is to seek joint funding from existing organizations. Other regional organizations like the Southern African Development Community, the Association of Southeast Asian Nations, or Mercosur should promote and implement this idea. Even without truly supranational decision and funding structures, such regional organizations can facilitate and mobilize international funding schemes with a set of willing member countries aligning around a common challenge or knowledge area. This is a matter of political will, not a matter of lack of integration or supranational structures.

Another way forward is for existing international organizations that are already aligned around missions and challenges [e.g., International Energy Agency (IEA) and World Health Organization (WHO)] to break the mold of rather small-scale international research funding and build the supporting platform for variable-geometry approaches, working toward a most appropriate inclusion of ministries and funding agencies from member countries—and, if appropriate, even beyond. They could support the set up of variable schemes that have different layers of involvement and commitment around a common theme. This would

allow ministries and agencies from weaker countries to mobilize, avoid an “insider-outsider” problem, and utilize existing national resources for transnational activities in most flexible ways.

The global challenges ahead will not be tackled effectively with the existing focus on national funding and will not wait for truly global, fully integrated approaches to emerge. We need pragmatic flexible collaboration, and form needs to follow function.

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## ASTRONOMY

## Galactic Archaeology

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Galactic archaeologists sift through stellar fossil records to uncover the history of our nearby universe. Akin to archaeologists interpreting the customs of ancient civilizations, clues are assembled of past events in the galaxy's lifetime based on the remnants observable today. In most galactic archaeology studies deciphering the histories of spiral galaxies like our own Milky Way, a critical assumption made for the past 30 years was that stars born in disks do not venture far from their original birthplace. The demographics of a population in a given part of the disk (see the figure) then yield a complete record of the galaxy's past, turning star counts into cosmologically relevant tools. The assumption that stars mostly stay put throughout their lives therefore carries enormous implications and wields considerable power. However, recent work on galactic disk dynamics shows that this critical assumption is likely flawed and instead many stars are expected to wander considerable distances throughout their lives. This process of radial migration affects the entire galactic disk, and if theoretical expectations are confirmed by observations, its consequences are integral to fields as diverse in scope and scale as solar system science and extragalactic galaxy formation. It was with this in mind that a workshop dedicated specifically to the issues related to radial migration in disk galaxies took place earlier this year (1).

The orbits of the oldest stars we observe in the solar neighborhood oscillate radially by less than 20% (the amplitude of these oscillations increases with time as a star's orbit is perturbed by irregular structure in the disk). This observational fact led to the conclusion that a given star always occupies the same part of the galaxy. However, in 1996 Wielen *et al.* (2) postulated that because our Sun's atmosphere shows a higher fraction of heavy elements than other similar stars in our immediate vicinity, it may have been born in a different part of the galactic disk, thereby implying that stellar wanderings may not be uncommon. Some years later, Sellwood and Binney (3) showed that the earlier estimates were



**Star spotting.** Properties of populations of stars, such as these observed in the nearby galaxy NGC300 by the Hubble Space Telescope, provide clues to the formation history of their host galaxies.

likely too conservative and that radial migrations over many kiloparsecs are expected for stars in Milky Way-type disks. They showed that such migrations are possible when stars can match the speed of a passing spiral wave and use it to surf rapidly inward or outward in the galaxy.

Furthermore, although this process causes orbits to shrink or expand on short time scales, it does not disproportionately affect the oscillations about their orbital radii. In fact, the radial migration mechanism most drastically affects stellar orbits that are close to circular, meaning that the most distant migrants remain inconspicuous among the local population. Yet, they contaminate the demographic signatures of their new environment and cannot be ignored when digging around the galaxy for clues of past events.

We are presently in the middle of a boom in observational data on galactic structure, with large surveys such as the Sloan Digital Sky Survey and its extensions, as well as other similar projects, nearing completion. Additional efforts are commencing over the next several years, some of which are cornerstone missions like Gaia and high-profile observatories like the Large Synoptic Survey Telescope. Understanding the radial mixing process will be critical in interpreting the data from these surveys in the context of Milky Way disk structure and history.

A fundamental question is how efficient we expect the radial mixing process to be. To approach an answer, the engines of radial migration, nonaxisymmetric structures such as spiral arms, need to be understood. Are they steady or transient? How do their pattern

Theoretical insights combined with planned surveys of stellar populations will provide a clearer understanding of the evolution of spiral galaxies.

speeds change with radius and evolve with time? Recent work has reinvigorated the discussion on the subject but has also revealed a daunting range of possibilities highlighting the lack of a robust working theory, although some kind of transience seems to be required (4). Observational studies have measured spiral pattern speeds as a function of radius (5), and new data sets are mapping out disk structures in all tracers, from molecular and atomic gas to multiwavelength stellar photometry, in unprecedented detail. Integral field unit spectrographs are now being used to survey large numbers of galactic disks (6), providing structural, kinematic, and stellar population information. These new data sets will provide leverage for theorists to determine whether the spirals of computer simulations agree with those produced by nature in order to assess the robustness of their predictions for the radial mixing process.

Although the details remain elusive, it is difficult to explain existing observations of the solar neighborhood without invoking radial migration. For example, the local interstellar medium (ISM) metallicities are close to solar, but the metallicity distribution function reveals a substantial presence of super-solar metallicity stars across a range of ages. Unless the local ISM contained more heavy elements in the past and was subsequently diluted through the infall of pristine gas, these stars must have originated in the inner parts of the disk. Nevertheless, although migration helps explain many nuances of observed galactic disks, there are currently no unique predictions resulting from the process that could prove or refute its influence.

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The efficiency of migration in the Milky Way could in principle be measured by identifying families of stars that formed out of the same protostellar cloud based on their detailed chemical composition. Their radial distribution in the galaxy today would then give a measure of the diffusion process (7). Such chemical tagging measurements are among the primary goals of imminent spectroscopic surveys like the High Efficiency and Resolution Multi-Element Spectrograph (HERMES) project. Another enticing possibility for constraining migration is to look beyond the solar neighborhood, which has not been feasible with current data. However, with the unprecedented reach of Gaia and its complemen-

tary projects like the Gaia-European Southern Observatory survey (8), detailed structure of the disk at other radii will be probed for the first time in position-velocity-abundance space.

Radial migration is a dynamical effect in galactic disks that has over the past few years led to a conceptual shift in our approach to galactic archaeology. Major open questions remain with regard to the importance this process may have played in the growth of our own galaxy. If upcoming observations confirm that radial mixing has been influential in the history of the Milky Way, understanding it in detail will become increasingly critical in order to place it in a wider context of galaxy formation.

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## SYSTEMS BIOLOGY

# How Information Theory Handles Cell Signaling and Uncertainty

Matthew D. Brennan, Raymond Cheong, Andre Levchenko

Intracellular biochemical networks have traditionally been studied by stimulating populations of genetically identical cells and measuring the aggregate response. However, such population-based measurements may obscure the idiosyncrasies of individual cells and therefore suggest deceptively precise input-output relationships. Consequently, signaling pathways have been viewed as the finely tuned circuitry that programs the cell to behave in a predefined manner (1). Detailed studies of cellular biochemistry at the single-cell level now show that cells responding en masse may have quite varied behaviors when examined individually (2), raising the question of how precisely signaling pathways can control a cell's actions (3).

It is likely that under most circumstances, cells in populations constantly diversify their states (e.g., the response of their signaling networks) (4). Thus, there is an uncertainty in signaling outcomes, and responses of cells randomly selected from a population are unpredictable. In this sense, the match between the environmental input and the cellular output can no longer be predefined and stereotypically precise (5). This hampers the ability of individual cells or small cell ensembles to make appropriate decisions in fluctuating

environments, and requires a fundamentally different view toward analyzing signaling systems. Hence, rather than relying on seemingly robust and sensitive signaling input-output dependencies to analyze networks and cell behavior, we should instead seek to learn the limits to how well cell signaling can enable decision-making, given a cell's uncertain response to changes in the environment.

Variability in cell response is frequently referred to as "noise," and current metrics to characterize noise report on its magnitude but do not quantify how the noise limits the cell's decision-making abilities (6). Indeed, performance of a signaling network depends on more than just the level of noise in its underlying chemistry. For instance, signaling may allow a population of cells to simultaneously sample several distinct classes of behavior—a type of cellular bet hedging—which can improve some aspects of decision-making but with a cost of increased variability (7). Therefore, a new "language" may be needed to understand and quantify the impact of noise (variability) on a cell's functionality.

Mathematics turns out to have just the right theory. This theory has already been adopted to understand the workings of another type of "noisy" signaling network, the nervous system (8). Created to analyze uncertainty in human communication, information theory enables the limits of decision-making fidelity to be rigorously defined and measured (9).

Information theory allows analyses of cell signaling capabilities without necessarily requiring detailed knowledge of the signaling networks.

Conveniently, its general formulation permits analysis of many complex systems, including those found in biological signaling (10). Within this theory and in the context of signaling, information is quantified as the uncertainty about the environment that is removed by signaling activity (which is equivalent to the knowledge gained by the signaling system). The amount of information depends on both the amount of variability in the environment (the initial level of uncertainty) and noise in the signaling process itself (affecting the amount of uncertainty remaining). Extending this definition, we can also determine the information capacity of a system, which is the maximum information that a signaling system can obtain about some aspect of the environment under ideal conditions. This capacity is an intrinsic property of the signaling system, as much as the underlying chemistry, in that it is the key determinant of achievable decision-making fidelity (11).

As an example, consider a signaling pathway whose output measures the concentration of an extracellular ligand (i.e., a dose response). Signaling noise prevents a cell from determining the precise ligand concentration. However, does the noise also prevent a cell from resolving different concentrations of the ligand, and if so, how many and how accurately? Information theory states that it is possible to use the noisy signaling output to accurately discriminate different input doses (11).

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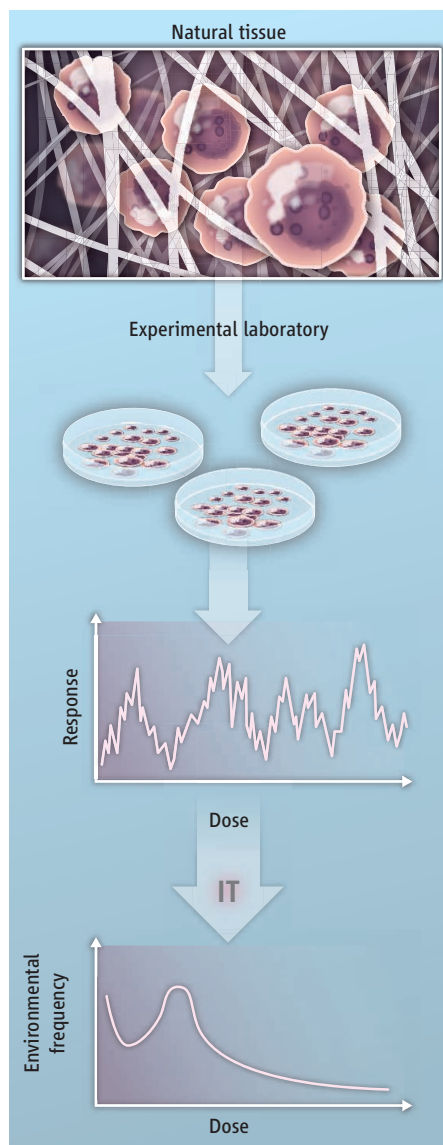


Furthermore, the number of resolvable concentrations is limited and is a simple function of the pathway capacity (12). Alternatively, if mistakes do occur, the capacity determines the minimum amount of error that a cell must tolerate, with higher capacity unambiguously allowing for lower error (13). Information theory allows such categorical statements without necessarily requiring detailed specifics of the signaling network organization and operation, and thus can be used to analyze the capabilities of complex and incompletely characterized biological systems.

Currently, we do not understand the decision-making limits of the vast majority of signaling systems, even those affected by variability. Consequently, the factors that affect and regulate those limits are also generally unknown. Thus, from the standpoint of information transfer, it is essential to determine the capacities of these signaling pathways and networks and the relationships between system structure and capacity. For instance, information lost at each step of processing should prevent information sources and destinations from being separated by more than a few intermediates (14, 15). Simultaneously, it is often necessary to integrate multiple pieces of information within a cell. Both of these considerations drive the formation of so-called small world networks that are widespread in biological systems and other networks, in which a relatively short path connects any two signaling nodes (16). Such networks are configured so that multiple signals pass through central nodes, thereby raising the information theoretic question of how the signals are multiplexed through the hub so as to minimally interfere with one another (17).

Acquiring information typically costs the cell energy, time, or opportunity, so a signaling system that collects more information than is necessary or ignores information that is easily obtained wastes valuable resources. Therefore, under evolutionary pressure, it's expected that signaling systems are optimally matched to the sources of information they have evolved to process. Indeed, examples from neuroscience (such as sensory perception) and developmental biology (such as embryonic patterning) show that biological systems usually have a capacity that is minimally sufficient for the information they process (18, 19).

This optimality principle can answer longstanding questions that cannot currently be addressed through models or direct experiments. One example is determining which of several putative aspects of environmental input (e.g., ligand dose, rate of dose change, or duration of ligand presentation) are biologically



ically relevant. Information-processing optimality suggests that the aspect of the input associated with a higher capacity is the more pertinent one. This concept further implies that the conditions that maximally utilize the information capacity of a sensory system should reflect the natural fluctuations in the environment. These conditions can be computed from controlled laboratory observations, enabling a form of “inverse ecology” that is sometimes the only feasible way to gain insight into a cell's natural surroundings (20) (see the figure). Similar arguments can be used to infer which aspect of a cell's response to an environmental input is most relevant to that input (21).

As biologists are finding more uses for information theory, it is evolving to handle more complex biological networks (22–25). By rigorously quantifying the properties and limits of cellular information transfer, new

**Inverse ecology.** To determine the frequency with which a stimulus (such as a ligand) occurs in a cell's natural environment, a population of cells (genetically identical) can be exposed to the ligand in an experimental setting. Individual cell responses can be measured at a range of doses; that information can be used to discover information about a cell's natural environment. There is variation in response at each dose (noise). Mathematically, such experimental measurements provide a conditional probability distribution [ $P(\text{response}|\text{signal})$ ]. It is assumed that a cell is “optimized” to obtain the most information about the frequency of doses it encounters in its natural environment [ $P(\text{signal})$ ]. Information theory (IT) determines the  $P(\text{signal})$  that maximizes the information capacity of the signaling network that is responsive to the particular ligand; this reflects the dose frequency in the cell's natural environment.

questions will be formulated and answered within the single-cell paradigm shift that is already underway. As the ability to quantify the functionality of signaling networks improves, we will hopefully gain insight into the details of their underlying chemistry while gaining a deeper understanding of their higher-level organization and functionality.

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## GEOCHEMISTRY

# Life in the Early Triassic Ocean

David J. Bottjer

In the next 100 years, it is projected that Earth will move to a greenhouse climate state (1). The future ocean will not only be hotter but also more acidic and will contain extended zones with reduced oxygen (2, 3). Study of past periods of global warming helps to project what Earth and its biota will be like in this new state and what the journey to that state will entail. On page 366 in this issue, Sun *et al.* (4) show that beginning with the end-Permian mass extinction (~252.6 million years ago) and continuing for the next 5 million years, Earth's oceans were extremely hot, with stressful and commonly lethal effects on ocean life.

An ancient global warming event, the Paleocene-Eocene Thermal Maximum (PETM) (~55 million years ago), has been used as a model for predicting the outcomes of current global warming. However, the PETM may have lacked the severity of the current rate of change (5). Sun *et al.* instead study what is perhaps the most severe biotic crisis in Earth history: the end-Permian mass extinction, when biota did not recover until almost 5 million years later (6, 7). During the Late Permian and subsequent Early Triassic, eruption of the Siberian Traps igneous province through coal and other organically enriched deposits led to vastly increased greenhouse gas concentrations in the atmosphere (6). Sun *et al.* show just how hot the oceans were during this interval and elucidate the lethal effects of this warming on ocean life.

The unusually warm Early Triassic ocean that Sun *et al.* depict had equatorial sea surface temperatures that may at times have exceeded 40°C (for comparison, annual mean modern equatorial sea surface temperatures range from ~25° to ~30°C). This hot ocean was typically characterized by a widespread oxygen minimum

zone that commonly expanded from deep into shallow environments (6). The shallow anoxic waters were rich in hydrogen sulfide, as indicated by evidence for green sulfur bacteria (8). Along with increased temperatures, anoxia and sulfide toxicity are hypothesized as contributing factors to the end-Permian mass extinction and prolonged recovery (6, 8).



**Characteristic features of hot Early Triassic oceans.** (A) The abundance of calcimicrobial carbonates, exemplified by this reef composed of stromatolite domes from strata of earliest Triassic age in the western Taurus Mountains (Turkey) (10), was possibly due to high ocean temperatures, as suggested by Sun *et al.* The scale card shows centimeters on the left. (B) Bedding plane assemblage of earliest Triassic bivalves from near Dixon Mountain (Montana), representing a sea floor with primarily *Eumorphotis*, a common cosmopolitan and abundant sessile benthic marine mollusk of this time. Sun *et al.* interpret the abundance of such organisms as indicating that they were better adapted to conditions of high temperatures and low oxygen. Scale bar, 1 cm.

The processes that caused mass extinction on land greatly increased weathering and decreased plant life, leading to a substantial increase in the supply of land-derived sediments to shallow ocean regions (9). Microbial structures in marine sedimentary rocks, such as stromatolites and wrinkle structures, were unusually abundant, implying an ocean more dominated by microbes than is typical for the past 500 million years (see the figure, panel A) (6, 10).

These stressful environmental conditions, including episodes of ocean acidification (11), also caused restricted metazoan reef building, including a lack of corals, during the Early Triassic (6, 12, 13). Similarly, other shallow sea-floor environments were characterized by low biodiversity and cosmopolitan taxa (see the figure, panel B), many with reduced size (4, 6, 14), as well as a reduced depth and extent of bioturbation by infaunal organisms (6).

Sun *et al.* provide an elegant view on how increased ocean temperatures led to restriction of fish, marine reptiles (ichthyosaurs), and calcareous algae to high latitudes. Bryo-

About 250 million years ago, extremely hot ocean temperatures had lethal effects on ocean life.

zoans (colonial organisms that are a common benthic element in ancient and modern oceans) were also restricted to high northern latitudes during the Early Triassic (15), which may similarly be a reaction to increased sea temperatures. As also shown by Sun *et al.*, the lethal effects of increased temperature can operate over millions of years, whereas the lethal effects of ocean

acidification, increased anoxia, and high sulfide concentrations in shallow waters typically operate over shorter time intervals (6). It is thus likely that the sustained lack of corals in the Early Triassic was not mainly due to acidification or increased anoxia and high sulfide concentrations in ocean ecosystems, but rather to increased ocean temperatures.

The predominance of a few abundant and cosmopolitan benthic taxa in shallow sea-floor environments during the Early Triassic (see the figure, panel B) provides an opportunity to understand the effects of increased temperature and other factors on such widespread communities. The effects of a resurgent microbial world on surviving metazoans can also be addressed, as well as the character of microbial and metazoan/microbial reefs under these conditions. Sun *et al.* have also pointed to increased ocean temperatures as the mechanism for the reduced size of organisms in Early Triassic ocean ecosystems, and their analysis should stimulate further research into what factors affect variability in this size reduction phenomenon.

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CREDITS: (PANEL A) USED WITH PERMISSION FROM S. B. PRUSS/SMITH COLLEGE; (PANEL B) USED WITH PERMISSION FROM E. PETIOS AND K. A. RITTERBUSH/UNIVERSITY OF SOUTHERN CALIFORNIA

At the end of the Permian, Earth's continents were assembled into one giant supercontinent, Pangaea, which would have affected climate differently from today's dispersed continents (5). The physiological capabilities of marine organisms at the end of the Permian were different from those of the organisms that dominate today's oceans (6). Plankton with calcium carbonate skeletons had not yet evolved, and the extent (and hence the calcium carbonate buffering capacity) of seafloors covered in carbonate sediments was therefore more restricted than in modern oceans (5).

Despite these differences between then and now, this ancient global warming inter-

val can provide insights that are relevant to our understanding of the future global warming ocean. In particular, the effects of increased temperature, oxygen minimum zone development, and ocean acidification on different components of the marine fauna can be better understood. A goal of future research should be to discern which environments fared better, and which worse, during this time of heightened environmental stress. Of particular interest is the nature of linkages between benthic and pelagic ecosystems and how they were affected by increased temperatures, anoxia, and high sulfide levels, as well as linkages between marine and terrestrial environments.

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## ATMOSPHERIC SCIENCE

# Refining the Radiocarbon Time Scale

Paula J. Reimer

The annually formed layers of sediment from Lake Suigetsu have the potential to reveal information about environmental change in Japan over the past ~50,000 years with chronological precision as good as, or better than, the Greenland ice cores (1). Such precision is invaluable for synchronizing records and evaluating leads and lags in the global climate system. Perhaps even more important, the fragile leaves and seeds hidden within these layers (see the figure) provide a record of past atmospheric concentration of  $^{14}\text{C}$ , the radioactive isotope of carbon. As reported by Bronk Ramsey *et al.* on page 370 of this issue (2), this record stretches back over the full length of the radiocarbon age scale. The results are invaluable for improving the accuracy with which radiocarbon dates can be converted to the calendar time scale.

Radiocarbon ( $^{14}\text{C}$ ) is one of the main dating methods in archaeology and Earth science for the late Quaternary period back to ~50,000 years ago. The method is based on the predictable radioactive decay of  $^{14}\text{C}$ , which forms when neutrons generated from cosmic ray bombardment collide with nitrogen in the upper atmosphere. However, the  $^{14}\text{C}$  concentration in the atmosphere is not constant, because varying amounts of cosmic

rays reach the atmosphere and carbon storage and release from the ocean and biosphere change with time. To estimate the true age of an unknown sample, its radiocarbon age must be corrected for these changes.

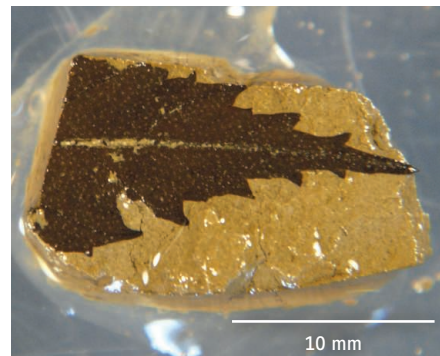
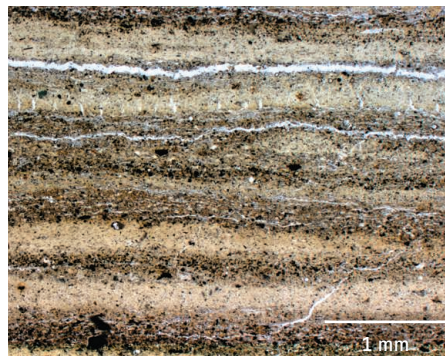
To ensure that radiocarbon dates are corrected consistently, scientists have been building internationally recognized calibration curves for many decades. The latest such curve, IntCal09, covers the whole radiocarbon age scale to ~50,000 years ago (3).

IntCal09 relies heavily on tree rings, which are ideal for building a correction (or calibration) curve, because the year of growth of the ring can be determined precisely, provided that the tree section to be measured can be matched to a master chronology. The old-

A highly resolved lake sediment record is set to improve the accuracy of calibrated radiocarbon dates between 12,600 and 50,000 years ago.

est securely dated master chronology goes back to 12,593 calendar years before the present (cal B.P., where "present" is set as 1950 A.D.). For calibration beyond this date, IntCal09 had to rely on  $^{14}\text{C}$  measurements of corals and the tiny shells of planktonic organisms found in marine sediments (3).

However, using the marine data to calibrate the atmospheric radiocarbon record is not straightforward. There is an apparent radiocarbon age difference of ~400 years on average between an organism living in the surface ocean and one living on land at the same time. This discrepancy arises because the ocean is a large carbon reservoir that responds more slowly than the atmosphere to changes in  $^{14}\text{C}$  production. The deep ocean also contains car-



**Radiocarbon correction beyond tree rings.** Bronk Ramsey *et al.*'s atmospheric  $^{14}\text{C}$  record, derived from measurements on terrestrial macrofossils (right) from the laminated sediments (left) of Lake Suigetsu, will help to improve the IntCal calibration curve used to estimate the true age of radiocarbon dated samples.

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bon that has become depleted in  $^{14}\text{C}$  through radioactive decay since it was entrained into the global circulation. Eventually, deep water upwells and mixes with the surface ocean, leaving it depleted in  $^{14}\text{C}$  relative to the atmosphere. Because changes in ocean circulation can result in variable upwelling of this deep water, the  $^{14}\text{C}$  record in marine organisms is attenuated and offset from the atmosphere by an amount that changes with time and location. To use the marine records in the terrestrial calibration curve for periods before the tree rings, the offset must be estimated, increasing the uncertainty of the calibration.

A direct atmospheric  $^{14}\text{C}$  record like that from Lake Suigetsu can be used to decrease the uncertainty in the calibration curve. It will also be invaluable for capturing higher-frequency oscillations in atmospheric  $^{14}\text{C}$ , which are not resolved in the IntCal09 curve for the time period before the tree rings. For stratigraphical series of radiocarbon ages—such as those used to date the earliest pottery from the Xianrendong Cave, China, to 20,000 years ago (4)—matching the radiocarbon dates from sediment layers to these oscillations will improve age estimates.

However, laminations in lake sediments are seldom, if ever, perfect. In the dark clay sediments of Lake Suigetsu, white layers are formed by the deposition of silica cases of algae (diatoms) in spring and summer; each layer is counted as 1 year (5). However, not all layers are preserved, and, in some cases, two diatom layers may be produced in a single year. Counting the layers thus has an associated uncertainty, which accumulates with depth. Bronk Ramsey *et al.* circumvent this problem to an extent by matching the  $^{14}\text{C}$  of uranium-thorium-dated stalagmites from the Bahamas (6) and China (7) to the Suigetsu  $^{14}\text{C}$  record, thereby reducing the uncertainty on the Suigetsu time scale.

The IntCal09 calibration curve assumes a constant atmospheric-surface ocean age offset ( $R$ ) to estimate the atmospheric  $^{14}\text{C}$  levels from the marine calibration records, which were selected from regions where ocean currents are believed to have been stable over the past 12,000 years. Through comparison with the Lake Suigetsu record, Bronk Ramsey *et al.* show that for these oceanographically stable regions,  $R$  was larger ~20,000 years ago than during the past 12,000 years, but not to the extent estimated by some authors (8, 9). This estimated variation in  $R$  will provide more realistic values for the marine data in the calibration curves.

Earth's geomagnetic field shields against cosmic rays, which produce  $^{14}\text{C}$  and other isotopes such as  $^{10}\text{Be}$  in the atmosphere. About

40,000 years ago, a substantial decrease of the geomagnetic field strength, the Laschamp event, nearly doubled the production rate of  $^{10}\text{Be}$  (10) and would have had a similar effect on  $^{14}\text{C}$ . The  $^{14}\text{C}$  production spike is attenuated in the IntCal09 marine records due to the large carbon reservoir. This has led to debates about whether radiocarbon calibration for atmospheric samples would be invalid for this time period (11, 12). In the Suigetsu record, the Laschamp effect is within 2 standard deviations of the IntCal09 calibration curve. Given radiocarbon measurement uncertainties of hundreds to even a few thousand years in this time period, the Laschamp effect will have minimal effect on calibration.

The Lake Suigetsu  $^{14}\text{C}$  record reported by Bronk Ramsey *et al.* does not have sufficient data density to provide a stand-alone calibration curve, but it will substantially augment and improve the atmospheric radiocarbon calibration curve. Work is in progress to update the IntCal calibration curve, which will include the Lake Suigetsu data. The

resulting improvement in the accuracy of calibrated radiocarbon dates will greatly affect studies of past climate and environmental change and human response to these changes.

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## CIRCADIAN RHYTHMS

# Circadian Surprise—It's Not All About Transcription

Colleen J. Doherty<sup>1</sup> and Steve A. Kay<sup>2</sup>

Posttranscriptional regulation plays a substantial role in controlling the mammalian circadian clockwork.

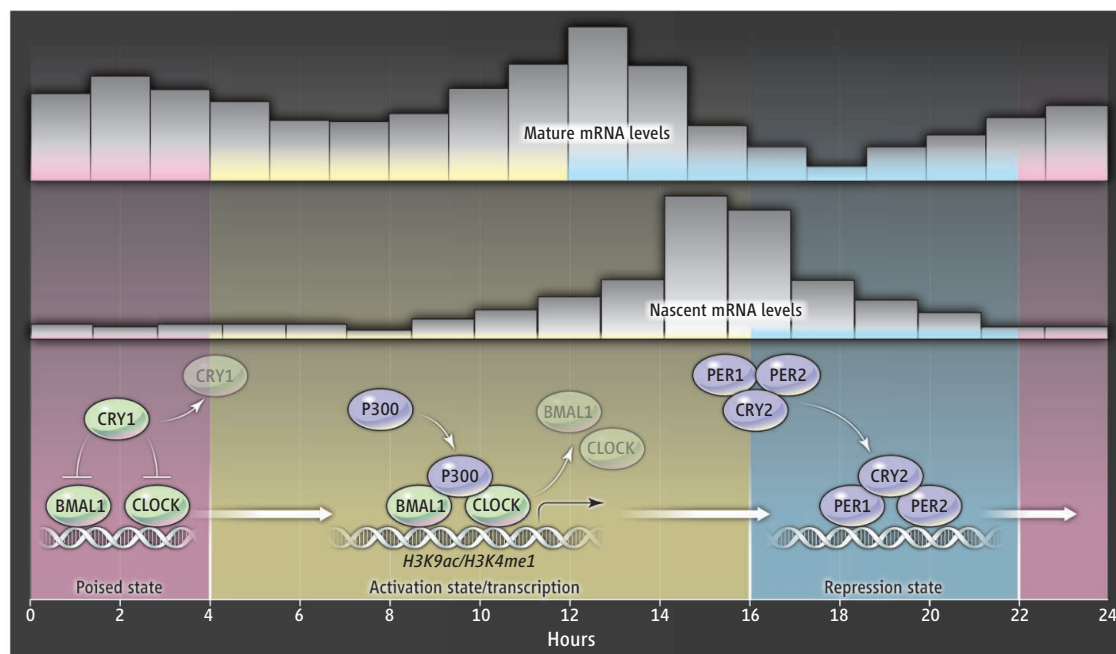
The integral role of the circadian clock in numerous aspects of health has prompted extensive study of the molecular architecture of clock networks. Understanding how the clock controls downstream processes has widespread clinical impacts, as it affects many diseases and biological processes including immune responses, metabolism, and aging (1–3). The primary molecular components of the mammalian oscillator and a detailed understanding of the regulatory interactions among them have been well characterized (4, 5). However, one critical question remains unanswered: How do the cogs of this clock translate the rhythmic regulatory relationship among themselves into the plethora of outputs that are under circadian

control? On pages 349 and 379 of this issue, Koike *et al.* (6) and Morf *et al.* (7) investigate this problem from opposite ends of the spectrum—a genome-wide discovery approach and the functional characterization of a specific factor, respectively—yet they converge to emphasize the importance of posttranscriptional regulation of messenger RNA (mRNA) levels on the clock.

Although a few specific connections have been identified (8–10), the direct global orchestration that occurs between transcriptional loops of the clock and the myriad of circadian-regulated phenotypic responses has been elusive. Koike *et al.* mapped in both time and genome-space the targets of many transcriptional components of the mammalian circadian clock. As they compared the regulation of these targets to their expression by RNA sequencing, the authors found a surprising disconnect between the nascent transcripts and the amounts of steady-state mRNA. This

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**RNA rhythmicity.** The model of the mammalian circadian clock depicts the temporal binding sequence of transcriptional proteins of the clock to regulatory DNA and the corresponding cyclic expression of nascent and mature mRNA.

implication of posttranscriptional control is echoed by Morf *et al.*, who identified a factor involved in circadian posttranscriptional regulation, cold-inducible RNA-binding protein (CIRP), and characterized its regulatory effects on the clock.

For more than a decade, circadian researchers have examined the cycling transcriptome in many tissues (11, 12). Consistently, the rhythmically expressed genes exhibited expression peaks throughout the entire day, with an enrichment for transcripts peaking at the dawn and dusk transitions. How is this wide daily range of expression achieved from a handful of circadian regulators that are themselves only present at specific times? The core transcriptional machinery of the mammalian circadian clock includes BMAL1, CLOCK, and NPAS2 proteins that positively regulate gene targets that contain the circadian cis-regulatory sequence known as an enhancer box (E box), or a slight sequence variant the E' box, in their promoters or enhancers. The circadian regulators PER1, PER2, PER3, CRY1, and CRY2 have a repressive role on these same targets. Koike *et al.* ascertained the exact temporal binding sequence of these factors on their targets by examining (in murine liver cells) changes in binding activity of six of the seven E/E' box regulators over a 2-day period by chromatin immunoprecipitation sequencing (ChIP-seq). These results were combined with an assessment of the transcriptional readiness of the chromatin, including occupancy of

DNA by RNA polymerase II, the presence of coactivators, and the occurrence of various active and repressive marks (biochemical modifications of chromatin). Their observations confirm the narrow phase of activity for each of these circadian regulatory factors. The simultaneous comparison of chromatin occupancy confirmed that arrival of factors in a “repressor precedes activator” manner contributes to the delay in expression (13), albeit through a different mechanism than previously proposed. The transcriptional activity takes place in three phases (see the figure). First, a transcriptionally poised state occurs as both the repressor, CRY1, and the activators, BMAL1 and CLOCK, occupy regulatory sequences. As the amount of CRY1 binding slowly decreases, the transition to activation (the second phase) occurs as the coactivator protein p300 and marks of active chromatin [histone acetylation (H3K9ac) and methylation (H3K4me1)] are established. In the final repressive state, BMAL1 and CLOCK occupancy decreases and accumulation of the repressive factors PER1, PER2, and CRY2 is observed on target genes. It will be interesting to see how this transition occurs at each individual promoter, whether the same DNA region can retain both repressors and activators in the poised state, or whether this observation reflects an average of multiple cells in different states of transition.

Consistent with this model, the amounts of nascent transcripts, as measured by intron amounts, appear to peak about 8 hours after

with an RNA polymerase inhibitor (15).

Morf *et al.* further explore this posttranscriptional regulation of rhythmic expression in their analysis of CIRP, an RNA binding protein regulated by systemic signals. In response to temperature cycles that resemble daily oscillations in body temperature, both the CIRP transcript and protein concentrations are rhythmic. However, unlike transcripts that are direct targets of the core transcriptional clock, CIRP fails to cycle in cultured mouse fibroblasts after cells are exposed to a sudden increase in serum concentration, a treatment that synchronizes the circadian phase of the individual cells. Cells with reduced amounts of CIRP can readjust their clocks faster than wild-type cells after being exposed to a mimic of jet lag that is induced by changing the temperature cycles. Such accelerated adaptation is predicted theoretically for low-amplitude oscillators. The loss of CIRP does indeed result in decreased mRNA cycling amplitude in many of the components of the molecular clock. In fact, the reduction of CIRP levels mimics the effects observed for CLOCK depletion, with a decrease in mRNA of many core circadian components: *Clock*, *Ncor1*, *Per2*, *Per3*, and *Dbp*. CIRP directly interacts with many of the circadian mRNAs, including *Clock*, and the loss of CIRP reduces amounts of cytoplasmic *Clock* mRNA and CLOCK protein throughout the day. This is likely through a direct mechanism as CIRP binds to *Clock* mRNA, as well as the mRNA of several other circadian factors.

the activation phase and prior to the repressive state. This sharp phasing of nascent transcriptional activity to a specific time in the organism's internal clock of 24 hours—circadian time (CT) 15—is in contrast to the range of phases observed for mRNA accumulation. Only 22% of the cycling mRNAs also cycle transcriptionally. These findings indicate that the majority of cycling transcripts are likely rhythmic as a result of posttranscriptional regulation, consistent with previous observations (14) including the continuation of cycling rhythms in cells treated

The extensive contribution of post-transcriptional regulation to cyclic mRNA abundance might explain why the connections between core clock mechanisms and circadian-regulated processes have been challenging to define. But at the same time, it opens up several additional questions. If transcriptional regulation is not driving the rhythmicity for the majority of cycling mRNAs, is the rhythmic transcription required for function? To what extent does CIRP regulate the difference in nascent and steady-state mRNA levels observed by Koike *et al.*? In addition to the phase of expression, examination of the interplay between transcriptional and posttranscriptional regulation may elucidate aspects of rhythmic expression such as amplitude of mRNA cycles. Under-

standing the regulation of amplitude can aid in identifying clinical targets to address the reduction in circadian amplitude observed with aging and some metabolic disorders.

Although the studies of Koike *et al.* and Morf *et al.* will likely shift the focus in mammalian circadian outputs to post-transcriptional regulation, the question remains of how the wide range of phases of circadian expression can be achieved. Steps toward answering this will include identifying the mechanisms by which RNA-regulatory processes, such as those involving CIRP, achieve specificity for their targets. Certainly, the models in which generation of overt rhythms is controlled primarily by transcriptional mechanisms require serious revision.

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## CHEMISTRY

# Unconventional Chemistry for Unconventional Natural Gas

Eric McFarland

Making most of our fuels and chemicals from fossil hydrocarbons is unsustainable. However, until cost-effective renewable sources are developed, natural gas could provide a secure economical alternative to petroleum. Proven reserves of natural gas have doubled in the last decade (1), mainly from increases in “unconventional” gas found with shale (shale gas), coal (coal bed methane), and in low-permeability “tight” sandstones (tight gas) (see the first figure). Today, because it is not easy to convert methane into heavier molecules, natural gas (composed largely of methane) is mostly burned for heating and electrical power generation; a tiny fraction is used in vehicles. Cost-effective conversion of natural gas into higher-value chemical intermediates and liquid products could reduce our need for oil and help lower its shipping costs, which are higher than those of petroleum or coal on an energy-delivered basis. In addition, such processes might recover the large quantities of gas now flared or vented from fossil reservoirs (2).

The challenge in converting natural gas into liquids, olefins, alcohols, ethers, and

other high-value products is to exploit reactions that only partially oxidize alkanes and stop short of complete combustion to CO<sub>2</sub>. Improvements in process efficiency and economics will come when new chemistry is integrated into novel engineering systems. Process chemistry—including free-radical routes or catalytic partial oxidation—already allows a fraction of the heavier alkanes in natural gas (ethane, propane, and butane) to be converted into more-valuable chemical products. Further innovations should increase the efficiency and expand the uses of these products and may also lead to ways to harness methane, which is typically between 70 and 90% of the carbon in natural gas.

Today, we activate most light alkanes by homolytically cleaving bonds using high temperatures (cracking) to create a complex radical reaction network that produces mixtures of coproducts and hydrogen (H<sub>2</sub>). Almost all industrial chemical processing of natural gas is performed with steam. In “steam crackers,” the steam acts as a relatively unreactive diluent that favors fragmented radical formation and minimizes solid carbon (coke). Direct reaction with water is kinetically limited by very short reaction times. A high yield of ethylene and propylene can be achieved under properly selected reaction conditions when the primary feed is ethane; but steam crack-

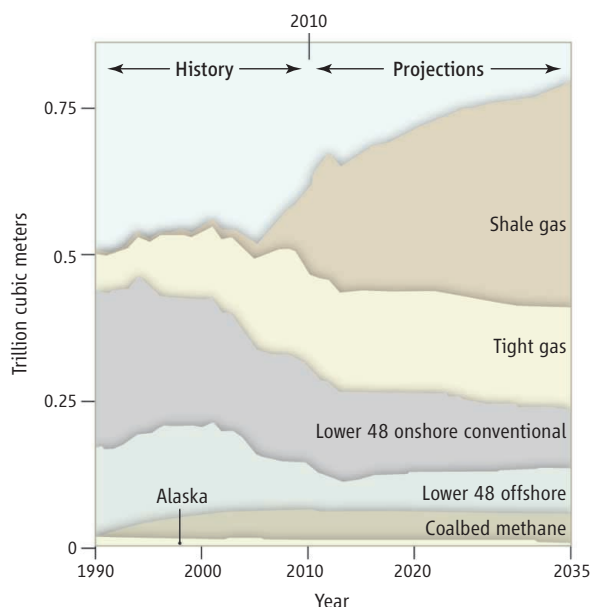
New process chemistries will be needed to replace petroleum with abundant natural gas as the most economical feedstock for fuels and chemicals.

ing still produces a mixture of olefin products along with other hydrocarbons and H<sub>2</sub>. Adjusting the conditions can bring the reaction toward equilibrium in “steam reforming,” where synthesis gas (H<sub>2</sub> and CO) is produced. Reforming of light alkanes accounts for ~50% of the world’s H<sub>2</sub> production used to produce ammonia and other important commodity chemicals. Synthesis gas made by reforming natural gas is used to make over 45 billion kg of methanol per year and high molecular weight linear alkanes (3).

It would seem that between cracking for olefin production and reforming to make synthesis gas, all of the chemistry needed to make use of natural gas is already available. However, steam cracking of ethane produces a mixture of products, ~15% of which are not high-value chemicals. Steam reforming is costly; it requires high operating pressures and temperatures (15 to 40 atm, 700° to 1000°C) in expensive reactors with heat transfer and coke management challenges. Overall efficiencies are 75% or lower. New low-temperature, high-yield alkane conversion chemistries that are relatively insensitive to common natural gas impurities are needed if we want to do better than cracking and reforming.

The problem is to both activate the strong C–H bonds and, under the same conditions,

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**Changing production sources.** Worldwide, about half of the known natural gas is in “conventional,” highly permeable geologic formations, often together with liquid petroleum. The other half is the “unconventional gas” found in more diffuse, low-permeability geologic formations. In the United States, conventional gas production peaked in the 1990s and today represents less than half of the annual U.S. gas production (15).

maintain control of the subsequent reaction intermediates and elementary steps—the sites for activation of C–H bonds must be relatively inactive for further oxidation. In general, greater selectivity comes at the cost of lower reaction rates. In nature, methane monooxygenase systems can have tremendous selectivity to partial oxidation products such as methanol; however, the conditions and overall volumetric rates cannot be scaled up for large chemical production volumes.

Under milder conditions than required for cracking or reforming, catalytic dehydrogenation of light alkanes selectively produces olefins and  $H_2$ . Worldwide, more than 100 billion kg of olefins are produced per year, and demand for propylene is increasing faster than it can be produced from traditional sources. Catalytic dehydrogenation provides selectivity to a single olefin product much higher than from cracking and can make use of the abundant propane in shale gas. The endothermic reaction requires substantial energy input, low reaction pressures, and a high temperature ( $\sim 500^\circ\text{C}$ ) to achieve even a low conversion. The alkane/olefin separation is difficult, requiring high pressure and low temperature. New engineering solutions such as low-cost hydrogen-membrane reactors to selectively remove hydrogen can help improve yields by driving the equilibrium-limited reaction forward. Use of inno-

vative chemistry to selectively remove (react) the hydrogen produced from alkane dehydrogenation without affecting the partial oxidation product is a major challenge for chemists. A long-sought goal is high yields from oxidative dehydrogenation (ODH) using oxygen to react away the hydrogen. Selective ODH of butane in DuPont’s maleic anhydride process uses a solid oxidizing agent, vanadium phosphorus oxide, to limit butane’s oxidation. The solid is reoxidized in a separate reaction step. The overall process is profitable and shows that cost-effective partial oxidation is possible.

Although there has been commercial success in making butadiene for rubber by ODH of butene with  $O_2$ , the high-yield production of olefins from alkanes by ODH has proven more challenging. Some progress for partial ox-

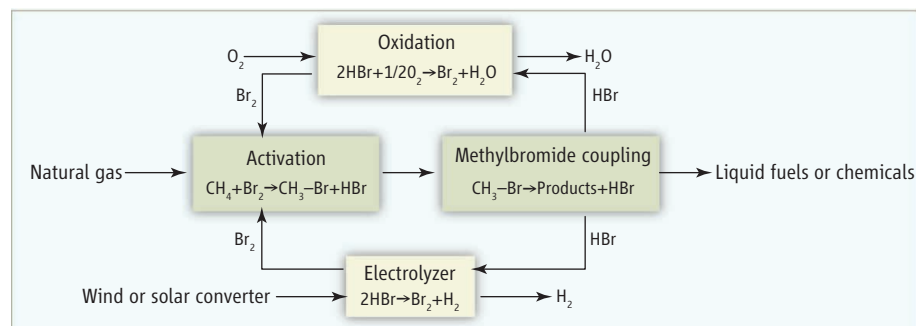
idation of other alkanes has been made with vanadium and molybdenum oxides (4). A single commercial plant in the Middle East uses a molybdenum-based oxide catalyst to convert very low-cost ethane to acetic acid; substantial improvements in ODH catalysts are still needed for widespread adoption.

A small amount of iodine facilitates the selective ODH of butane over metal oxide catalysts (5). Iodine may participate in the same kinetic steps occurring in oxyhalogenations whereby hydrogen halides are oxidized by  $O_2$  to form reactive halogens, which in turn oxidize the alkanes, resulting in readily separated alkyl halide products. Recent

work combining theory and experiment has improved our understanding of methane oxyhalogenation as a potentially selective partial oxidation pathway (6). Oxyhalogenation has been evaluated by industrial scientists as the lowest-cost natural gas-to-liquids (GTL) conversion process for converting methane to liquid fuels (7). Oxychlorination of ethane to produce vinyl chloride is a commercial process competing successfully with the less direct conversion of an ethylene intermediate to vinyl chloride.

In all the oxyhalogenation processes, corrosive components must be safely managed, which can increase the capital and maintenance costs substantially. With advances in corrosion science, lower cost, corrosion-resistant materials are increasingly available and may enable large-scale processes to be implemented safely and economically. One example is a platinum-catalyzed homogeneous system for conversion from methane to methyl bisulfate ( $CH_3OSO_3H$ ) in sulfuric acid, which can achieve  $\sim 90\%$  selectivity (8). The reactivity of the methyl bisulfate on the Pt catalyst active site is lower than that of the methane reactant, overcoming a major limitation of other alkane activation catalysts, and resulting in a single-pass yield of more than 70%.

Non-oxygen-containing oxidizing agents that might allow more control over the thermodynamics are promising alternatives that can eliminate water and carbon oxides as potential by-products. Oxidizing light alkanes with bromine or chlorine is facile under mild conditions and produces stable alkylhalides that are readily converted to halogen-free products. Bromine is used commonly for functionalization in organic synthesis because it is relatively easy to add and easy to remove (the C–Br bond is weaker than the C–Cl bond). Furthermore, bromine’s high molecular weight makes the alkylbro-



**Natural gas conversion with halogens.** Bromine reacts with methane to produce methylbromide, which behaves chemically like methanol and can be reacted over zeolite catalysts to produce aromatic-rich liquid fuels or olefins. The HBr coproduct can be oxidized in air (above) or electrochemically converted to bromine and hydrogen (below). The electrochemical step might use intermittent renewable electricity sources (such as solar or wind).



mides easy to separate by distillation from nonbrominated hydrocarbons. Ethyl or propyl bromides form with high selectivity and are readily dehydrobrominated to ethylene or propylene or oligomerized to high molecular weight hydrocarbons (9). Under moderate conditions, methane reacts with bromine to produce a high yield of methylbromide and HBr. By-products are readily separated, and dibromomethane reacts on transition metals with H<sub>2</sub> to produce similar products, as observed from Fischer-Tropsch chemistry with synthesis gas (10). Methylhalides, especially methylbromide, behave chemically like methanol on zeolites (see the second figure) to produce olefins or liquid fuels (11–13). Much less heat is generated, and there is no need to first produce methanol.

Ultimately, the bromine used for activation must be regenerated, either by oxidation of the HBr or by electrolysis to produce Br<sub>2</sub> and H<sub>2</sub> (see the second figure). Electrochemical recovery of bromine could make use of renewable process integration whereby tem-

porally varying wind or solar electricity is used to drive electrolysis of stored HBr to produce a bromine (a liquid at room temperature) reservoir used continuously in the hydrocarbon process.

Low-cost natural gas availability has beaten all expectations. In 2005, the U.S. government and chemical industry were preparing to import massive quantities of liquefied natural gas because of expected shortfalls in domestic supplies, and in 2008, coal was announced as the future hydrocarbon feedstock of choice (14). It is likely that natural gas, and possibly methane hydrates, will provide ample supplies of light alkanes for chemical processing for decades to come. A major challenge for chemical science is to invent new efficient and cost-effective processes for making use of alternative resources to replace those now made from oil. Such processes using natural gas are part of a stepwise transition toward a future where fuels and chemicals are produced from economically and environmentally sustainable sources.

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## NEUROSCIENCE

# Preventable Forms of Autism?

Arthur L. Beaudet

Autism has attracted enormous attention in recent years primarily because of the high incidence and the impact it has on patients' lives and their families, given its medical, social, financial, and emotional burdens (1, 2). There is growing acceptance that the incidence and prevalence of autism have dramatically increased over the past 20 years (3), although some of the increase relates to changing diagnostic terminology and criteria. The use of DNA microarrays and exome sequencing to detect pathological copy number variants (CNVs) and point mutations, respectively, is making progress in identifying genetic causes of autism. The recent confirmation that paternal age is a risk factor for autism relates to the occurrence of de novo point mutations that can now be discovered through next-generation sequencing (4). However, for virtually all of the pathological CNVs and point mutations causing autism, there is no definitive or curative therapy, although CNVs can be detected using invasive prenatal diagnosis.

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On page 394 of this issue, Novarino *et al.* (5) use exome sequencing in consanguineous families to discover an inborn metabolic error associated with autism, epilepsy, and intellectual disability, in which the clinical manifestations are likely to be treatable or, even better, preventable.

Empirical results reveal that the more severe and complex the autistic phenotype, the greater the likelihood that a causative mutation can be found. Most of the patients at the more severe end of the spectrum also have intellectual disability, and there is substantial overlap in the etiology for this group compared to that for intellectual disability in general. It has emerged from recent genetic studies that the number of different CNV loci and individual genes that—when mutated—can cause autism ranges into the hundreds, reflecting extreme genetic heterogeneity (6) (<https://gene.sfari.org/>). Another recurring observation is that the same genotype, whether CNV or point mutation, can give rise to a broad spectrum of phenotypes including autism, intellectual disability, schizophrenia, bipolar disorder, and epilepsy with various combinations of these being present in a single patient (variable expressivity), but with

Inborn errors of metabolism underlying some cases of autism present possibilities for prevention and treatment.

complete lack of penetrance (no symptoms) also occurring with such genotypes (7).

Novarino *et al.* identified a point mutation in a gene encoding the branched-chain keto acid dehydrogenase kinase (BCKDK). BCKDK inactivates an enzyme complex that converts branched-chain amino acids to the corresponding keto acids. Thus, defective BCKDK results in unchecked degradation and depletion of branched-chain amino acids. This is effectively the reverse of the metabolic problem present in maple syrup urine disease, where deficiency of the same enzyme complex leads to toxic accumulation of branched-chain amino acids and their metabolites. In a mouse model of BCKDK deficiency, similar metabolic and neurobehavioral abnormalities are found, and the animals improve upon dietary supplementation with branched-chain amino acids.

Assuming that it would be desirable to prevent rather than reverse symptoms of BCKDK deficiency, newborn screening could be explored. Over 50 inborn errors of metabolism are detectable by newborn screening, and its use in treatable disorders is particularly beneficial. It is not clear whether BCKDK deficiency would be easily detected

by newborn screening. It could involve measuring the plasma ratio of branched-chain amino acids to other amino acids or perhaps the ratio of branched-chain amino acids to their respective keto acids. If necessary, sequence analysis could be used. At this time, it is assumed that BCKDK deficiency will be quite rare, but newborn screening might prove to the contrary, and milder forms of the disorder may exist. Another rapidly evolving genetic strategy is universal carrier detection for autosomal recessive or X-linked mutations (8). Using universal carrier testing, couples might be alerted to their one-in-four risk of having an affected child even before conception. Then, they could consider preimplantation genetic diagnosis whereby only unaffected embryos would be

of trimethyllysine and reductions of other metabolites, although most infants identified would be expected to become normal adults even without intervention. The authors suggest that abnormalities of carnitine metabolism including intake, loss, transport, or synthesis may be important in a larger fraction of nondysmorphic autism cases.

The role of carnitine might be relevant for explaining part of the male predominance of autism because there is an estrogen-inducible, X-linked carnitine transporter at the blood-brain barrier (10, 11). The sex ratio in autism is quite noteworthy. For the severe end of the spectrum, the male:female ratio is between 2 to 1 and 4 to 1, but at the milder end of the spectrum, including Asperger syndrome, the male:female ratio is often 8

There may be a common thread for both of these newly discovered inborn errors of metabolism. For both disorders, physical examinations are generally normal with no dysmorphic features described (as is true for most inborn errors of metabolism). Both could involve neuronal deficiency of essential nutrients. Malnutrition is widely accepted to contribute to intellectual disability, and perhaps specific nutritional deficiencies (e.g., of branched-chain amino acids or carnitine) in the brain can lead to abnormal synaptic development. Maternal exposure to famine increases the risk of schizophrenia. At the time that solid foods are introduced into the diet, fruits, cereals, and vegetables are usually offered for several weeks and months. These foods are especially low in carnitine and branched-chain amino acid content, and neuronal deficiency of any of these nutrients could provide a mechanism to explain the regression that can occur in autism. From this perspective, dietary intake, infection, other factors causing loss of nutrients, and altered transport across the blood-brain barrier all become interesting variables. A recent study on twin pairs increased the evidence for a role of shared environmental factors in addition to genetic heritability in the etiology of autism (14). The level of metabolites in the cerebrospinal fluid would be of great interest for both disorders, but no data are yet available. Intensive metabolomic analyses of cerebrospinal fluid (15) in cases of autism would be very worthwhile and might uncover other treatable or preventable forms of autism.

| HYPOTHESIS FOR A Milder, MORE HOMOGENEOUS FORM OF AUTISM    |                                               |
|-------------------------------------------------------------|-----------------------------------------------|
| Severe, complex, syndromic with genetic etiology            | Milder, sole autism with unknown etiology     |
| Lower mean IQ                                               | Higher mean IQ                                |
| Mix of de novo and inherited mutations                      | Unknown genetic inheritance                   |
| CNVs and point mutations                                    | Unknown genetic mutations                     |
| Extreme genetic heterogeneity                               | Possibly less heterogeneity                   |
| Environment less important                                  | Environment more important                    |
| Paternal age relevant                                       | Paternal age less relevant                    |
| Sex ratio 2–4 to 1                                          | Sex ratio 4–8 to 1                            |
| Often dysmorphic/complex                                    | Nondysmorphic                                 |
| Microcephaly or extreme macrocephaly                        | Mild transient macrocephaly                   |
| Regression rare                                             | Regression more common                        |
| Treatment or prevention difficult except prenatal diagnosis | Possible optimism for treatment or prevention |

**Hypothesis for an alternative subtype of autism.** Characteristics of a proposed form of autism (right column) that is predominantly male and represents 10 to 20% of all autism.

implanted as an additional option.

BCKDK deficiency is not the first inborn error of metabolism to be linked with autism. Deficiency of carnitine biosynthesis was also reported recently as a likely risk factor for autism (9). The *TMLHE* (trimethyllysine hydroxylase, epsilon) gene maps to the X chromosome and encodes the first enzyme in the pathway for the biosynthesis of carnitine. Carnitine is an amino acid derivative necessary for transport of fatty acids from the cytoplasm into mitochondria. About 1 in 350 healthy males of European descent are deficient for *TMLHE*, and the penetrance for autism on a British or American diet would be only 2 to 4%, making replication studies essential to confirm the link to autism. In this case, toxic accumulation of trimethyllysine—or, more likely, neuronal carnitine deficiency—might cause the autism. Thus, carnitine supplementation might be useful for treatment or prevention. For *TMLHE* deficiency, newborn screening is straightforward, based on increased concentrations

to 1 or greater. One group found a sex ratio of 23 to 1 in patients with a normal physical examination and a normal MRI of the brain (12). Part or all of the sex ratio at the severe end of the spectrum may be explained by CNVs or point mutations affecting causative genes located on the X chromosome; but identification of causative mutations is rare for the milder end of the spectrum. The basis of the sex ratio and the etiology of the autism are both very enigmatic for the milder end of the spectrum, but the sex ratio suggests that there may be a substantial group of at least 10 to 20% with a more homogeneous pathophysiology or etiology within the milder autism population that depends highly on the biological differences between males and females (see the table). Regression in behavioral or learning skills is common in autism, and there is evidence linking regression to transient milder macrocephaly (13). The putative increase in the incidence of autism is more prominent in children with higher levels of functioning (3).

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# Anticipating Critical Transitions

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Tipping points in complex systems may imply risks of unwanted collapse, but also opportunities for positive change. Our capacity to navigate such risks and opportunities can be boosted by combining emerging insights from two unconnected fields of research. One line of work is revealing fundamental architectural features that may cause ecological networks, financial markets, and other complex systems to have tipping points. Another field of research is uncovering generic empirical indicators of the proximity to such critical thresholds. Although sudden shifts in complex systems will inevitably continue to surprise us, work at the crossroads of these emerging fields offers new approaches for anticipating critical transitions.

About 12,000 years ago, the Earth suddenly shifted from a long, harsh glacial episode into the benign and stable Holocene climate that allowed human civilization to develop. On smaller and faster scales, ecosystems occasionally flip to contrasting states. Unlike gradual trends, such sharp shifts are largely unpredictable (1–3). Nonetheless, science is now carving into this realm of unpredictability in fundamental ways. Although the complexity of systems such as societies and ecological networks prohibits accurate mechanistic modeling, certain features turn out to be generic markers of the fragility that may typically precede a large class of abrupt changes. Two distinct approaches have led to these insights. On the one hand, analyses across networks and other systems with many components have revealed that particular aspects of their structure determine whether they are likely to have critical thresholds where they may change abruptly; on the other hand, recent findings suggest that certain generic indicators may be used to detect if a system is close to such a “tipping point.” We highlight key findings but also challenges in these

emerging research areas and discuss how exciting opportunities arise from the combination of these so far disconnected fields of work.

## The Architecture of Fragility

Sharp regime shifts that punctuate the usual fluctuations around trends in ecosystems or societies may often be simply the result of an unpredictable external shock. However, another possibility is that such a shift represents a so-called critical transition (3, 4). The likelihood of such transitions may gradually increase as a system approaches a “tipping point” [i.e., a catastrophic bifurcation (5)], where a minor trigger can invoke a self-propagating shift to a contrasting state. One of the big questions in complex systems science is what causes some systems to have such tipping

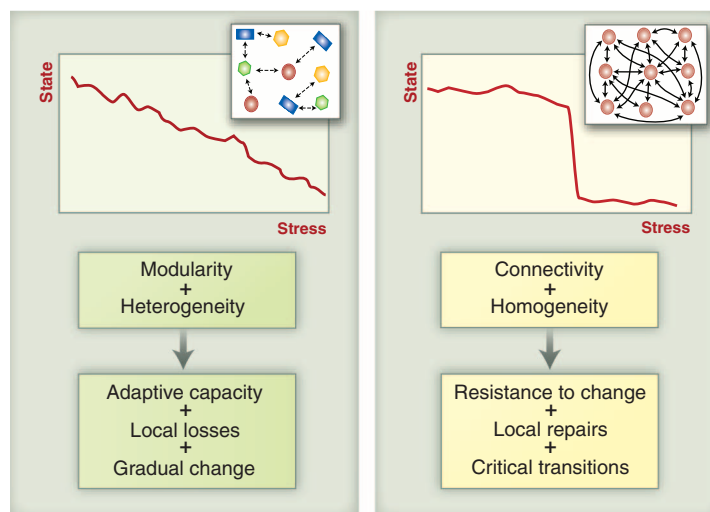
points. The basic ingredient for a tipping point is a positive feedback that, once a critical point is passed, propels change toward an alternative state (6). Although this principle is well understood for simple isolated systems, it is more challenging to fathom how heterogeneous structurally complex systems such as networks of species, habitats, or societal structures might respond to changing conditions and perturbations. A broad range of studies suggests that two major features are crucial for the overall response of such systems (7): (i) the heterogeneity of the components and (ii) their connectivity (Fig. 1). How these properties affect the stability depends on the nature of the interactions in the network.

**Domino effects.** One broad class of networks includes those where units (or “nodes”) can flip between alternative stable states and where the probability of being in one state is promoted by having neighbors in that state. One may think, for instance, of networks of populations (extinct or not), or ecosystems (with alternative stable states), or banks (solvent or not). In such networks, heterogeneity in the response of individual nodes and a low level of connectivity may cause the network as a whole to change gradually—rather than abruptly—in response to environmental change. This is because the relatively isolated and different nodes will each shift at another level of an environmental driver (8). By contrast, homogeneity (nodes being more similar) and a highly connected network may provide resistance to change until a threshold for a systemic critical transition is reached where all nodes shift in synchrony (8, 9).

This situation implies a trade-off between local and systemic resilience. Strong connectivity

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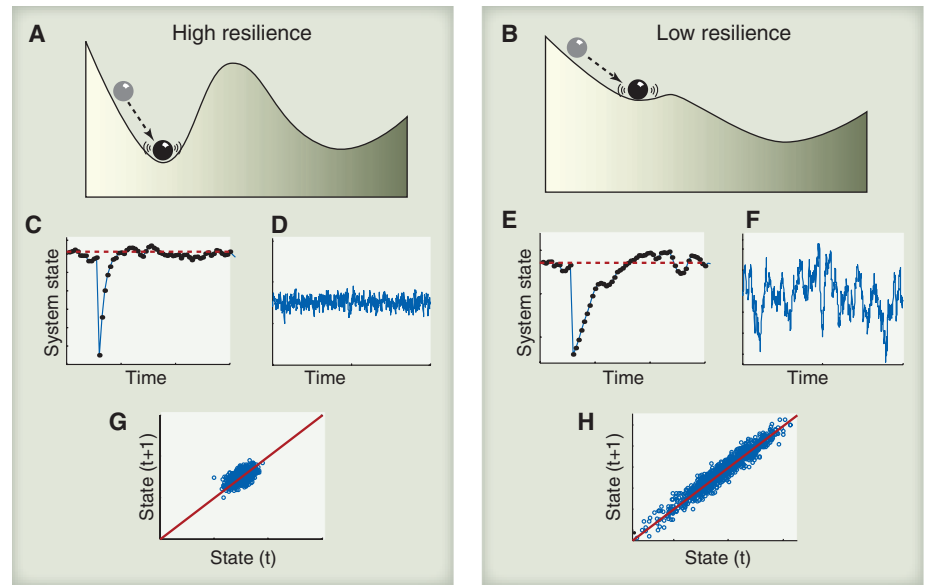
**Fig. 1.** The connectivity and homogeneity of the units affect the way in which distributed systems with local alternative states respond to changing conditions. Networks in which the components differ (are heterogeneous) and where incomplete connectivity causes modularity tend to have adaptive capacity in that they adjust gradually to change. By contrast, in highly connected networks, local losses tend to be “repaired” by subsidiary inputs from linked units until at a critical stress level the system collapses. The particular structure of connections also has important consequences for the robustness of networks, depending on the kind of interactions between the nodes of the network.



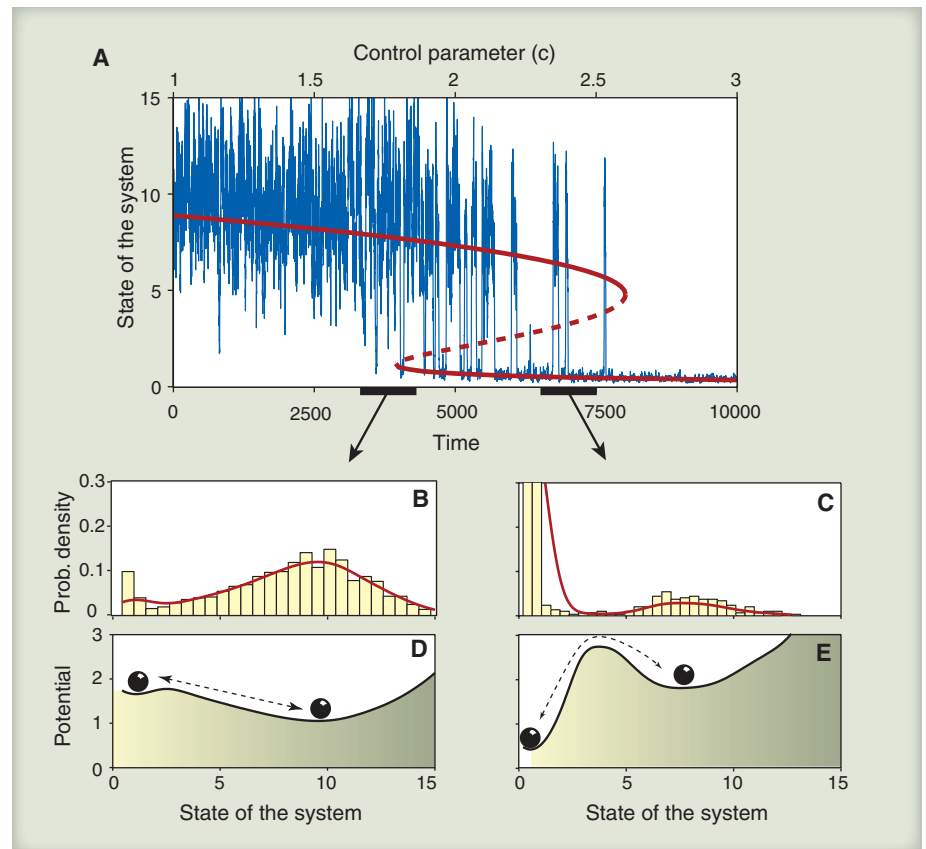
promotes local resilience, because effects of local perturbations are eliminated quickly through subsidiary inputs from the broader system. For instance, local damage to a coral reef may be repaired by “mobile link organisms” from nearby reefs, and individual banks may be saved by subsidiary inputs from the larger financial system (10). However, as conditions change, highly connected systems may reach a tipping point where a local perturbation can cause a domino effect cascading into a systemic transition (8). Notably, in such connected systems, the repeated recovery from small-scale perturbations can give a false impression of resilience, masking the fact that the system may actually be approaching a tipping point for a systemic shift. For example, before the sudden large-scale collapse of Caribbean coral systems in the 1980s evoked by a sea urchin disease outbreak, the reefs were considered highly resilient systems, as they recovered time and time again from devastating tropical storms and other local perturbations (11). In summary, the same prerequisites that allow recovery from local damage may set a system up for large-scale collapse.

**Robustness in different kinds of networks.** In addition to the work on systems where units can switch between alternative states in a contagious way, there has been an increasing interest in understanding robustness of webs of other kinds of interactions. For instance, species in ecosystems can be linked through mutualistic (+/+) interactions such as in pollinators and plants, or by competition (-/-) or predation (+/-). Rather than asking what causes the overall systems response to be catastrophic or gradual, most of these studies have focused on what topology of interaction structures makes the overall system less likely to fall apart when components are randomly removed. The answer turns out to depend on the kind of interactions between the units. Overall, networks with antagonistic interactions (e.g., competition) are predicted to be more robust if interactions are compartmentalized into loosely connected modules, whereas networks with strong mutualistic interactions (e.g., pollination) are more robust if they have nested structures where specialists are preferentially linked in their mutualism to generalists that act as hubs of connectivity (12, 13). Empirical studies in ecology suggest that the structures predicted to be more robust are also found most in nature (13–15), but this is an active field of research where new insights are still emerging (16) and much remains to be explored.

**The challenge of designing robust systems.** Work on ecological networks has led to the idea that we might apply our insights in the functioning of natural systems when it comes to designing structures that are less vulnerable to collapse. For instance, about half a year before the collapse of global financial markets in 2008, it was pointed out (17) that it could be helpful to analyze the financial system for the generic structural features that were found by ecologists to affect the risk



**Fig. 2.** Critical slowing down as an indicator that the system has lost resilience and may therefore be tipped more easily into an alternative state. Recovery rates upon small perturbations (C and E) are slower if the basin of attraction is small (B) than when the attraction basin is larger (A). The effect of this slowing down may be measured in stochastically induced fluctuations in the state of the system (D and F) as increased variance and “memory” as reflected by lag-1 autocorrelation (G and H).



**Fig. 3.** (A) Flickering to an alternative state as a warning signal in highly stochastic systems. In such situations, the frequency distribution of states (B and C) can be used to approximate the shape of the basins of attraction of the alternative states (D and E). The data in this example are generated with a model of overexploitation (38):  $\frac{dx}{dt} = x(1 - \frac{x}{K}) - \frac{cx}{1+x}$  with different additive and multiplicative stochastic terms (30) (we used  $K = 11$ ).

of systemic failure. Building on such parallels between the architecture of ecological and financial systems, Haldane and May (18) have made specific recommendations to encourage modularity and diversity in the financial sectors as a way to decrease systemic risk. There are still obvious challenges in bridging from ecosystems and conceptual models to societal structures, and much will be beyond our reach when it comes to “design.” For instance, the extremely fast global spread of information is an important feature of current social systems, and the worldwide connection of social-ecological systems through markets implies a daunting level of complexity (19). Nonetheless, this line of thinking about features that affect robustness across systems clearly offers fresh perspectives

on the question of how we can make the complex networks on which we depend more robust.

Early-Warning Signals for Critical Transitions

Although such insight into structural determinants of robustness and fragility can guide the design of systems that are less likely to go through sharp transitions, there are so far no ways in which these features can be used to measure how close any particular system really is to a critical transition. A new field of research is now emerging that focuses on precisely that (20).

*Critical slowing down near tipping points.* One line of work is based on the generic phenomenon that in the vicinity of many kinds of tipping points, the rate at which a system recovers from small perturbations becomes very slow, a

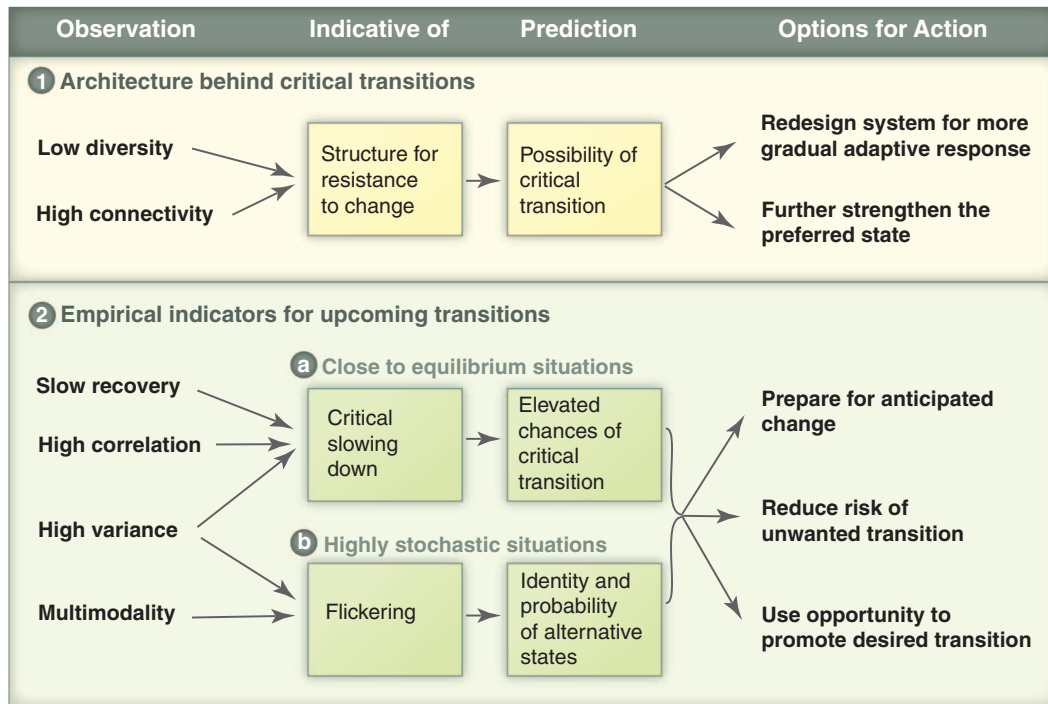
phenomenon known as “critical slowing down” (Fig. 2). This happens, for instance, at the classical fold bifurcation, often associated with the term “tipping point,” as well as more broadly in situations where a system becomes sensitive so that a tiny nudge can cause a large change (20). The increasing sluggishness of a system can be detected as a reduced rate of recovery from (experimental) perturbations (21, 22). However, the slowness can also be inferred indirectly from rising “memory” in small fluctuations in the state of a system (Fig. 2), as reflected, for instance, in a higher lag-1 autocorrelation (23, 24), increased variance (25), or other indicators (26, 27).

Not all abrupt transitions will be preceded by slowing down. For instance, sharp change may simply result from a sudden big external impact. Also, slowing down of rates can have causes other than approaching a tipping point (e.g., a drop in temperature). Therefore, slowing down is neither a universal warning signal for shifts nor specific to an approaching tipping point. Instead, slowing down should be seen as a “broad spectrum” indicator of potential fundamental change in the current regime. Further diagnosis of what might be coming up requires additional information.

*Changing stability landscapes in stochastic systems.* In highly stochastic systems, transitions will typically happen far from local bifurcation points. This makes it unlikely that in such stochastic situations slowing down is a useful characteristic to measure. Nevertheless, the behavior of systems exposed to strong perturbation regimes can hint at features of the underlying stability landscape. When an alternative basin of attraction begins to emerge, one may expect that in stochastic environments, systems will occasionally flip to that state, a phenomenon referred to as “flickering” (20). Rising variance can reflect such a change. Moreover, under certain assumptions, the probability density distribution of the state of a system can even be used to estimate how the potential landscape reflecting the stability properties of the system changes over time (28) or is affected by important drivers (29) (Fig. 3). The idea behind this approach is that even if stochasticity is large, systems will more often be found close to attractors than far away from them. The scope of this approach is different from that implied in work on critical slowing down. Slowing down suggests an increased probability of a sudden transition to a new unknown state. By contrast, the information extracted from more wildly fluctuating systems suggests a contrasting regime to which a system may shift if conditions change. Just as in the detection of critical slowing down, patterns in the data should be interpreted with caution. For instance, multimodality of the frequency distribution of states over a parameter range may be caused by nonlinear responses to other, unobserved drivers or from a multimodality of the distribution of such drivers. Also, the character of the perturbation regime may have a large effect.

**Table 1.** Studies of early-warning indicators for critical transitions in different complex systems. (+) Cases in which early warning signals were detected by indicators; (0) cases in which transitions were not preceded by indicators; (–) cases of unknown or opposite effect.

| Field                    | Phenomenon             | Indicator                               | Signal | References       |
|--------------------------|------------------------|-----------------------------------------|--------|------------------|
| Chemistry                | Critical slowing down  | Recovery rate/<br>return time           | +      | (39)             |
| Physics                  | Critical slowing down  | Return time/<br>dominant eigenvalue     | +      | (40)             |
|                          |                        | Rate of change<br>of amplitude          | +      | (41)             |
|                          |                        | Autocorrelation at lag 1                | +      | (42)             |
| Engineering<br>Tectonics | Not specified          | Autocorrelation/<br>spatial correlation | +      | (43)             |
| Climate                  | Critical slowing down  | Autocorrelation at lag 1                | +      | (23, 44, 45)     |
|                          |                        |                                         | 0      | (44, 46)         |
|                          |                        | Detrended fluctuation analysis          | +      | (27, 44)         |
|                          | Increasing variability | Variance                                | -      | (44)             |
|                          |                        |                                         | +      | (44, 46)         |
|                          |                        |                                         | 0      | (44)             |
| Ecology                  | Skewed responses       | Skewness                                | 0      | (47)             |
|                          |                        |                                         | +      | (22, 48–50)      |
|                          |                        |                                         | +      | (22)             |
|                          | Critical slowing down  | Return time/dominant eigenvalue         | +      | (48)             |
|                          |                        | Autocorrelation at lag 1                | +      | (48, 49, 51, 52) |
|                          |                        | Spectral reddening                      | +      | (48, 49, 52, 53) |
| Microbiology             | Increasing variability | Variance                                | 0      | (22, 54)         |
|                          |                        |                                         | +      | (48, 49, 55, 56) |
|                          |                        | Spatial variance                        | +      | (48, 49)         |
|                          | Skewed responses       | Skewness                                | +      | (57)             |
|                          |                        |                                         | +      | (57)             |
|                          |                        |                                         | +      | (57)             |
| Physiology               | Critical slowing down  | Return time                             | +      | (57)             |
|                          |                        | Skewness                                | 0      | (57)             |
|                          |                        | Recovery rate/<br>return time           | +      | (58)             |
| Epilepsy                 | Critical slowing down  | Correlation                             | +      | (59, 60)         |
|                          | Increasing variability | Variance                                | +      | (61)             |
| Behavior                 | Critical slowing down  | Recovery rate/<br>return time           | +      | (62, 63)         |
| Sociology                | Critical slowing down  | Autocorrelation at lag 1                | +/0    | (64)             |
|                          |                        | Variance                                | +/0    | (64, 65)         |
|                          |                        | Fisher information                      | +      | (66)             |
| Finance                  | Not specified          | Correlation                             | +      | (60)             |
|                          | Not specified          | Shannon index                           | +      | (67)             |
|                          | Not specified          | Variance                                | +      | (68)             |



**Fig. 4.** Different classes of generic observations that can be used to indicate the potential for critical transitions in a complex system.

*Prospects, challenges, and limitations.* Although research on empirical indicators of robustness and resilience is just beginning, there is already a fast-growing body of modeling as well as empirical work (Table 1). Nonetheless, major challenges remain in developing robust procedures for assessment. One problem is that methods for detection of incipient transitions from time series tend to require long, high-resolution data (23, 30). As a picture of a spatial pattern can carry much more information than a single point in a time series, the interpretation of spatial patterns is a potentially powerful option. Like increased memory in time series, correlation between neighboring units can reflect slowing down (31). Similarly, spatial data can be used to infer how resilience of alternative states depends on key drivers (29). Various aspects of spatial patterns may also change in specific ways near a critical point (31–36), but these patterns and their interpretation differ across systems in ways that are not yet entirely understood.

A fundamental limitation is that the indicators cannot be used to predict transitions, as stochastic shocks will always play an important role in triggering transitions before a bifurcation point is reached. Also, interpreting absolute values of indicators as signaling particular levels of fragility so far remains beyond reach. Thus, indicators should be used to rank situations on a relative scale from fragile to resilient. Detecting early-warning signals in monitoring time series may seem an obvious application. However, this requires the rare situation of having high-resolution data for a system that moves toward a tipping

point gradually (37). In addition to such challenges in detection, there are still gaps in our understanding of how indicators will behave in more complex situations. Given these limitations, there is no “silver bullet” approach. Instead, a diverse collection of complementary indicators and methods of applying them is emerging. A state-of-the-art overview linked to a Web site with open-source software for data analysis is published elsewhere (30) ([www.early-warning-signals.org](http://www.early-warning-signals.org)).

#### Toward an Integrative Approach for Anticipating Critical Transitions

So far, research on network robustness and work on empirical indicators of resilience have been largely segregated. However, connecting these fields opens up obvious new perspectives. First, there is complementarity in the existing approaches. The structural features that create tipping points and the different empirical indicators for their proximity offer alternative angles for diagnosis and potential action (Fig. 4). A smart combination of approaches in a unified framework may therefore greatly enhance our capacity to anticipate critical transitions.

At the same time, linking these two vital fields may generate exciting new directions for research. For instance, an intriguing question is how early-warning signals for loss of resilience may best be detected in a complex network (e.g., of species, persons, or markets). Will particular nodes in the network reveal critical slowing down or other early-warning indicators more than others? Can we know a priori which nodes

would carry such a clear signal? Or would some integrative indicator over the entire network be best? Clearly, this is an open area of research, and much may be gained by developing the different lines of work into an integrative science for understanding and predicting fragility and transitions in complex systems. Occasional radical transitions will continue to surprise us. However, the emerging field of research that we have sketched may reduce the realm of surprise in transitions related to tipping points.

Perhaps the most exciting aspect of this work is that it is uncovering generic features that should in principle hold for any complex system. This implies that we may use these approaches even if we do not understand all details of the underlying mechanisms that drive any particular system. This is the rule rather than the exception, as we are far from being able to construct accurate predictive mechanistic

models for most, if not all, complex systems. So far, most work on generic indicators of resilience has been carried out in ecology and climate science (Table 1). However, social sciences and medicine might well be particularly rich fields for exploration.

Developing sound predictive systems based on these generic properties poses major challenges. However, the potential gains are formidable. Empirically detecting opportunities where positive transitions in social or ecological systems can be invoked with minimal effort may be of great value. On the risk side, guidelines for designing financial systems that are less prone to systemic failure, or ways to foresee critical transitions ranging from epileptic seizures to the collapse of fish stocks or tipping elements of the Earth climate system, rank high in their importance to humanity.

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# Transcriptional Architecture and Chromatin Landscape of the Core Circadian Clock in Mammals

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The mammalian circadian clock involves a transcriptional feedback loop in which CLOCK and BMAL1 activate the *Period* and *Cryptochrome* genes, which then feed back and repress their own transcription. We have interrogated the transcriptional architecture of the circadian transcriptional regulatory loop on a genome scale in mouse liver and find a stereotyped, time-dependent pattern of transcription factor binding, RNA polymerase II (RNAPII) recruitment, RNA expression, and chromatin states. We find that the circadian transcriptional cycle of the clock consists of three distinct phases: a poised state, a coordinated de novo transcriptional activation state, and a repressed state. Only 22% of messenger RNA (mRNA) cycling genes are driven by de novo transcription, suggesting that both transcriptional and posttranscriptional mechanisms underlie the mammalian circadian clock. We also find that circadian modulation of RNAPII recruitment and chromatin remodeling occurs on a genome-wide scale far greater than that seen previously by gene expression profiling.

The circadian clock in mammals is cell autonomous and is composed of an autoregulatory transcriptional network with interlocked feedback loops (1, 2). At the core, the basic helix-loop-helix–PER–ARNT–SIM (PAS) transcriptional activators BMAL1, CLOCK, and NPAS2 activate the *Period* (*Per1* and *Per2*) and *Cryptochrome* (*Cry1* and *Cry2*) genes, whose transcripts and proteins slowly accumulate during the daytime (3–7). The PER and CRY proteins associate and translocate into the nucleus during the evening and physically interact with the CLOCK/NPAS2:BMAL1 complex to repress their own transcription (5, 7, 8). As the PER and CRY proteins are progressively phosphorylated during the night, they are targeted for ubiquitination by specific E3 ligases and are eventually degraded by the proteasome (9–12). The waxing and waning of this transcriptional feedback loop takes ~24 hours to complete and represents the core mechanism of the circadian clock in mammals. Biochemical analysis on a small set of target genes has shown that CLOCK, BMAL1, and CRY1 bind in a diurnal manner to regulatory regions; interact with p300 and CREB-binding protein (CBP); and are accompanied by rhythmic changes in histone H3 Lys<sup>4</sup> trimethylation (H3K4me3) and H3 Lys<sup>9</sup> acetylation (H3K9ac) (13–19) characteristic of active promoter regions (20–23).

Although the majority of the core components of the circadian gene network are likely known

(1) and many thousands of transcripts have been shown to express circadian oscillations in various tissues (24), the genome-wide architecture of the transcriptional network regulated by the core circadian clock remains to be defined (25, 26). Recent work has explored the genome-wide cis-acting targets (cistromes) of two sets of circadian transcription factors, BMAL1 (27, 28) and REV-ERB $\alpha$  and  $\beta$  (29–31). In liver, BMAL1 binds to ~2000 genomic targets in a diurnal pattern with peak DNA binding occurring during the day (28). REV-ERB $\alpha$ / $\beta$  bind to thousands of sites in the genome and have 28% overlap with BMAL1 peaks (30). Despite these initial genome-wide views of BMAL1 and REV-ERB $\alpha$ / $\beta$  cistromes, a comprehensive analysis of both the activators and the repressors within the core circadian transcription factor network has not been reported under constant conditions.

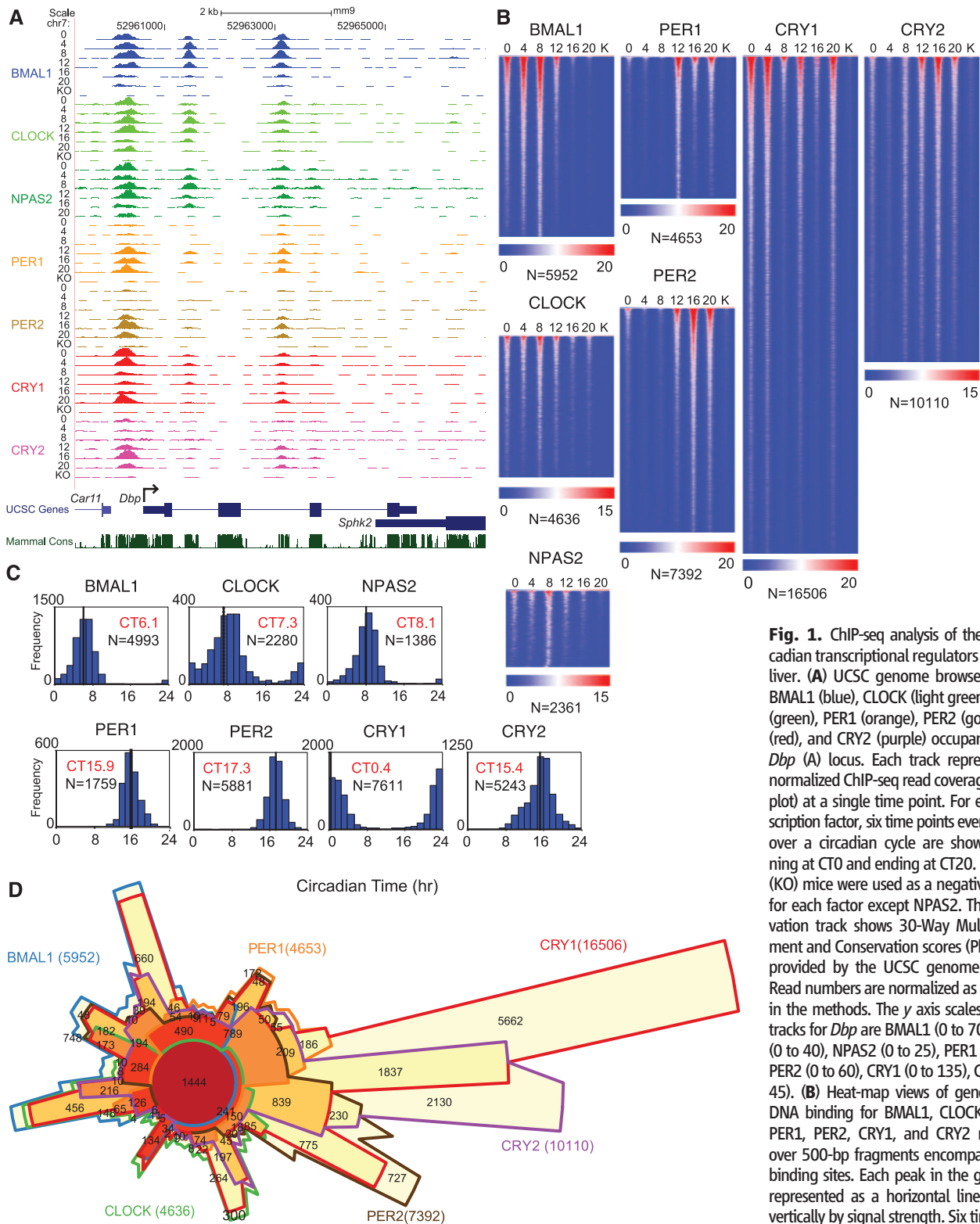
**Circadian cistrome of the liver.** We used chromatin immunoprecipitation sequencing (ChIP-seq) to locate DNA binding sites for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, and CRY2 in vivo in murine liver. We report here the results of ~200 ChIP-seq samples encompassing more than 9 billion sequence reads (see Materials and Methods, fig. S1A, and table S1). The cistromic landscape of the core circadian transcriptional regulators is shown at the *Dhbp* locus as a University of California Santa Cruz (UCSC) genome browser view (32) (Fig. 1A). Similar to that reported under diurnal lighting conditions (14, 28), BMAL1 binds in a circadian manner between circadian time (CT) 0 and 12 to three sites in *Dhbp* with peak binding phases occurring at CT4 to 8. We also confirmed that rhythmic binding of BMAL1 is sustained over two cycles in constant darkness (fig. S2). The heterodimeric partners of BMAL1, CLOCK and NPAS2, also bind in a time-dependent manner

similar to BMAL1 (Fig. 1A). The repressors PER1, PER2, and CRY2 then bind the same sites between CT12 and 20 (Fig. 1A). In contrast, CRY1 exhibits maximal binding at CT0, which extends the repression phase into the beginning of the next cycle, when BMAL1, CLOCK, and NPAS2 occupancy increases again at CT0 (Fig. 1A). Heat-map representations of genome-wide peak location analysis illustrate the dynamics of binding during the circadian cycle in constant darkness (Fig. 1B). Because the DNA binding of these factors is time-dependent, the number of peaks detected at each time point varies. Thus, we developed a method to create a master peak list for each transcription factor that compares the overlap of peaks detected by the MACS and PeakSplitter peak-finding algorithms (33, 34) among the six time samples (fig. S3 and table S2). The genome-wide distributions of the peaks for each factor show that 31 to 36% of sites are intergenic and the remaining sites are enriched at promoter and intronic regions of genes (fig. S4). We find ~5900 BMAL1 sites, ~4600 CLOCK sites, and ~2300 NPAS2 sites for the activators and ~4600 PER1, ~7300 PER2, ~16,000 CRY1, and ~10,000 CRY2 sites for the repressors. Motif analysis shows that all seven factors are enriched for E-box sites as well as nuclear receptor binding sites (fig. S5). Similar to that seen at the *Dhbp* locus (Fig. 1A), the genome-wide occupancy rhythms of BMAL1, CLOCK, and NPAS2 peak at CT6 to 8; whereas PER1, PER2, CRY1, and CRY2 show binding rhythms that peak at CT15.9, CT17.3, CT0.4, and CT15.4, respectively (Fig. 1C). Gene ontology analysis shows that the target genes for all seven core circadian transcriptional regulators are highly enriched for metabolic pathways, pathways in cancer, and insulin signaling, as seen previously (28, 30, 35, 36) (tables S3 and S4).

To analyze the overlap of the binding sites of the circadian regulators genome-wide, we compared the six-way overlap of the factors BMAL1, CLOCK, PER1, PER2, CRY1, and CRY2 and show the results in a Chow-Ruskey diagram (Fig. 1D). At the core, 1444 sites in the genome are bound by all six factors. Binding site coverage plots of these common sites are shown in fig. S6A. Motif analysis of the common binding sites shows an enrichment for the canonical CLOCK:BMAL1 E-box motif (CACGTG) as well as CEBPA and a number of nuclear receptor motifs (fig. S7). Of particular interest are the large numbers of CRY1, CRY2, and PER2 sites that are uniquely bound by each factor and appear to be independent of BMAL1:CLOCK sites (Fig. 1D). Motif analysis of these unique sites shows a reduction in E-box sites and enrichment for nuclear hormone receptor sites (fig. S7), consistent with recent reports that PER2 and CRY1 interact with nuclear receptors (37, 38). To visualize the dynamics of activator and repressor binding on a genome-wide level, we analyzed the overlap of the activators (BMAL1, CLOCK, and NPAS2) in comparison to the repressors (PER1, PER2, CRY1, and CRY2) during the circadian cycle and see a reciprocal

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**Fig. 1.** ChIP-seq analysis of the core circadian transcriptional regulators in mouse liver. **(A)** UCSC genome browser view of BMAL1 (blue), CLOCK (light green), NPAS2 (green), PER1 (orange), PER2 (gold), CRY1 (red), and CRY2 (purple) occupancy at the *Dbp* (A) locus. Each track represents the normalized ChIP-seq read coverage (wiggle plot) at a single time point. For each transcription factor, six time points every 4 hours over a circadian cycle are shown beginning at CT0 and ending at CT20. Knockout (KO) mice were used as a negative control for each factor except NPAS2. The conservation track shows 30-Way Multiz Alignment and Conservation scores (PhastCons) provided by the UCSC genome browser. Read numbers are normalized as described in the methods. The y axis scales between tracks for *Dbp* are BMAL1 (0 to 70), CLOCK (0 to 40), NPAS2 (0 to 25), PER1 (0 to 35), PER2 (0 to 60), CRY1 (0 to 135), CRY2 (0 to 45). **(B)** Heat-map views of genome-wide DNA binding for BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, and CRY2 measured over 500-bp fragments encompassing the binding sites. Each peak in the genome is represented as a horizontal line, ordered vertically by signal strength. Six time points are shown beginning at CT0 and ending

at CT20 from left to right. Knockout (K) mouse control is shown at the far right. The number of peaks in the genome is indicated at the bottom. The blue-red gradient indicates the coverage or signal strength (normalized uniquely mapped reads per 10 million reads) for all binding sites in the genome. **(C)** Histograms showing circadian phase distributions of BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, and CRY2 binding rhythms. Cyclic binding was determined by ARSER ( $P < 0.05$ ), and the mean circular phases of peak binding as indicated in red are indicated by using Oriana. The number of cycling peaks is indicated in black. **(D)** Chow-Ruskey diagram showing the six-way overlap of BMAL1, CLOCK, NPAS2, PER1, PER2, CRY1, and CRY2 peaks. Master peak lists were used to determine peak overlaps, and an overlap was called if two peak summits were within 120 bp of each other. The red circle in the middle represents the overlap of all six factors. Lighter shades of red, orange, and yellow represent fewer overlaps of subsets. The boundaries for each protein are color-coded: BMAL1 (blue), CLOCK (green), PER1 (orange), PER2 (brown), CRY1 (red), and CRY2 (purple). The areas of each domain are proportional to the number of binding sites.

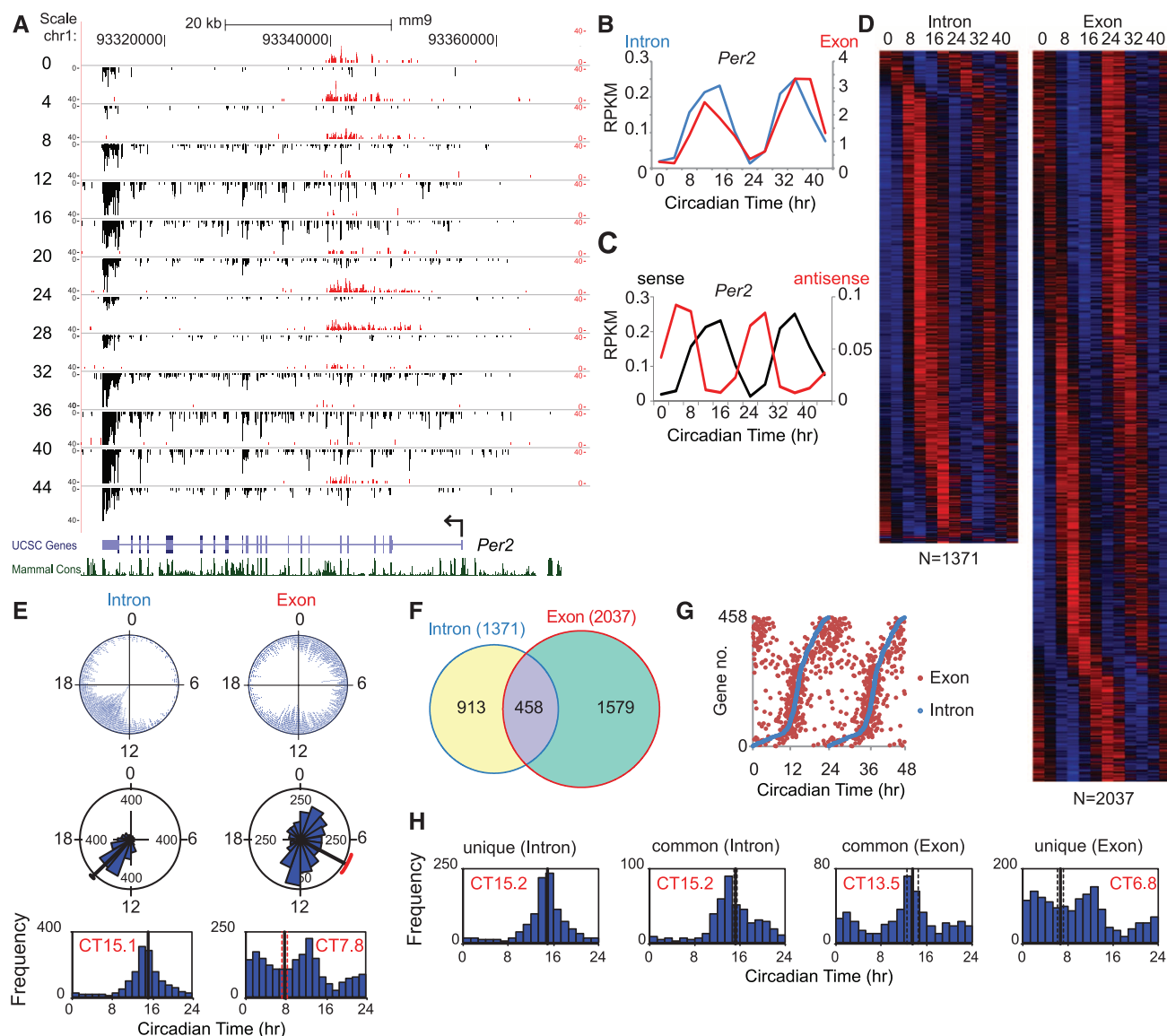


binding pattern between activators and repressors (fig. S6, B and C).

**Whole-transcriptome analysis of circadian gene expression.** In order to assess transcriptional readouts on a genome level, we used whole-transcriptome RNA-seq to measure circadian gene expression in the liver over a 48-hour interval sampled every 4 hours in constant darkness. A high-amplitude RNA rhythm can be seen at the *Per2* locus with a peak at CT12 and CT36 (Fig. 2, A and B). In addition, there is a long noncoding antisense RNA transcript that oscillates in antiphase

in a manner similar to that seen at the *Neurospora frequency* locus (39) (Fig. 2C and table S5). To quantitate RNA expression levels across the genome, we calculated the RNA-seq read coverage in reads per kilobase per million reads (RPKM) in exons and introns separately for the UCSC canonical gene set (28,661 total; 21,789 with introns). We interpret the intron signal as a representation of pre-mRNA expression or nascent transcription (40) and the exon signal as a representation of mRNA expression, which can reflect not only transcriptional activity but also posttranscriptional pro-

cessing events. At the *Per2* locus, the intron and exon signals both oscillate and their phases are similar (Fig. 2B), as seen previously using quantitative polymerase chain reaction measurements of pre-mRNA and mRNA (12, 14) (fig. S8). In genome-wide analyses, we detected 1371 intron and 2037 exon RNA cycling transcripts (tables S6 and S7), which are shown as heat maps ordered by the phase of RNA expression (Fig. 2D). Gene ontology analysis of both classes of cycling RNAs shows highly significant enrichment for metabolic pathways, pathways in cancer, and many other



**Fig. 2.** Whole-transcriptome RNA-seq analysis of circadian gene expression. (A) UCSC genome browser views of RNA-seq data at the *Per2* locus. Sense strand reads are shown in black and reverse strand reads in red as normalized average reads per 50 million total reads in 10-bp bins. The y-axis scales are 0 to 40 for the positive *Per2* strand and -40 to 0 for the negative *Per2* strand. (B) RNA-seq read coverage in RPKM reads in exons and introns. The intron (blue) and exon (red) RNA expression of *Per2* is circadian (ARSER;  $P < 0.01$ ). (C) Cyclic expression of *Per2* sense and antisense transcripts. The *Per2* antisense transcript is circadian (ARSER;  $P < 0.05$ ) and antiphasic (phase of sense, CT14.4; phase of antisense, CT7.1). (D) Heat-map views of intron- (left) and exon-

cycling genes. Each gene is represented as a horizontal line, ordered vertically by phase determined by ARSER. (E) The phase distribution of cycling genes. The phase of each transcript rhythm is represented in a dot plot (top), rose plot (middle), and histogram plot (bottom). The mean circular phase of the rhythms is indicated in red. (F) Venn diagram of intron and exon cycling genes. (G) Comparison of the phase of intron and exon cycling transcripts for common genes. The RNA peak phase of intron (blue) and exon (red) is double plotted and ordered by intron phase. (H) Histograms of intron phase in intron-only cycling genes, intron phase in common genes, exon phase in common genes, and exon phase in exon-only cycling genes (mean circular phase is shown in red).

pathways (tables S8 and S9). A comparison of the phase distribution of the intron and exon cycling transcripts reveals a difference: The intron cycling transcripts are clustered and peak at CT15.1, whereas the exon cycling transcripts have three peak phases and are distributed across all phases (Fig. 2E), as seen in previous microarray experiments (35, 41–43). Comparing the genes in the intron and exon cycling transcript classes shows that only 458 genes (22% of the exon cycling class) are in common (Fig. 2F), and this set of common genes is enriched for known circadian clock genes and high-amplitude cycling target genes reported previously (28, 43). The phases of the common intron and exon cycling transcripts are correlated, suggesting that transcriptional cycles primarily drive these mRNA rhythms (Fig. 2G). In the intron cycling/exon not cycling class, the cycling pre-mRNA transcripts are clustered at the same phase as the overall intron cycling class, but the steady-state mRNAs are likely to have long half-lives (>24 hours), which would damp oscillations generated at the transcriptional level as described previously (44, 45) (Fig. 2H). By contrast, in the intron not cycling/exon cycling class, the phases are widely distributed as seen in the overall exon cycling class, and these rhythms likely arise from posttranscriptional regulatory processes such as circadian changes in RNA splicing, polyadenylation, or mRNA stability (Fig. 2H) (46, 47). Taken together, the analysis of intron- and exon-specific cycling transcripts shows that circadian regulation of gene expression can occur at both transcriptional and posttranscriptional levels. The genome-wide phase clustering of the intron cycling transcripts is unexpected and suggests that a global circadian regulation of *de novo* transcription may exist.

**Circadian expression of RNAPII and coactivator occupancy.** To explore the origins of the global rhythms in nascent transcription, we analyzed the genome-wide occupancy of RNA polymerase II (RNAPII) as well as p300 and CBP coactivators as a function of the circadian cycle. The C-terminal domain (CTD) of the large subunit of RNAPII is modified at various stages of transcription (48, 49). RNAPII is recruited into the preinitiation complex with a hypophosphorylated CTD that is recognized by the 8WG16 antibody (50). The CTD is then phosphorylated on serine 5 (Ser5P) during initiation and is recognized by the 3E8 Ser5P antibody (51). We used ChIP-seq with these two antibodies to measure RNAPII occupancy and to estimate RNAPII recruitment and initiation (Fig. 3 and fig. S1B). On a genome-wide basis, we detected over 7300 RNAPII-8WG16 peaks and 20,000 RNAPII-Ser5P peaks, and RNAPII occupancy is circadian with 8WG16 signal peaking at CT14.5 and Ser5P signal peaking at CT0.6 (Fig. 3A). The peak of RNAPII-8WG16 at CT14.5 coincides closely with the peak of intron-cycling transcripts at CT15.1 (Fig. 2E), further supporting the idea that intron-cycling transcripts represent nascent or *de novo* transcription events. On the other hand, the peak of RNAPII-Ser5P occupancy

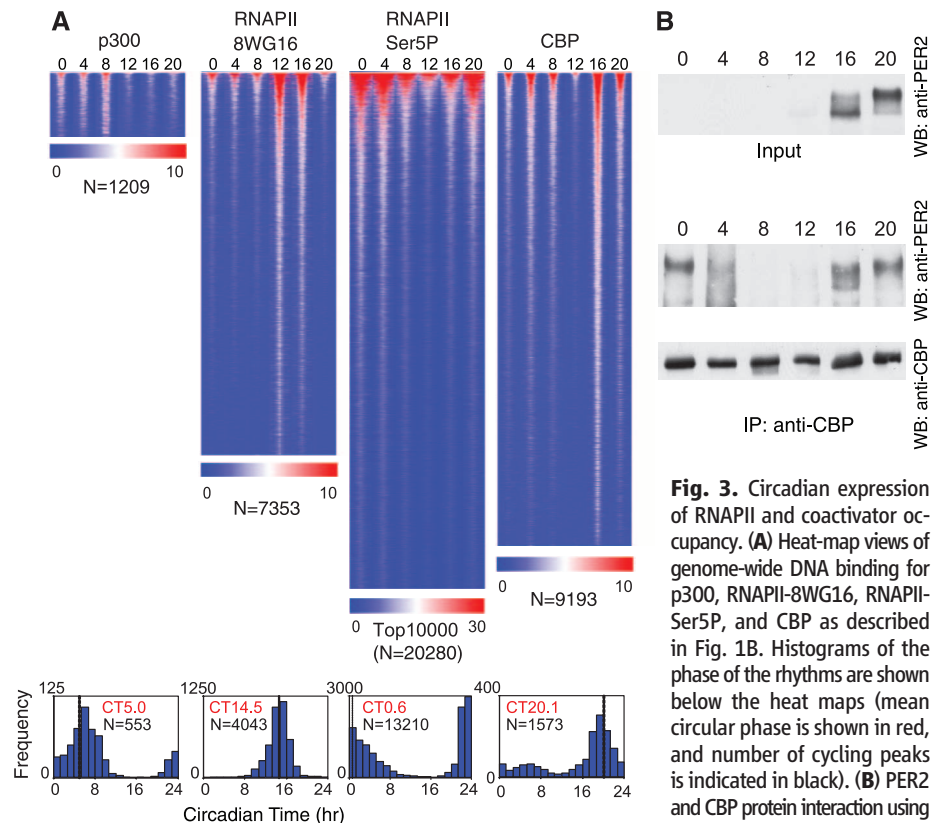
at CT0.6 coincides with the peak of CRY1 occupancy at CT0.4. At this time, CLOCK and BMAL1 are beginning a new cycle of binding but are transcriptionally silent, likely because CRY1 is bound at the same sites. One possible scenario is that RNAPII can be recruited by CLOCK:BMAL1 and that RNAPII initiation occurs but then pauses or stalls and accumulates at CT0. An example of this can be seen at the *Nr1d1* locus where RNAPII-Ser5P occupancy initiates at CT0, but RNA expression and RNAPII-8WG16 occupancy begin later at CT4 (fig. S9A; a 4-hour lag between RNAPII-Ser5P and transcription can be seen at many CLOCK:BMAL1 targets). Alternatively, RNAPII-Ser5P occupancy at CT0 could be independent of CLOCK:BMAL1 and could reflect a peak of transcription at CT0. For example, RNAPII-Ser5P occupancy at CT0 overlaps with REV-ERB $\alpha/\beta$  peaks (30) in the *Nfil3*, *Adck3*, and *Ppp1r3c* genes (along with ~300 intron cycling genes) in which peak RNA expression occurs between CT20 and CT4 (fig. S9, B to D). In these cases, CLOCK:BMAL1 and CRY1 sites are present nearby but do not appear to overlap with RNAPII-Ser5P. In these cases, RNAPII-8WG16 occupancy tends to occur in antiphase to RNAPII-Ser5P.

After this CRY1-repressed phase at CT0, CLOCK and BMAL1 occupancy increases at CT4 to 8 along with p300 occupancy (peak at

CT5) (Fig. 3A), and global nascent transcription begins at CT8 and peaks at CT15. Examination of the time-dependent pattern of CBP occupancy reveals that CBP can have either a 24-hour or a 12-hour binding rhythm with the 24-hour rhythm peaking at CT16 to 20 and the 12-hour rhythm peaking at CT4 and CT16 (Fig. 3A and fig. S10). Because CBP can interact at E-box sites either at CT4 or CT16, this suggests that CBP may be able to function as either a coactivator at CT4 or a co-repressor at CT16. In support of this hypothesis, we find that CBP can interact with the repressor, PER2, in native extracts in a time-dependent manner with maximal interaction occurring at CT16 to 20 when PER2 levels are elevated (Fig. 3B).

#### Circadian regulation of chromatin remodeling.

Given the genome-wide circadian rhythms of RNAPII and coactivator occupancy, we next assessed chromatin states associated with transcription initiation and elongation by using ChIP-seq during the circadian cycle (20, 22, 23, 52–55). Histone H3K4me3, H3K9ac, and H3K27ac are enriched at promoters and show robust circadian rhythms in occupancy at transcription start sites (TSSs) (Fig. 4A and fig. S1C). Histone H3K4me1, a mark that is characteristic of enhancer sites and gene bodies, exhibits a very subtle circadian modulation (Fig. 4B). Noticeably, there is an antiphase rhythm in H3K4me1 and H3K4me3



**Fig. 3.** Circadian expression of RNAPII and coactivator occupancy. (A) Heat-map views of genome-wide DNA binding for p300, RNAPII-8WG16, RNAPII-Ser5P, and CBP as described in Fig. 1B. Histograms of the phase of the rhythms are shown below the heat maps (mean circular phase is shown in red, and number of cycling peaks is indicated in black). (B) PER2 and CBP protein interaction using coimmunoprecipitation (IP) of

native extracts during the circadian cycle. Coimmunoprecipitation was carried out with antibody against CBP (anti-CBP) and mouse cerebellum protein lysate. The immunoprecipitates were visualized on Western blots (WB) with anti-PER2 or anti-CBP.

occupancy at the *Dbp* intron 1 site (fig. S1C, a and b). Consistent with recent reports (54, 55), histone H3K27ac is also highly enriched at both intragenic and intergenic enhancer sites. The elongation marks, H3K36me3 and H3K79me2, also express very low-amplitude circadian modulation (Fig. 4B). On a genome-wide level, circadian rhythms in RNAPII-8WG16, RNAPII-Ser5P, H3K4me3, H3K9ac, and H3K27ac occupancy at TSSs can be seen in all classes of expressed genes as compared with unexpressed genes (Fig. 4, A and B). These rhythms in RNAPII occupancy and histone modifications are stronger in intron RNA cycling genes (Fig. 4A), and rhythmic RNAPII-Ser5P elongation past the TSS can be seen prominently in cycling genes relative to all expressed genes (Fig. 4A and fig. S11). Unexpectedly, noncycling intron expressed genes also show circadian modulation of RNAPII occupancy and histone modifications similar to that seen in all expressed genes (Fig. 4A bottom). In contrast to the observation reported from the analysis of human embryonic stem cells that inactive genes harbor paused RNAPII (22, 56, 57), we see no evidence of RNAPII (8WG16 and Ser5P) pausing

at the promoters of unexpressed genes (Fig. 4A and fig. S11). Genome-wide analysis of the periodicity and phase of these histone marks reveals that large numbers of genes exhibit circadian rhythms in histone modifications (Fig. 4B). The overall number of histone modification sites does not appear to vary on a circadian basis; rather, the recruitment (and initiation) of RNAPII appears to underlie the variation in the amplitude of histone marks (fig. S12A). Thus, our analysis reveals that the majority of expressed genes are undergoing circadian histone modifications irrespective of whether RNA cycling can be detected.

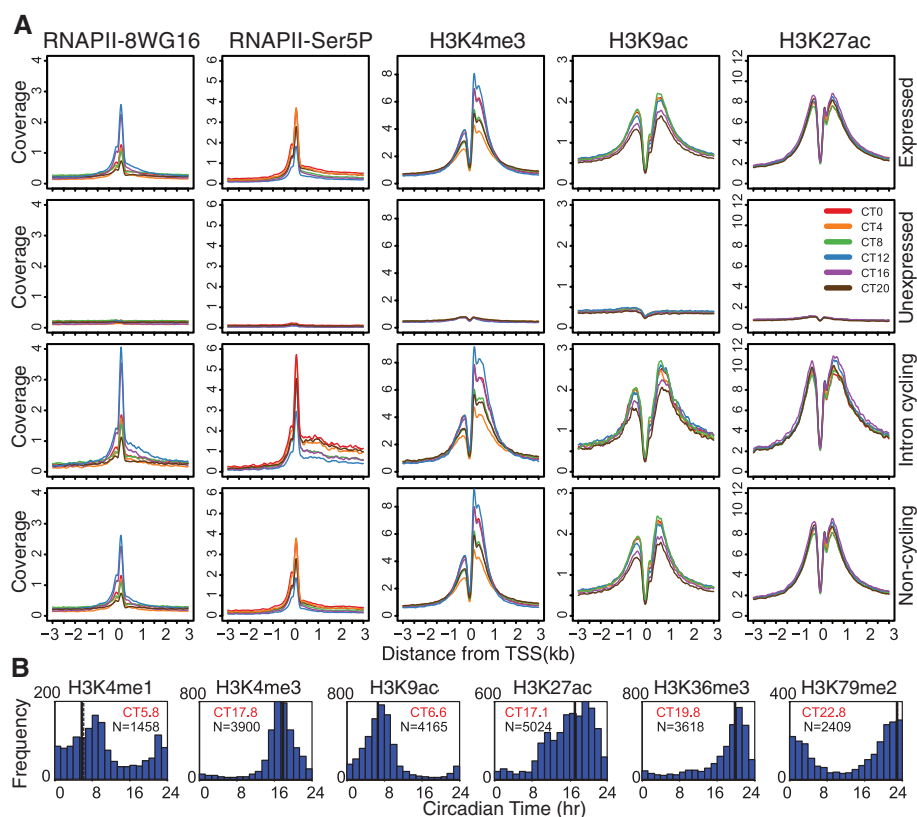
#### Circadian regulation at intergenic enhancers.

To determine whether circadian regulation of RNAPII occupancy and histone modifications also occurs at enhancer sites, we identified intergenic enhancer sites bound by either BMAL1 or CBP and compared these sites to BMAL1 or CBP promoter sites, respectively (fig. S12, B and C). Coverage profiles for BMAL1 show circadian occupancy that is similar at both promoter and enhancer sites with peaks at CT8. RNAPII-8WG16 profiles show circadian modulation at

BMAL1- and CBP-bound promoter sites as well as enhancer sites, with a peak at CT12 except that occupancy was much lower at enhancer sites. H3K4me1 showed enrichment at enhancer sites and depletion at promoter sites as expected and also showed a subtle circadian modulation in occupancy that peaked at CT8. In contrast, H3K4me3 was enriched at promoter sites and was lower at enhancer sites, but in this case the timing of the circadian modulation was different: Promoter sites peaked at CT12 and enhancer sites peaked at CT20. H3K9ac was enriched at promoter sites, but a circadian modulation can be seen at both promoter and enhancer sites with peaks at CT8. Last, H3K27ac was equally enriched at both promoter and enhancer sites and showed low-amplitude circadian modulation with peaks at CT16. Thus, overall we observe circadian modulation of BMAL1 occupancy, RNAPII occupancy, and histone modifications at both promoter proximal sites as well as distal intergenic enhancer sites.

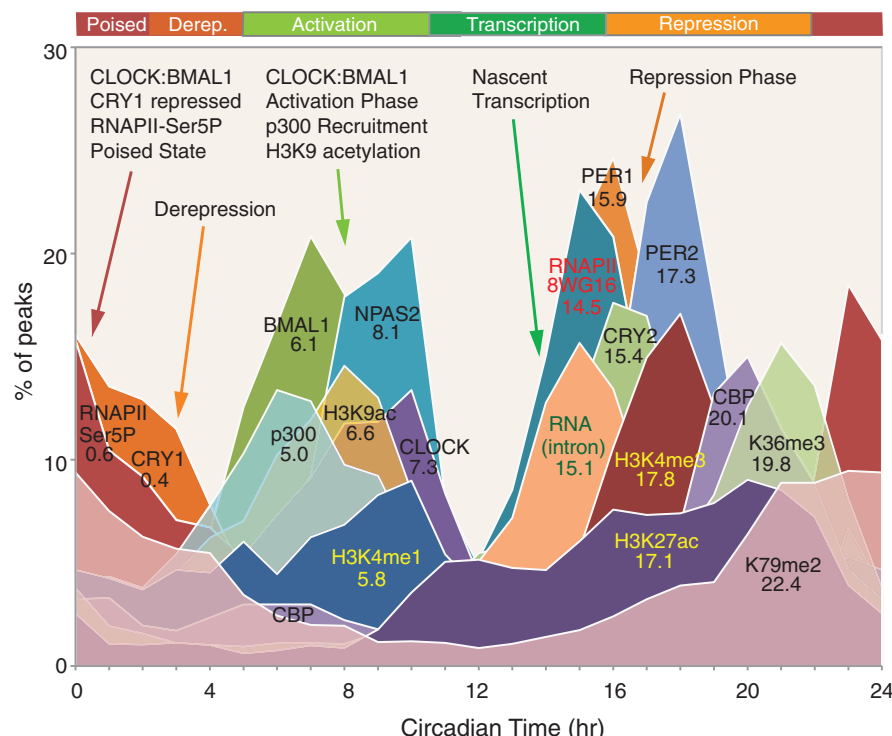
**Discussion.** We have defined the cis-regulatory network of the entire core circadian transcriptional feedback loop under constant conditions coupled with whole-transcriptome RNA expression and RNAPII recruitment and initiation, as well as chromatin states on a genome-wide basis. We find three distinct phases of the circadian transcriptional cycle of the clock in the murine liver: (i) a transcriptionally poised state, (ii) a global peak of de novo transcription that peaks at CT15, and (iii) a repressed state in which CLOCK:BMAL1 occupancy decreases at CT16 to 20 (Fig. 5). The poised state of CLOCK:BMAL1 bound with CRY1 is consistent with that seen in vitro (58) and in vivo (19) and is followed by derepression (59) as the occupancy of CRY1 declines and recruitment of the coactivator, p300, occurs. The details of this cis-regulatory cycle of circadian transcriptional regulators differ from that reported in *Drosophila*, in which PER sequesters CLOCK both on and off DNA and RNAPII appears to be paused and is regulated by elongation at the *period* locus and by recruitment at the *timeless* locus (60).

The dynamics of nascent transcription, RNAPII occupancy, and histone modifications provide important insights into the relations among these events. Although coactivator recruitment of p300 by CLOCK:BMAL1 and histone H3K9ac precede nascent transcription, there is a lag in histone H3K4me3 relative to RNAPII recruitment as measured by 8WG16 (Fig. 5). Histone H3K27ac also peaks well after nascent transcription, as do the elongation marks H3K36me3 and H3K79me2, and thus may reflect processes involved in the consequences of elongation rather than elongation events themselves (Fig. 5). Last, it will be of interest to explore the role of histone deacetylases (HDACs) and co-repressor complexes in circadian regulation. Both SIN3A/HDAC1 (61) and HDAC3 (29) have been found in PER2 and REV-ERBa complexes, respectively, as well as RNAPII accumulation at the termination sites of *Per* and *Cry* genes (62).



**Fig. 4.** Circadian regulation of histone modifications. **(A)** Binding profiles of RNAPII-8WG16, RNAPII-Ser5P, H3K4me3, H3K9ac, and H3K27ac at the TSS  $\pm$  3 kb in 12,680 expressed genes (top row), 8,945 unexpressed genes (second row), 1,371 intron cycling genes (third row), and 5,839 noncycling genes (bottom row). The y axis represents the average signal in a 10-bp bin (normalized uniquely mapped reads). **(B)** Histograms showing circadian-phase distributions of histone modification rhythms (ARSER;  $P < 0.05$ ). Mean circular phase is shown in red. The signal in TSS  $\pm$  1 kb was used to determine H3K4me1, H3K4me3, H3K9ac, and H3K27ac binding rhythm phases. The signal in the gene body was used for H3K36me3 and H3K79me2. Genes without MACS peaks in the corresponding area (TSS or gene body) were filtered out from the analysis.





**Fig. 5.** Circadian landscape of the cistrome and epigenome of the liver. Phase distributions of circadian transcriptional regulators, intron cycling RNA transcripts, and histone modifications as shown in Figs. 1C, 2E, 3A, and 4B. The mean circadian phase of peak binding is indicated under the name.

Although global circadian rhythms in steady-state mRNA levels have been known for a decade (35, 41, 42), here we find that only ~22% of cycling mRNA transcripts are driven by transcription (Fig. 2). Therefore, posttranscriptional regulatory events must contribute significantly to the generation of steady-state cycling mRNA levels (46, 47). Unexpectedly the most pervasive circadian regulation observed on a genome scale are rhythms in RNAPII recruitment and initiation, H3K4me3, H3K9ac, and H3K27ac, which occur at thousands of expressed genes whether or not RNA cycling was detectable. What accounts for these genome-wide circadian rhythms in RNAPII occupancy and histone modifications? Examination of the correlation between circadian transcription factor occupancy and gene expression shows that about 90% of genes bound by these factors are expressed whereas only 1 to 5% of unexpressed genes are similarly bound (table S10). These results demonstrate that gene expression per se, rather than rhythmicity of gene expression, is tightly correlated with circadian transcription factor binding. Rhythmic circadian transcription factor occupancy in turn could then be responsible for RNAPII recruitment and initiation on a genome-wide basis, which would then lead to the global rhythmic histone modifications seen here. Thus, circadian transcriptional regulators appear to be involved in the initial stages of RNAPII recruitment and initiation and the histone modifications associated with these events to set the stage for gene expression on a global

scale, but additional control steps must then determine the ultimate transcriptional outputs from these sites.

#### References and Notes

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#### Supplementary Materials

[www.sciencemag.org/cgi/content/full/science.1226339/DC1](http://www.sciencemag.org/cgi/content/full/science.1226339/DC1)  
Materials and Methods  
Figs. S1 to S13  
Tables S1 to S11  
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# Jet-Launching Structure Resolved Near the Supermassive Black Hole in M87

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Approximately 10% of active galactic nuclei exhibit relativistic jets, which are powered by the accretion of matter onto supermassive black holes. Although the measured width profiles of such jets on large scales agree with theories of magnetic collimation, the predicted structure on accretion disk scales at the jet launch point has not been detected. We report radio interferometry observations, at a wavelength of 1.3 millimeters, of the elliptical galaxy M87 that spatially resolve the base of the jet in this source. The derived size of  $5.5 \pm 0.4$  Schwarzschild radii is significantly smaller than the innermost edge of a retrograde accretion disk, suggesting that the M87 jet is powered by an accretion disk in a prograde orbit around a spinning black hole.

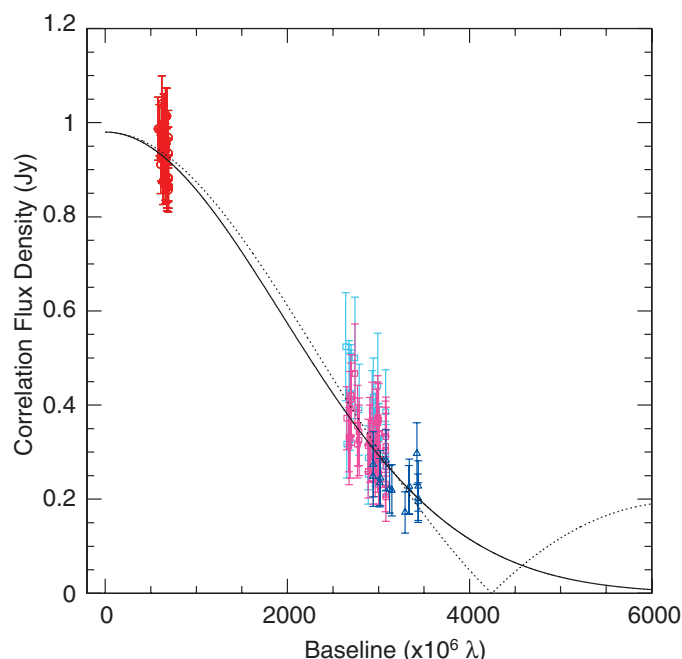
The compact central regions of some galaxies are so luminous that they outshine the combined output of all other energy sources in the galaxy. The small size and high power output of these active galactic nuclei (AGN) are most plausibly explained by the conversion of gravitational energy through accretion onto a supermassive black hole. These black holes have masses of  $M \gtrsim 10^6 M_\odot$  ( $M_\odot$ , solar mass), in contrast with lower-mass black holes ( $M \lesssim 10 M_\odot$ ) that result from the gravitational collapse of evolved stars. Many AGN produce powerful col-

limited jets of relativistic particles that can extend for hundreds and thousands of light-years, providing an important mechanism for redistributing matter and energy on large scales that affect galactic evolution (1). Jets are thought to form through magnetic acceleration processes located within the accretion flow or at the central black

hole itself (2–4), but no observations to date have had the angular resolution required to detect and confirm structure on these scales for extragalactic jet sources. High-resolution radio interferometry of these sources at centimeter wavelengths is limited by optical depth effects that obscure the innermost accretion region. For these reasons, it remains unclear if jet formation requires a spinning black hole (5, 6), and if so, whether jets are more likely to be formed when the orbital angular momentum of the accretion flow is parallel (prograde) or antiparallel (retrograde) to the black hole spin (7, 8). To address these questions, we have assembled a very long baseline interferometry (VLBI) array operating at a wavelength of 1.3 mm, the Event Horizon Telescope (9), where AGN start to become optically thin and angular resolutions necessary to resolve the inner accretion disks of nearby AGN are obtained.

Using a distance to M87 of  $16.7 \pm 0.6$  Mpc (10) and adopting the corresponding recent mass measurement of  $(6.2 \pm 0.4) \times 10^9 M_\odot$  (11), the Schwarzschild radius of the M87 black hole [ $R_{\text{SCH}} = 2 GM/c^2 = (5.9 \pm 0.4) \times 10^{-4}$  pc  $= (1.9 \pm 0.12) \times 10^{15}$  cm ( $G$ , gravitational constant;  $c$ , speed of light)] subtends an angle of  $7.3 \pm 0.5$  micro-arc sec, presenting us with the best known opportunity for studying the formation of relativistic jets on scales commensurate with the black hole and accretion disk. Radiating via synchrotron emission, the relativistic jet from M87 extends for hundreds of kiloparsecs and terminates in extended lobes of emission as it slows and interacts

**Fig. 1.** Measuring the size of the M87 core with 1.3-mm VLBI. Correlated flux density data from 3 consecutive days of observing are plotted as a function of baseline length (in units of observing wavelength). The CARMA-SMT baselines are shown in red; the two baselines from CARMA dishes to the JCMT are shown in magenta and teal; the SMT-JCMT baseline is shown in blue. Calibration errors of 5% have been added in quadrature to the  $1\sigma$  random errors associated with the incoherent fringe search performed on each baseline. The weighted least-squares best-fit circular Gaussian model is shown as a solid line and has a total flux density of 0.98 Jy and a FWHM size of 40.0 micro-arc sec. A hybrid model (dotted line) combines a circular Gaussian of the same size with a thin ring of diameter 40.0 micro-arc sec, which represents the expected shadow feature due to illumination of the central supermassive black hole from behind by a counterjet in M87. In the hybrid model, the Gaussian and ring components each contribute one-half of the flux density. On VLBI baselines shorter than the null spacing, the VLBI interferometric phase is zero, but on baselines beyond the null spacing, the phase is 180°.



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with the intergalactic medium. Closer to the galaxy's core, on a scale of hundreds of parsecs, the radio jet is remarkably well collimated, with an opening angle of less than  $5^\circ$  (12), and is also clearly seen in the optical, ultraviolet, and x-ray (13, 14), where the emission is primarily confined to knots along the central "spine" of the jet. VLBI observations at wavelengths ranging from 3.5 mm to 20 cm show that the jet opening angle, delineated by edge brightening in the outflow, continually increases as the core is approached, reaching  $\sim 60^\circ$  within 1 milli-arc sec of the core (15–19). This wide opening angle is a signature of the launch point for a magnetohydrodynamically (MHD) powered jet that has not yet had time to collimate (2), and it identifies the VLBI core as the most likely site of the central black hole.

We observed M87 over 3 consecutive days with a 1.3-mm wavelength VLBI array consisting of four telescopes at three geographical locations: the James Clerk Maxwell Telescope (JCMT) on Mauna Kea in Hawaii, the Arizona Radio Observatory's Submillimeter Telescope (SMT) in Arizona, and two telescopes of the Combined Array for Research in Millimeter-wave Astronomy (CARMA, located  $\sim 60$  m apart) in California. On Mauna Kea, the JCMT partnered with the Submillimeter Array (SMA), which housed the hydrogen maser atomic frequency standard and wideband VLBI recording systems; the SMT and CARMA were similarly equipped. These special-purpose systems allowed two frequency bands, each of 512 MHz bandwidth, to be sampled at 2-bit precision and recorded at an aggregate rate of 4 gigabits/s. The two bands, labeled "low" and "high," were centered on 229.089 GHz and 229.601 GHz, respectively. Data recorded at all sites were shipped to the MIT Haystack Observatory for processing on the Mark4 VLBI correlator. Once correlated, data for each VLBI scan (typically 10 min) were corrected for coherence losses due to atmospheric turbulence and were searched for detections using established algorithms tailored for high-frequency observations (20). M87 was clearly detected each day on all VLBI baselines, and the interferometric data were then calibrated to flux density units (20).

Clear detections on the long baselines to Hawaii (CARMA-JCMT and SMT-JCMT) represent the highest angular resolution observations of M87 reported in any waveband, and when combined with the CARMA-SMT baseline data, they provide a robust means to measure the size of the M87 core. The baseline between the two CARMA antennas corresponds to angular scales of  $\sim 4$  arc sec and is sensitive to extended and much larger-scale jet structure; these data were thus used to refine the calibration of the antennas but were excluded from the analysis of the core component. To extract a size for the core, we fitted a two-parameter circular Gaussian model to the 1.3-mm VLBI data, deriving a total flux density and full width at half maximum (FWHM) size for each day of observations. Sizes and flux densities fit separately for each day are consistent

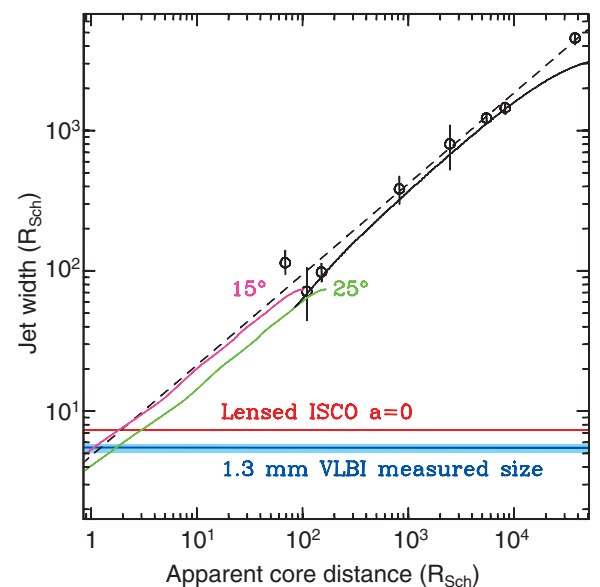
with each other at the  $3\sigma$  level, indicating no significant variation in the 1.3-mm core structure over the 3 days of observation (fig. S4). When data from all 3 days are combined, the weighted least-squares best-fit model for the compact component results in a flux density of  $0.98 \pm 0.04$  Jy and a FWHM of  $40 \pm 1.8$  micro-arc sec ( $3\sigma$  errors) (Fig. 1). The conversion to units of Schwarzschild radius yields a value of  $5.5 \pm 0.4 R_{\text{SCH}}$  ( $1\sigma$  errors), where the errors are dominated by uncertainties in the distance to M87 and the black hole mass. We adopt the circular Gaussian size derived using data from all 3 days for subsequent discussion.

Our VLBI observations cannot be used to fix the absolute position of this Gaussian component; however, in the case of M87, there is compelling evidence that this ultracompact 1.3-mm emission is in immediate proximity to the central supermassive black hole. Measurements of the jet width starting tens of  $R_{\text{SCH}}$  from the core and extending to core-separations of more than  $10^4 R_{\text{SCH}}$  are well fit by a parabola-like collimating profile (19) (Fig. 2). This fit matches similar profiles of general relativistic MHD (GRMHD) jet simulations (21, 22). When extrapolated to small scales, the empirical profile intersects the 1.3-mm emission component size within one  $R_{\text{SCH}}$  of the jet base. Because the angle of the M87 jet axis to our line of sight is estimated to be within the range of  $15^\circ$  to  $25^\circ$  (23), the de-projected distance of this intersection point from the jet base lies in the range of 2.5 to  $4 R_{\text{SCH}}$ . A second method of locating the 1.3-mm emission derives from observations of the position shift

of the M87 core as a function of wavelength. Multiwavelength astrometric VLBI observations (15 cm to 7 mm) confirm that the absolute position of the core moves asymptotically toward the jet base with a  $v^{-0.94}$  dependence (23). Extrapolation to 1.3 mm wavelength places the 1.3-mm VLBI component coincident with the jet base to within the uncertainty of the core-shift relation, which is  $\sim 1.5 R_{\text{SCH}}$ . In isolation, these observational trends would be unable to clearly link the jet base with the central engine. In blazar sources, for example, the relativistic jets are closely aligned to our line of sight, and the jet base is illuminated hundreds of thousands of  $R_{\text{SCH}}$  from the central engine (24). In contrast, M87 is now the only known case where the jet base has a size ( $\sim 5.5 R_{\text{SCH}}$ ) that is consistent with scales on which energy is extracted from the black hole and accretion disk to feed the jet (2). It is thus most natural to spatially associate the 1.3-mm VLBI component with the central engine, further guided by GRMHD simulations (21) that exhibit jet widths, within a few  $R_{\text{SCH}}$  of the black hole, that match the 1.3-mm VLBI size (Fig. 2). In M87, the favorable geometry of a misaligned jet and increased transparency of the synchrotron emission at millimeter wavelengths (25) have allowed us direct access to the innermost central engine with 1.3-mm VLBI.

The most plausible mechanisms for powering extragalactic jets involve conversion of the black hole rotational energy through the Blandford-Znajek (BZ) process (3), whereby magnetic field lines cross the black hole event horizon and launch

**Fig. 2.** Width profile of the M87 jet as a function of distance from the core. Measurements of the jet opening angle from the literature (33) were converted to projected jet width and fit with a power law (dashed black line) of the form  $\theta \propto r^\beta$ , where  $\theta$  is jet width and  $r$  is separation from the core. The best fit  $\theta \propto r^{0.69}$  indicates a parabola-like profile for the jet, with collimation increasing at larger distances as expected from MHD theory, and is in agreement with recent detailed measurements of the M87 jet profile (19). The dark blue horizontal line indicates the size reported in this paper, with the light blue band corresponding to  $1\sigma$  uncertainties. To estimate the position relative to the central black hole, we extrapolated the power law fit, which intersects the 1.3-mm VLBI size at an apparent core distance of  $\sim 1 R_{\text{SCH}}$ . GRMHD models, tailored to simulate jet emission from the black hole out to distances of  $\sim 100 R_{\text{SCH}}$ , are shown as the solid magenta and green lines for jet axis angles to our line of sight of  $15^\circ$  and  $25^\circ$ , respectively (21). These close-in simulations also intersect the 1.3-mm VLBI size between 1 and  $2 R_{\text{SCH}}$  from the black hole. For comparison and illustration, jet width values derived from a separate GRMHD simulation of the larger-scale M87 jet (22) are shown as a solid black line for distances larger than  $\sim 100 R_{\text{SCH}}$ . The red horizontal line indicates the apparent size of the ISCO for a nonspinning black hole when strong gravitational lensing effects near the black hole are properly accounted for (20).





Poynting flux–dominated outflows. The inner portion of the accretion disk is not only the source of the magnetic fields threading the black hole, but also launches a disk-wind via the Blandford-Payne (BP) mechanism (4), which serves to collimate the jet. This BZ/BP combination forms a spine/sheath morphology, in which a narrow, electromagnetic, and initially nonradiative jet from the black hole is surrounded by a slower and quickly mass-loaded outflow originating from the inner disk (6, 26). In the case of M87, the broad opening angle and existence of a counterjet (17, 18) indicate that the dominant contribution to the 1.3-mm VLBI emission is from the slower-moving sheath, anchored within the accretion disk (6). In this model of jet genesis, which we adopt here, the critical size scale associated with the jet footprint is the innermost stable circular orbit (ISCO) of the black hole, within which matter quickly plunges to the event horizon. The ISCO marks the peak density and rotational speed of the accretion flow (27) and is the location where particles are most efficiently accelerated from the disk (5) and begin to radiate. Strong beaming effects, which might produce small and high-brightness features unrelated to the ISCO, are not expected to be a factor on the scales probed by the 1.3-mm VLBI observations, because the detection of a counterjet in M87 and the parabolic profile of the jet both indicate that the outflow near the black hole is subrelativistic.

Based on this understanding of the M87 jet, and taking the 1.3-mm VLBI size as the ISCO diameter, we estimated the black hole spin and determined whether the accretion disk orbits in a prograde or retrograde sense. This is possible because the intrinsic ISCO diameter ( $D_{\text{ISCO}}$ ) is sensitively dependent on black hole spin, with a value of  $D_{\text{ISCO}} = 6 R_{\text{SCH}}$  for a nonspinning (Schwarzschild) black hole [spin ( $a$ ) = 0], and ranging from  $D_{\text{ISCO}} = 9 R_{\text{SCH}}$  to  $D_{\text{ISCO}} = 1 R_{\text{SCH}}$  for retrograde and prograde orbits, respectively, around a maximally spinning (Kerr) black hole ( $a = 1$ ). Strong lensing effects due to the Kerr

spacetime metric near the rotating black hole magnify the apparent size of the ISCO, with the relationship between observed ISCO size and black hole spin shown in Fig. 3 (20, 28). The measured 1.3-mm VLBI size corresponds to a prograde ISCO around a black hole with  $a > 0.2$  ( $3\sigma$ ). This result explicitly excludes the possibility of a retrograde ISCO orbit in the accretion flow, because all such orbits would be larger than the core size derived here. The smallest possible retrograde ISCO orbit would present an apparent diameter of  $7.35 R_{\text{SCH}}$ , which is more than  $4\sigma$  larger than the observed size. This result is consistent with generally accepted theories that the spin axes of the accretion disk and black hole will be brought into alignment through gradual angular momentum transfer from the orbiting disk (29).

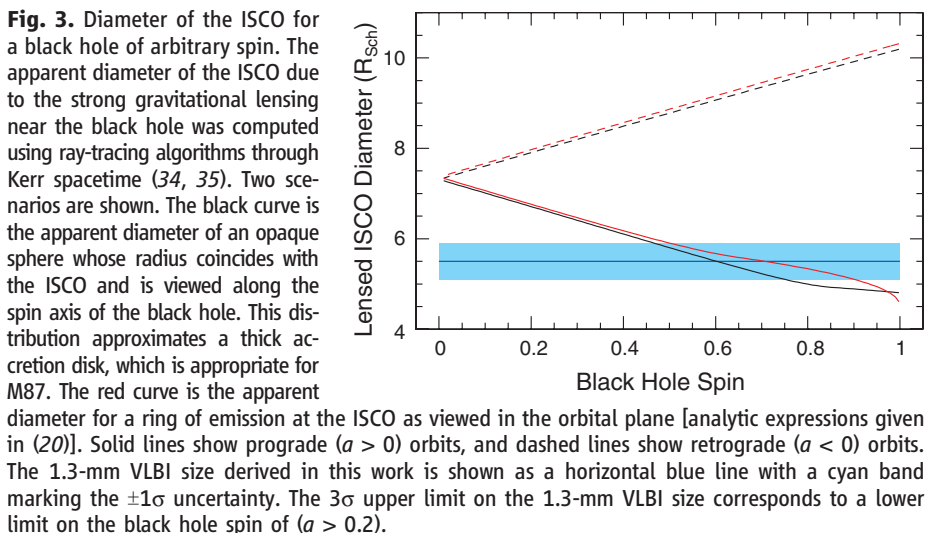
As the sensitivity and resolution of the 1.3-mm VLBI array improve, modeling of the data can move beyond simple Gaussian distributions to physically motivated models that include accretion physics, relativistic beaming, and full GR ray tracing. Recent modeling of the M87 jet on Schwarzschild radius scales indicates that in many cases, emission from a counterjet will illuminate the black hole from behind, creating a bright feature at the last photon orbit (25, 30). The relatively dim region interior to this ring is known as the black hole shadow or silhouette, and its dimensions are determined by black hole mass, spin, and inclination of the spin axis (31). Over a wide range of spin and inclination, the last photon ring of emission has a diameter of  $D_{\text{RING}} = 5.2 R_{\text{SCH}}$ , so that fitting for the ring size yields an estimate of the black hole mass (32). The presence of such a shadow feature is not ruled out by the 1.3-mm VLBI data presented here, which can be well fitted by a hybrid model combining a circular Gaussian representing jet emission launched from the ISCO, with a uniform annular ring at the last photon orbit (Fig. 1). Although the current VLBI array cannot be used to meaningfully constrain this more complex mod-

el, there are two distinguishing characteristics of this hybrid approach that can be readily tested with future 1.3-mm VLBI arrays. The first is a predicted null in correlated flux density near baseline lengths of  $4.5 \times 10^9 \lambda$  ( $\lambda$ , observing wavelength), and the second is a  $180^\circ$  flip in interferometric phase between VLBI baselines on either side of this null.

It is increasingly clear that strong gravity effects can dominate observed AGN structure on the scales accessible with short-wavelength VLBI. Included among these effects is the spin-dependent ISCO period, which ranges from 5 days ( $a = 1$ ) to 1 month ( $a = 0$ ) for the mass of the M87 black hole. The consistency of the 1.3-mm VLBI sizes presented here, spanning 3 days of observation, does not reflect dramatic structural changes in the jet that might be expected because of accretion disk inhomogeneity for a black hole spinning near the maximum rate (fig. S4). More-sensitive searches for such periodic features in the jet launch region can be carried out with the full Event Horizon Telescope. In general, this work signals that Earth-sized 1.3-mm VLBI networks are now able to provide angular resolutions that link observations of compact objects dominated by strong-field GR to outflows on the largest galactic scales.

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#### Supplementary Materials

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Methods

Figs. S1 to S4

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# Self-Assembled Colloidal Superparticles from Nanorods

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Colloidal superparticles are nanoparticle assemblies in the form of colloidal particles. The assembly of nanoscopic objects into mesoscopic or macroscopic complex architectures allows bottom-up fabrication of functional materials. We report that the self-assembly of cadmium selenide–cadmium sulfide (CdSe–CdS) core-shell semiconductor nanorods, mediated by shape and structural anisotropy, produces mesoscopic colloidal superparticles having multiple well-defined supercrystalline domains. Moreover, functionality-based anisotropic interactions between these CdSe–CdS nanorods can be kinetically introduced during the self-assembly and, in turn, yield single-domain, needle-like superparticles with parallel alignment of constituent nanorods. Unidirectional patterning of these mesoscopic needle-like superparticles gives rise to the lateral alignment of CdSe–CdS nanorods into macroscopic, uniform, freestanding polymer films that exhibit strong photoluminescence with a striking anisotropy, enabling their use as downconversion phosphors to create polarized light-emitting diodes.

**D**irectional bonding interactions dictate the structural complexity and functional specificity of naturally occurring materials at all length scales (1–4). On the nanometer scale, anisotropic nanoparticles are used to design directional bonding interactions through shape-specific (3–5) and surface-specific functionalization (6, 7). Because anisotropic nanoparticles exhibit shape-dependent physical and chemical properties (8, 9), the self-assembly of these nanoparticles can lead to metamaterials with important collective properties (3) such as spin-dependent electron transport (10), vibrational coherence (11), enhanced conductivity (12), and tandem catalysis (13). We report that anisotropy-driven self-assembly of CdSe–CdS core-shell semiconductor nanorods produces three-dimensional (3D) colloidal superparticles with multiple well-defined supercrystalline domains or needle-like superparticles with a single supercrystalline domain. The needle-like superparticles can be unidirectionally aligned and further assembled into centimeter-scale, uniform, freestanding polymer films, exhibiting a photoluminescence

(PL) anisotropy ratio larger than that of single CdSe–CdS nanorods.

Colloidal CdSe–CdS superparticles were prepared using a previously described method with minor modifications (14), including two major steps: (i) synthesis of water-soluble nanorod micelles and (ii) growth of superparticles from nanorod micelles in an aqueous solution of ethylene glycol (Fig. 1A). We used highly fluorescent CdSe–CdS nanorods primarily capped with octylamine and octadecylphosphonic acid (ODPA), prepared via a literature method (15, 16), as precursors for making nanorod micelles (figs. S1 to S3). In a typical superparticle synthesis (17), a clear nanorod-micelle aqueous solution was prepared by mixing a chloroform solution of CdSe–CdS nanorods with length  $l = 28.0 \pm 1.5$  nm and diameter  $d = 6.7 \pm 0.3$  nm (10 mg/ml, 1 ml) with an aqueous solution (1 ml) containing varying amounts of dodecyl trimethylammonium bromide (DTAB), followed by bubbling Ar to evaporate chloroform. Under vigorous stirring, the nanorod micelle solution was injected into a three-neck flask with ethylene glycol (5.0 ml), causing the decomposition of nanorod micelles as a result of the loss of DTAB molecules into the growth solution. This led to the aggregation of nanorods and the eventual formation of superparticles (Fig. 1A). After stirring for 10 min, an aqueous solution of

dithiol-functionalized Tween-20 (0.1 mM, 1.0 ml) was added into the growth solution to stabilize the superparticles (14). The resulting superparticles were isolated and purified by centrifugation and redispersed in polar solvents (such as water, ethanol, and ethylene glycol) at a variety of concentrations.

Previous studies have shown that the amount of DTAB affects the kinetics of superparticle formation from isotropic spherical nanocrystals and thus can be used to achieve a size-controlled synthesis of superparticles (18), and we apply this to the synthesis of superparticles from nanorods. Accordingly, we have prepared CdSe–CdS superparticles with an average size ranging from  $180 \pm 23$  nm to  $1100 \pm 150$  nm by varying the amount of DTAB in nanorod micelle solutions (18). Low-magnification transmission electron microscope (TEM) images show that the resulting superparticles are nearly spherical (fig. S4), whereas higher-magnification images from tilting experiments reveal that these superparticles exhibit multiple supercrystalline domains with configurations and sizes that are dependent on the total number ( $N$ ) of constituent nanorods inside each superparticle (Fig. 1, B to P, and fig. S5).

When  $N$  is less than  $\sim 80,000$ , superparticles typically exhibit a morphology of double-domed cylinders, wherein the cylindrical domain occupies a volume larger than that of the corresponding domes (Fig. 1, B to K). The cylindrical domain adopts a lamellar structure that consists of stacked multilayer disks formed from nanorods via lateral association, where the thickness of each disk is consistent with the length of a single nanorod. With an increase of  $N$ , the radius of the cylindrical domain increases, and so does the number of stacked-disk monolayers but with some fluctuations (Fig. 1, B to K). For example, the superparticle shown in Fig. 1G possesses more constituent rods than that shown in Fig. 1F, but the cylindrical domain of the superparticle in Fig. 1F has one more stacked-disk monolayer. When  $N$  is larger than  $\sim 80,000$ , CdSe–CdS superparticles appear as either irregular-multidomain particles (Fig. 1, L, M, and O) or double-domed cylinders (Fig. 1, N and P).

TEM tilting experiments further reveal that the domes in a double-domed cylinder typically consist of three supercrystalline domains together

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forming a curved, arc-shaped architecture (Fig. 2, A to D, and figs. S5 and S6). The middle domain possesses a lamellar structure that comprises stacked multilayer arches formed from close-packed nanorods lying perpendicular to the rods in the cylindrical domain, and the thickness of each arch is nearly identical to the length of a single nanorod (Fig. 2D). The two small side domains appear as closely packed dots, indicating that the constituent nanorods are aligned parallel to the direction of the electron beam (Fig. 2D).

These TEM observations are consistent with selected-area electron diffraction (SAED) measurements, which also provide structural information of nanorod packing inside a superparticle (19). The SAED data show that although the alignment of nanorods in this superparticle is random at the atomic level (largely because of the rotational freedom around their long axes), the long axes of the nanorod constituents within a given domain are parallel to each other, whereas the orientation of long axes of nanorods from neighboring domains is perpendicular (Fig. 2, E to H). Taken together, these results further suggest that a typical double-domed cylinder consists of seven supercrystalline domains where the nanorods of two neighboring domains are arranged

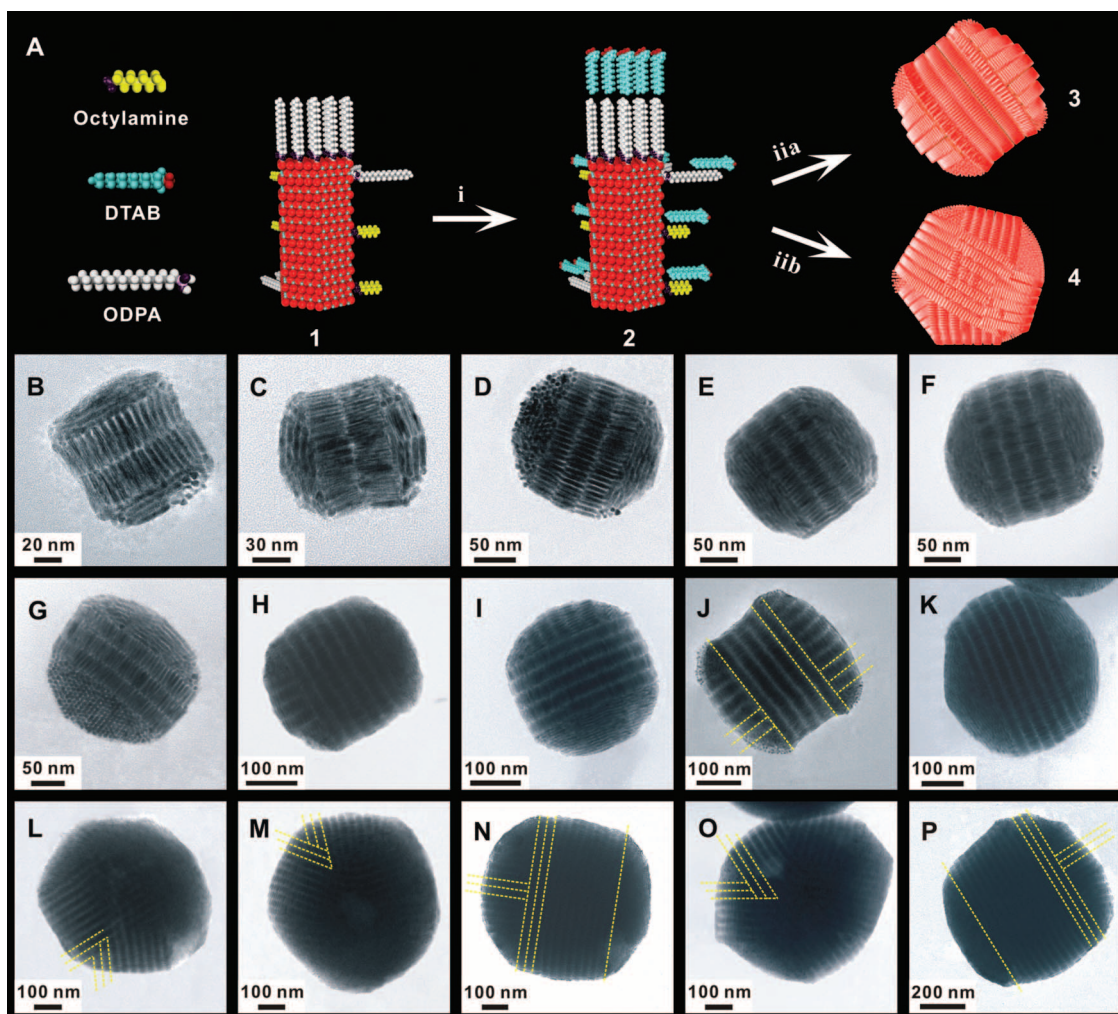
in an orthogonal configuration (Fig. 2, A to H). In contrast, although irregular-multidomain particles exhibit multiple lamellar supercrystalline domains, the long axes of constituent nanorods in two neighboring domains are not at right angles (Fig. 2, I to L).

TEM observations further show that the orientations of the nanorods in domains from different domes of a double-domed cylinder do not correlate with each other (Fig. 1, B to I), thereby suggesting that the domes have degenerate free energy levels for lying at different rotational angles along the double-domed cylinder's axis. The supercrystalline structure of double-domed cylinders was further characterized using synchrotron-based small-angle x-ray scattering (SAXS). The SAXS data show that these double-domed cylinders do not adopt perfect 3D superlattices, but instead exhibit both 1D and 2D supercrystalline orders along directions parallel and perpendicular to the long axis of constituent nanorods, respectively (Fig. 2, M and N). The 1D superlattice possesses a lamellar period of  $31.1 \pm 0.2$  nm, and the 2D ordered structure adopts a hexagonal lattice with a unit cell constant of  $8.7 \pm 0.1$  nm (17) (table S1). These results further suggest that the end-to-end distance between neighboring constituent nanorods is  $3.1 \pm 1.5$  nm, confirming

that the long axes of nanorods are parallel to the lamellar normal as observed from TEM. In addition, the side-by-side distance of neighboring rods is  $2.0 \pm 0.5$  nm, which is about twice the length of an octylamine ligand, thus indicating that the long chains of ODPA ligands are intercalated between neighboring nanorods.

Superparticles with multiple supercrystalline domains are mesoscopic analogs of multiply twinned particles with atomic lattices (20). The formation of multidomain superparticles may occur through two different mechanisms: (i) a nucleation-growth process in which larger supercrystalline particles are created from the growth of smaller particles or (ii) an embryo-crystallization process wherein nonsupercrystalline embryos are formed by decomposition of nanorod micelles, after which colloidal crystallization of nanorods occurs inside the embryos to form multiple supercrystalline domains (fig. S7A). To distinguish between these formation mechanisms, we performed a superparticle synthesis with two separate injections of nanorod micelles (17). Our results show that the second injection did not increase superparticle size, but instead increased the amount of particles (fig. S8), thus favoring the embryo-crystallization mechanism (17). This formation mechanism is

**Fig. 1.** (A) Scheme for the synthesis of superparticles from CdSe-CdS nanorods: (i) nanorod-micelle formation, (ii) superparticle formation. Numbered images: (1) Proposed model for a CdSe-CdS nanorod functionalized with ODPA and octylamine (17); (2) proposed model for a nanorod micelle prepared using DTAB; (3) proposed model for a double-domed cylinder; (4) proposed model for an irregular-multidomain particle. (B to P) TEM images of superparticles made from nanorods of  $l = 28.0 \pm 1.5$  nm and  $d = 6.7 \pm 0.3$  nm.





also consistent with our previous results from the synthesis of superparticles with spherical nanoparticles, showing that supercrystalline particles form via a crystallization process from particles without a long-range supercrystalline order (18).

We propose that the colloidal crystallization of nanorods inside an embryo is under thermodynamic control. Hence, the surface morphology and constituent nanorod packing configuration of a superparticle adopt an equilibrium structure proposed by a modified Wulff construction (21), wherein this superparticle has a minimized Gibbs free energy ( $G$ ):  $G = G_b + S \cdot \gamma$  (where  $G_b$  represents the bulk Gibbs free energy, and  $S$  and  $\gamma$  are the surface area and surface tension of the superparticle, respectively). The surface free energy is determined by the repulsive solvophobic interactions between the superparticle and the surrounding water and ethylene glycol molecules (14), whereas  $G_b$  is determined by anisotropic interactions between CdSe-CdS nanorods (including van der Waals and dipole interactions) that favor formation of a single-layer disk with hexagonal close-packed nanorods (14, 22). Because these anisotropic interactions exhibit an energy level comparable to the repulsive solvophobic interactions, the interplay of the bulk and surface free energy minimizations governs the formation of multiple supercrystalline domains that consist of stacked multilayer structures of hexagonal-close-packed nanorods (Figs. 1, 2, and 3A).

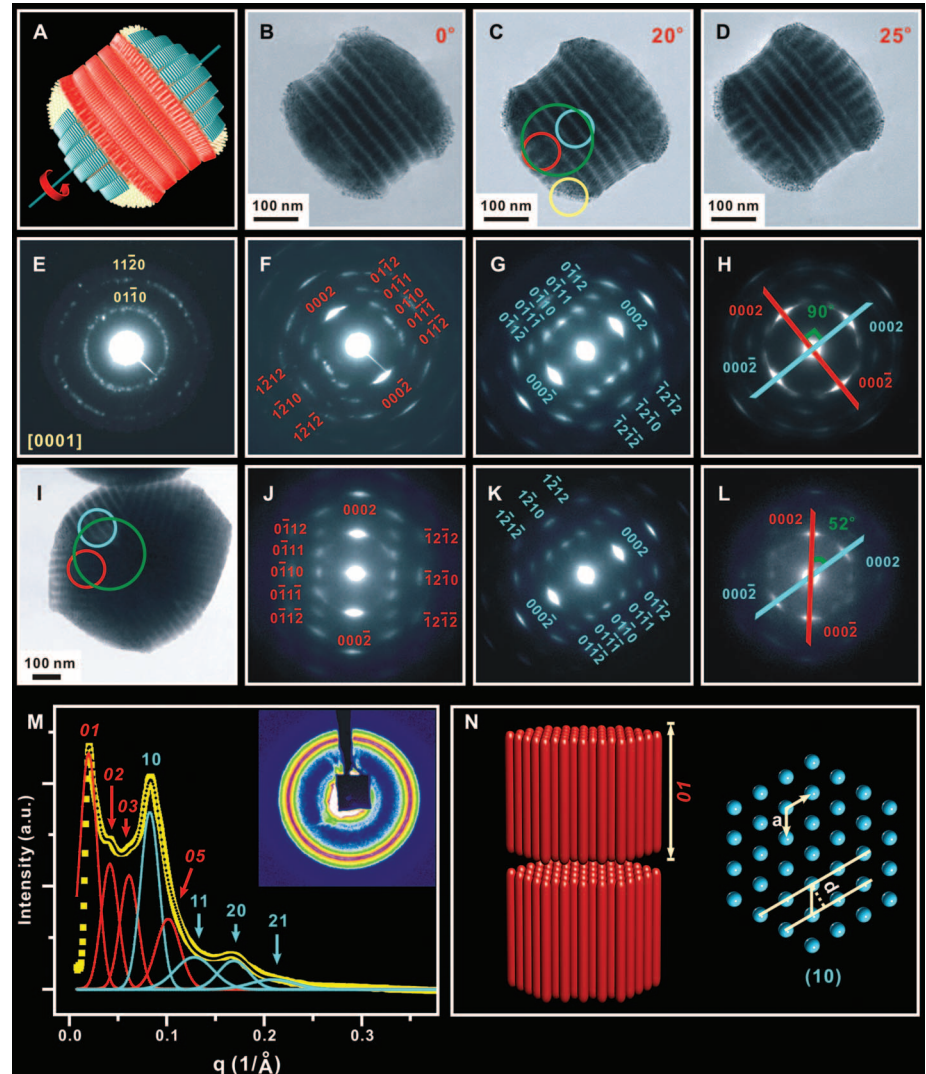
To explore how colloidal crystallization of nanorods leads to the formation of double-domed cylinders, we conducted numerical simulations based on the assumption that surface area minimization is the dominant factor in minimizing the Gibbs free energy of a superparticle. The 3D architecture of a double-domed cylinder is characterized by nine parameters (Fig. 3A and figs. S9 to S11): the number of lamellar layers in the cylinder ( $n$ ), the numbers of lamellar arch layers in the middle ( $i$ ) and two small side ( $j$ ) domains, the height ( $H$ ) and radius ( $R$ ) of its cylindrical domain, the length ( $l$ ) and diameter ( $d$ ) of constituent nanorods, the relative curvature of the domes ( $\kappa_d$ ), and the relative curvature of the cylinder wall ( $\kappa_c$ ). The minimization of the surface area of a double-domed cylinder requires maximization of the nanorod packing factor ( $\Phi$ ) on its cylinder base. In turn, this requires that the long axes of nanorods in the middle domain are aligned perpendicular to the long axes of nanorods in the side domains (Fig. 3A), which agrees with our experimental observations (Fig. 2, B to H). The nanorod packing factor ( $\Phi$ ) is given by

$$\Phi(i, j, R, l) = 1 - \frac{2 \cdot l}{\pi} (2 - \cos \varphi - \cos \theta) - \frac{2}{\pi} (\cos \varphi - \sin \theta)(\cos \theta - \sin \varphi) \quad (1)$$

where  $\theta$  and  $\varphi$  are the angles determined by  $i$  and  $j$ , respectively;  $\sin \theta = i \cdot l / (2 \cdot R)$ ; and  $\sin \varphi = j \cdot l / (2 \cdot R)$  (17) (fig. S9). Our numerical simu-

lations show that  $\Phi$  exhibits a parabola-like shape as a function of  $i$ , whereas for each  $i$  value, a corresponding  $j$  value is chosen to maximize the packing factor in the two small side domains (Fig. 3B). If the  $i$  values for the top three simulated packing densities are chosen for a superparticle of a given  $R$ , the trend for simulated  $i$  as a function of  $R$  encompasses all the experimental data (Fig. 3C and fig. S6).

Using Lagrange multipliers (17), we determined that there are two necessary conditions for a double-domed cylinder of a given size to have minimal surface area: (i) Its two domes must have identical  $H$  values, and (ii)  $H$  must be smaller than  $R$  (fig. S10). These mathematically determined conditions are consistent with our TEM observations of more than 400 individual superparticles, for which the two domes in a double-domed cyl-



**Fig. 2.** (A) Proposed double-domed cylinder model in TEM tilting experiments. (B to D) TEM images of a double-domed cylinder superparticle at different tilting angles, as indicated. (E to H) SAED patterns taken from the double-domed cylinder superparticle at the selected areas labeled with colored circles in (C). A [0001] zone ring pattern of a wurtzite CdS crystal shown in (E) indicates that the nanorods in the small side domain of the double-domed cylinder's dome are arranged randomly at the atomic level, whereas their long axes are aligned parallel to the electron beam. Two sets of diffraction dots from the [10 $\bar{1}$ 0] and [2 $\bar{1}$  $\bar{1}$ 0] zones, shown in (F) and (G), also suggest that the nanorods are arranged randomly at the atomic level in the selected areas, whereas their long axes are aligned in parallel along the direction of the two (0002) diffraction dots. (I) TEM image of an irregular-multidomain particle. (J to L) SAED patterns taken from this irregular-multidomain particle at the selected areas indicated in (I). (M) The integrated data from the SAXS pattern (inset), wherein the color scale indicates the intensity. This diffraction pattern characterizes a 1D lamellar structure with peak positions corresponding to Bragg reflections on planes specified by Miller indices 01, 02, 03, and 05 (indicated in red), and a 2D hexagonally packed structure with Bragg reflection peaks on planes specified by Miller indices 10, 11, 20, and 21 (indicated in blue). See table S1 for the values of interplanar spacing associated with each peak. (N) Schemes of the 1D lamellar and 2D hexagonally packed structures created from nanorods.

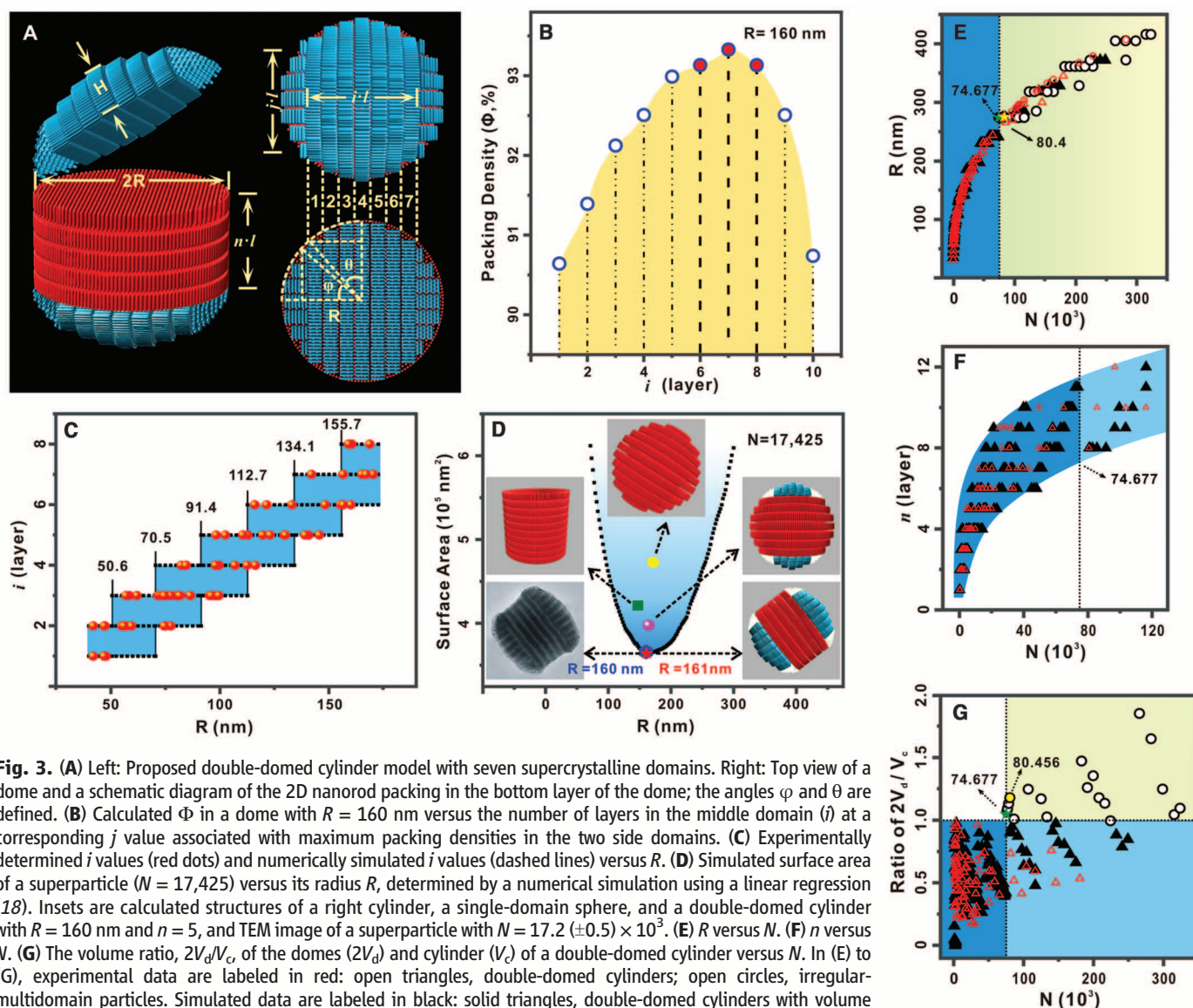
inder have nearly identical sizes (Fig. 1). In addition, the rule of surface area minimization allows for the two domes to rotate at different angles around the axis of a double-domed cylinder while still maintaining a minimized Gibbs free energy. As observed experimentally, nanorod packing directions in the domains of different domes are not necessarily related to each other (Fig. 1).

To further understand the formation of double-domed cylinders, we built a superparticle with hexagonal close-packed nanorods of a given size ( $l$  and  $d$ ). The superparticle structure is defined with seven variables ( $R$ ,  $H$ ,  $i$ ,  $j$ ,  $n$ ,  $\kappa_c$ , and  $\kappa_d$ ) that dictate the evolution of superparticle morphology and domain configuration during surface-area minimization (figs. S11 and S12). The global minimal

surface area of the superparticle as a function of  $N$  was determined using a method of mixed-integer linear programming of these variables (17) (figs. S13 to S16). As an example, we show that the surface area of a superparticle ( $N = 17,425$ ) is parabolically related to the value of its  $R$ . The superparticle having the minimal surface area adopts a double-domed cylinder structure with  $R$  and  $n$  values in good agreement with an experimentally observed particle, whereas the right cylinder and single-domain sphere display substantially larger surface areas (Fig. 3D). Although the simulated lamellar layer number ( $i = 7$ ) does not match the experimental values of 6 and 8 in the observed particle, these experimental values are still within the range of simulated values that

are associated with the top three nanorod packing densities on the base of cylinder (Fig. 3, C and D, inset). This minor deviation is likely caused by the small size difference in the cylinder diameters at the two bases adjacent to the domes (Fig. 2D and Fig. 3D, inset).

In addition, the simulated data—for  $R$  and  $n$  of superparticles as a function of their  $N$ —show strong consistency with the experimental values (Fig. 3, E and F). The simulations further reveal that the fluctuations of  $n$  as a function of  $N$ , as observed experimentally (Fig. 1), are attributable to the strong intercorrelations among  $R$ ,  $H$ , and  $n$  during the minimization of superparticle surface area (figs. S17 to S19). For instance, with an increase in  $N$ , the corresponding decrease in  $n$  is



**Fig. 3.** (A) Left: Proposed double-domed cylinder model with seven supercrystalline domains. Right: Top view of a dome and a schematic diagram of the 2D nanorod packing in the bottom layer of the dome; the angles  $\phi$  and  $\theta$  are defined. (B) Calculated  $\Phi$  in a dome with  $R = 160$  nm versus the number of layers in the middle domain ( $i$ ) at a corresponding  $j$  value associated with maximum packing densities in the two side domains. (C) Experimentally determined  $i$  values (red dots) and numerically simulated  $i$  values (dashed lines) versus  $R$ . (D) Simulated surface area of a superparticle ( $N = 17,425$ ) versus its radius  $R$ , determined by a numerical simulation using a linear regression (18). Insets are calculated structures of a right cylinder, a single-domain sphere, and a double-domed cylinder with  $R = 160$  nm and  $n = 5$ , and TEM image of a superparticle with  $N = 17.2 (\pm 0.5) \times 10^3$ . (E)  $R$  versus  $N$ . (F)  $n$  versus  $N$ . (G) The volume ratio,  $2V_d/V_c$ , of the domes ( $2V_d$ ) and cylinder ( $V_c$ ) of a double-domed cylinder versus  $N$ . In (E) to (G), experimental data are labeled in red: open triangles, double-domed cylinders; open circles, irregular-multidomain particles. Simulated data are labeled in black: solid triangles, double-domed cylinders with volume ratio smaller than 1; dots, double-domed cylinders with volume ratio larger than 1, wherein only irregular-multidomain particles were observed in the corresponding size regime experimentally. The smallest irregular-multidomain particle determined by numerical simulation, shown as a yellow star, has  $N = 74,677$ . The smallest observed irregular-multidomain particle (see Fig. 1L), shown as a yellow star, has  $N = 80,456$ , which is close to a simulated irregular-multidomain particle with  $N = 80,456$  shown in (G). The regime of  $N < 74,677$  is shown in blue. In the regime of  $N \geq 74,677$ , the subregime consisting of the “stable islands” for double-domed cylinders is shown in pale blue, and the subregime for the existence of irregular-multidomain particles is shown in yellow.



associated with an increase of  $R$  and/or  $H$ , and thus a substantial increase in the volume ratio of the domes and cylindrical domain (fig. S18). The consistency between experimental data and simulation results confirms that the formation of double-domed cylinder superparticles is a thermodynamically controlled process that minimizes double-domed cylinder surface area.

However, there are cases when the surface energy term does not dominate the minimization of superparticle free energy. Because of the strong anisotropic interactions between neighboring rods aligned side-by-side, the stacked arches in the domes of a superparticle can have a specific bulk free energy substantially higher than that of stacked disks in the cylindrical domain (fig. S20A). Thus, the increase in the volume of the two domes in a double-domed cylinder with a given  $N$  can increase the overall bulk free energy of this superparticle. When the volume percentage of the domes reaches a threshold, the minimization of superparticle surface energy no longer dominates the bulk free energy term, and the Gibbs free energy of the double-domed cylinder structure can be higher than that of an irregular-multidomain particle, thus leading to irregular-multidomain particle formation (fig. S20B).

If we assume this threshold to be a 1:1 volume ratio between the domes and the cylinder, our simulation results match all experimental data on the formation of double-domed cylinders and irregular-multidomain particles (Fig. 1, B to P, and Fig. 3G). Moreover, our simulations show that when  $N < 74,677$ , all superparticles have a volume ratio smaller than 1 between the domes and the cylinder, and thus only adopt a double-domed cylinder structure (Fig. 1, B to K). In the regime of  $N \geq 74,677$ , superparticles can have volume ratios larger than 1 and thus appear as irregular-multidomain particles (Fig. 1, L, M, and O). Our simulations also reveal that in this regime, an increase in  $N$  can periodically lead to superparticles having volume ratios smaller than 1, thereby creating “stable islands” for double-domed cylinders (Fig. 3G), which is in agreement with our experimental observations (Fig. 1, L to P).

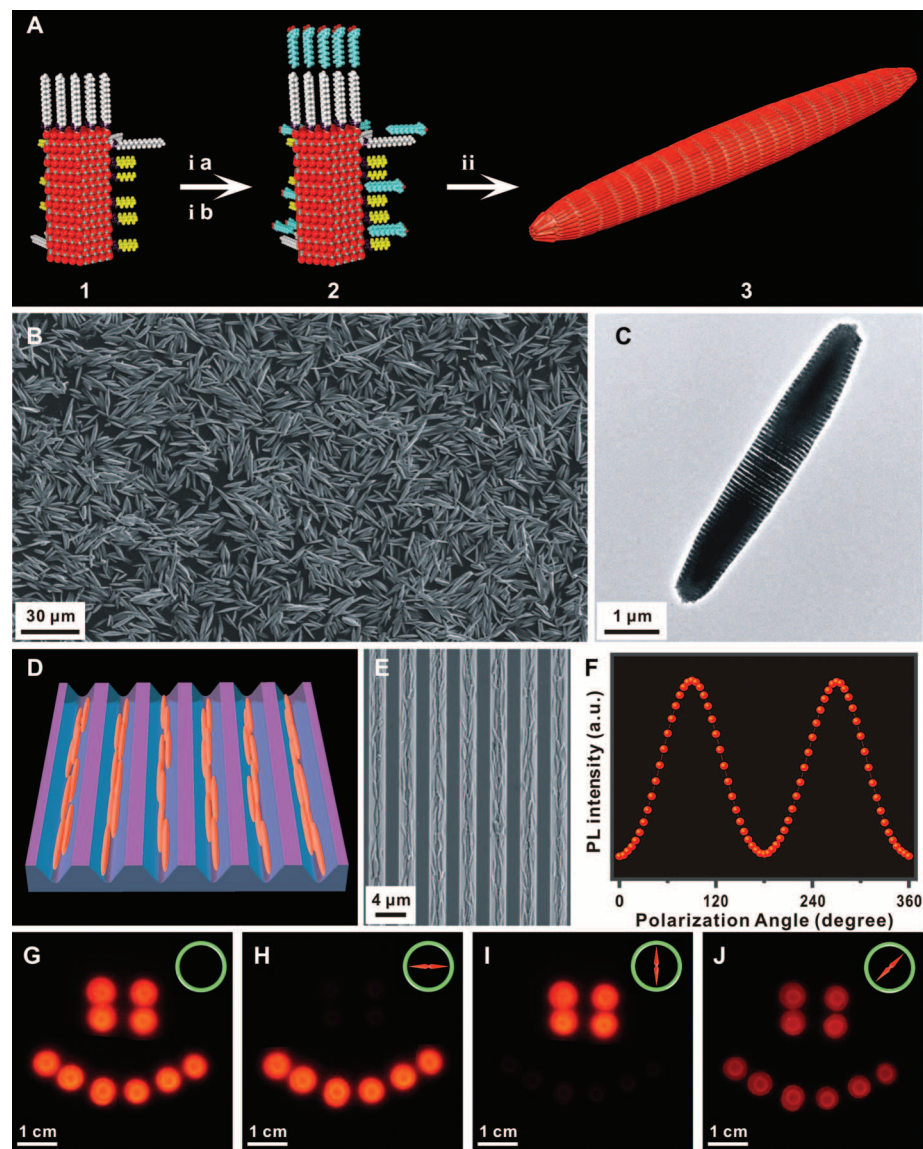
Our numerical simulations can also predict the morphology and supercrystalline domain configurations of superparticles made from constituent nanorods of other sizes (fig. S21). The excellent agreement between the numerical simulations and experimental data further suggests that (i) multiple supercrystalline domains in double-domed cylinders and irregular-multidomain particles are formed through a spontaneous process in a cooperative manner under thermodynamic equilibrium (23), and (ii) the anisotropic interactions between nanorods and their anisotropic geometry play critical roles in this process.

Moreover, the surfaces of CdSe-CdS nanorods exhibit atomic packing factor anisotropy, enabling their bottom and top {0001} faces to have a higher ligand packing density than their side faces, such as {10 $\bar{1}$ 0} and {11 $\bar{2}$ 0} (fig. S22). Therefore, appropriately functionalized nano-

rods may allow the intercalation of DTAB hydrocarbon chains into the surface ligand layer on the side faces but not on the bottom and top faces of CdSe-CdS nanocrystals (fig. S7B). As a result, during nanorod micelle decomposition, the rates for DTAB leaving from the side faces are slower than those from the bottom and top faces, thus kinetically creating anisotropic and crystal face-specific functionality on the nano-

rods whose bottom and top faces exhibit greater solvophobicity than the side faces. This kinetic process can, in turn, lead to a rapid growth of supercrystalline domains along the long axes of constituent nanorods during colloidal crystallization (Fig. 4A and fig. S7B).

Incubation of CdSe-CdS nanorods ( $l = 78.0 \pm 2.1$  nm,  $d = 5.4 \pm 0.3$  nm; 10 mg) with octylamine (1  $\mu$ l) for 6 days under Ar modified the particles,



**Fig. 4.** (A) Scheme of needle-like superparticle synthesis: (ia) incubation with octylamine, (ib) nanorod-micelle formation, and (ii) superparticle formation. (B) Scanning electron microscope (SEM) image of needle-like superparticles. (C) TEM image of a needle-like superparticle. (D) Scheme of the lateral alignment of superparticles into the unidirectional line patterns on a solid substrate. (E) SEM image of laterally aligned needle-like superparticles inside a line pattern on a  $\text{Si}_3\text{N}_4$  substrate made using photolithography (groove width = 2.0  $\mu\text{m}$ , depth = 1.2  $\mu\text{m}$ , gap = 2.0  $\mu\text{m}$ ). (F) PL intensity versus polarization angle as the polarization was manually rotated while measuring a typical superparticle-embedded PDMS thin film under excitation wavelength of 380 nm. (G to J) Optical images of a light panel consisting of 10 LEDs [ $\lambda = 380$  nm, bandwidth (full width at half maximum) = 12 nm, radiant power = 20 mW; Super Bright LEDs Inc., St. Louis, MO] with superparticle-embedded PDMS thin films as energy downconversion phosphors, taken without a polarizer (H) and with a polarizer [(I) and (J)] at the angles indicated by the direction of the compass needles in each panel. The LEDs emit orange lights at 579 nm with a bandwidth of 40 nm and a polarization ratio of  $\sim 0.88$ .



leading to the formation of single-domain, elongated needle-like superparticles with  $l = 11 \pm 4 \mu\text{m}$  and  $d = 1.1 \pm 0.3 \mu\text{m}$  (Fig. 4, B and C). These single-domain superparticles have a different morphology from those multidomain superparticles made from identical nanorods without incubation treatment (fig. S21, C to E).

The needle-like superparticles from unoptimized syntheses exhibit a PL quantum yield of ~40% and are indefinitely stable in solvents with strong polarity, such as water or ethanol. However, these particles can undergo intraparticle ripening in lower-polarity solvents such as ethylene glycol, demonstrating that the needle-like morphology is not an equilibrium shape (fig. S23). The mesoscopic sizes of these needle-like superparticles allow them to be easily aligned into unidirectional line patterns on  $\text{Si}_3\text{N}_4$  substrates through capillary forces (24) (fig. S24), which can be readily transferred into uniform and removable thin films of polydimethylsiloxane (PDMS) with sizes as large as  $5.0 \text{ cm} \times 5.0 \text{ cm}$  (Fig. 4, D and E, and fig. S25). The resulting thin films exhibit strong linearly polarized PL at 579 nm with a typical emission polarization ratio [ $p = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$ , where  $I_{\parallel}$  and  $I_{\perp}$  are the intensities parallel ( $I_{\parallel}$ ) and perpendicular ( $I_{\perp}$ ) to the nanorod long axis] of 0.88 (Fig. 4F and fig. S26), which is substantially higher than that of individual single CdSe-CdS nanorods [0.75 (25, 26)]. This PL anisotropy enhancement can be attributed to a combination of dielectric effect and collective electric dipole coupling effects among the CdSe-CdS nanorods inside the elongated needle-like superparticles embedded in PDMS films (26–28). In addition, we show that the superparticle-embedded PDMS films can be used as energy downconversion phos-

phors to build polarized light-emitting diodes (Fig. 4, G to J, and fig. S27).

Our results show that anisotropic interactions of CdSe-CdS nanorods can be used to synthesize colloidal superparticles with multiple well-defined supercrystalline domains under thermodynamic equilibrium. Functionality-based anisotropic interactions between these nanorods can be kinetically introduced during the superparticle synthesis, leading to the formation of single-domain, needle-like particles. We anticipate that these findings can be extended for the self-assembly of nano-objects having other anisotropic shapes, as well as the self-assembly of two or more types of anisotropic nano-objects into well-defined mesoscopic and macroscopic complex architectures (1–3).

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## Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6105/358/DC1  
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# Integrated Compact Optical Vortex Beam Emitters

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Emerging applications based on optical beams carrying orbital angular momentum (OAM) will probably require photonic integrated devices and circuits for miniaturization, improved performance, and enhanced functionality. We demonstrate silicon-integrated optical vortex emitters, using angular gratings to extract light confined in whispering gallery modes with high OAM into free-space beams with well-controlled amounts of OAM. The smallest device has a radius of 3.9 micrometers. Experimental characterization confirms the theoretical prediction that the emitted beams carry exactly defined and adjustable OAM. Fabrication of integrated arrays and demonstration of simultaneous emission of multiple identical optical vortices provide the potential for large-scale integration of optical vortex emitters on complementary metal-oxide–semiconductor compatible silicon chips for wide-ranging applications.

The discovery that photons in optical vortices—light beams with helical phase fronts and an azimuthal component of the wave vector—can carry orbital angular momentum (OAM) (1) may lead to wide-ranging applications in optical microscopy (2), micromanipulation (3), free-space communication (4, 5), and quantum information (6, 7). Techniques for generating optical

vortices involve passing free-space light beams through optical elements, including computer-generated holograms (4, 8), spiral phase plates (9), inhomogeneous birefringent elements (10), subwavelength gratings (11), and nanoantennas (12).

Photonic integration has been a major propellant for widespread application of photonic technologies due to advantages in reliability,

miniaturization, and scalability compared with bulk optics (13). Compact, robust, and efficient planar waveguide-based OAM emitters and receivers are critical elements, as they can be integrated in large numbers and interconnected via waveguides with each other and with lasers and detectors to form photonic integrated circuits (PICs). Recently, a waveguide-based device has been reported for multiplexing and demultiplexing of OAM beams as a means of realizing multi-channel optical communication (5, 14). However, its large size ( $2.5 \text{ by } 1.5 \text{ mm}^2$ ) and phase-sensitive arrayed waveguide structure do not yet support large-scale integration. Here, we report micrometer-sized silicon photonic waveguide OAM devices

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that emit vector optical vortices carrying well-defined, quantized, and tunable OAM, as well as integrated OAM emitter arrays that emit multiple optical vortices simultaneously.

Circular optical resonators, such as micro-rings or microdisks (15), support whispering gallery modes (WGMs) carrying high OAM (16, 17). To extract the confined WGMs into free-space emission, we embed angular grating structures into the WGM resonator (Fig. 1A) with a periodic modulation of refractive index in the azimuthal direction.

The working principle of the angular grating is analogous to that of second-order gratings widely used in straight waveguides as input/output couplers (18), in which the guided wave is scattered by the grating elements collectively and an appreciable fraction of power is diverted in a certain direction  $\phi$ , in which constructive interference occurs. The wavefront of the radiated light is a plane tilted at angle  $\phi$  (Fig. 1B). If the waveguide with grating is curved to form a loop so that the guided wave forms WGMs, by way of Huygens' Principle, the wavefront of the radiated light should skew in the azimuthal direction and transform into a helix, suggesting the creation of an OAM-carrying beam (Fig. 1C). Rigorous theoretical derivation shows that a WGM only emits into a free-space beam when the following angular phase-matching condition is satisfied (16)

$$v_{\text{rad}} = p - q \quad (1)$$

where  $p$  is the azimuthal order of the WGM involved, or the number of optical periods around the resonator, and  $q$  is the number of grating elements around the resonator.  $v_{\text{rad}}$  is the azimuthal propagation constant (phase shift per unit azimuthal angle) of the radiated beam, which gives rise to the azimuthal component of the wave vector and, hence, OAM. The  $z$  component of the propagation constant of the radiation mode is

given by  $\beta_{\text{rad},z} = \sqrt{(2\pi/\lambda)^2 - (v_{\text{rad}}R)^2}$ , where  $\lambda$  is the vacuum wavelength and  $R$  is the resonator radius (Fig. 1A).

Because the state of polarization (SOP) of the source WGMs and the angular grating structure are both cylindrically symmetric, the radiated beams should maintain this symmetry and should be cylindrical vector (CV) beams (19). In our devices (Fig. 1A), for quasi-transverse electric (TE) WGMs, the radiated near field is predominantly azimuthally polarized (16) with its Jones vector  $E_{\text{CV}}$  written as (20, 21)

$$\begin{aligned} E_{\text{CV}} &= \begin{pmatrix} -\sin\theta \\ \cos\theta \end{pmatrix} \exp(iv_{\text{rad}}\theta) \\ &= \begin{pmatrix} -\sin\theta \\ \cos\theta \end{pmatrix} \exp[i(p - q)\theta] \\ &= \begin{pmatrix} -\sin\theta \\ \cos\theta \end{pmatrix} \exp(il\theta) \end{aligned} \quad (2)$$

where  $\theta$  is the azimuthal angle, and  $i$  is the unit imaginary number (Fig. 1A). Following the

concept introduced in (20), we conclude that the radiated beams are vector vortices with a topological Pancharatnam charge of  $l = p - q$ . The topological Pancharatnam charge, similar to the topological charge in scalar vortices, is directly related to the OAM of vector vortices: The amount of OAM carried by the radiated beam is  $l\hbar$  per photon, where  $\hbar$  is Planck's constant  $h$  divided by  $2\pi$ .

We therefore have a very simple yet robust OAM emitter scheme, in which  $l$  can only take integer values, being solely determined by the difference between integers  $p$  and  $q$ . Equation 1 indicates that the angular grating diffracts the light confined in a  $p$ th order WGM [carrying a OAM of  $p\hbar$  per photon (16)] into a free-space beam, changing the OAM by an amount of  $q\hbar$  per photon in the process. For a fabricated device,  $q$  is a structural constant, whereas the value of  $p$  can be changed by exciting selected WGMs. Hence, variable OAM can be generated by tuning the injected laser wavelength to various cavity resonances or, alternatively, by tuning cavity resonances with respect to a fixed injection wavelength by changing the cavity refractive index. The integer  $p$  can also take negative values corresponding to the opposite WGM propagating direction.

We designed and fabricated two types of microring devices ( $R = 3.9 \mu\text{m}$ ,  $q = 36$  and  $R = 7.5 \mu\text{m}$ ,  $q = 72$ ) on the same silicon-on-insulator chip (16),

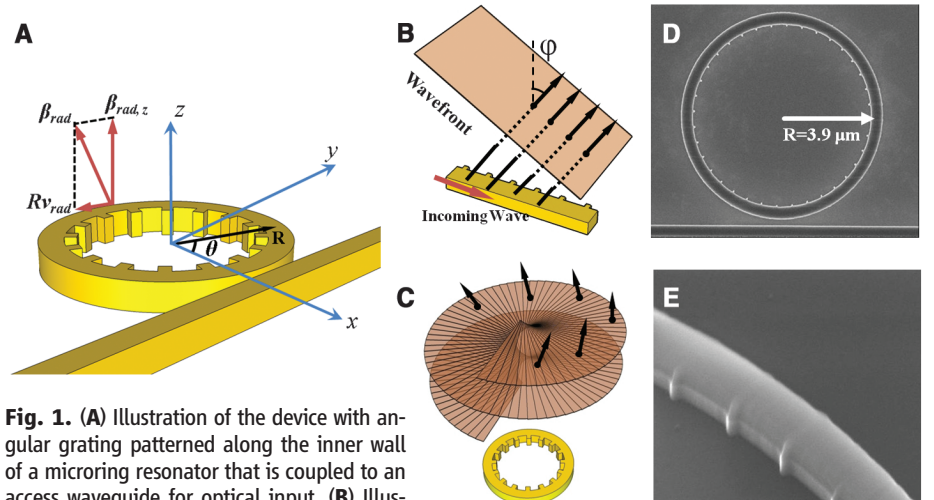
with their resonance associated with  $l = 0$  to be near the center of our tunable laser's wavelength range (1470 to 1580 nm). Figure 1, D and E, shows scanning electron microscopy (SEM) images of the device with  $R = 3.9 \mu\text{m}$ .

Both types of devices have been characterized by launching continuous-wave light from a tunable laser into the access waveguide to excite the quasi-TE mode. The near-field intensity of the radiated beam from the devices, with  $l = 0$ , is annular with a dark center, as imaged on an infrared camera (Fig. 2A), and is predominantly azimuthally polarized (Fig. 2, B to E).

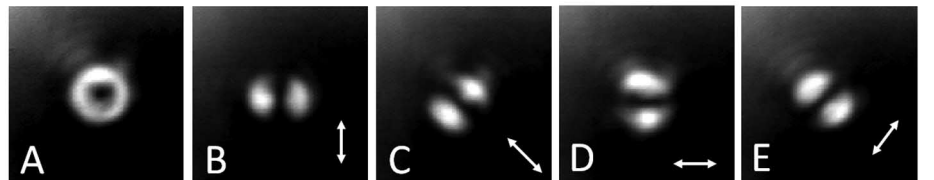
The emission spectrum of the device with  $R = 7.5 \mu\text{m}$  and  $q = 72$  is shown in Fig. 3A. Each resonance corresponds to a distinctive WGM ( $p$  value). The emission efficiencies at various resonances are measured to be 3 to 13% (16) and are higher at the longer wavelength side due to the wavelength-dependent coupling between the access waveguide and the resonator.

We used an interference scheme to characterize the wavefront structure. We observe that the radiated CV vortex beam can be described as the superposition of two orthogonal scalar vortices, as  $E_{\text{CV}}$  in Eq. 2 can be further decomposed into (22)

$$E_{\text{CV}} = \frac{i}{2} \begin{pmatrix} 1 \\ -i \end{pmatrix} \exp[i(l+1)\theta] - \frac{i}{2} \begin{pmatrix} 1 \\ i \end{pmatrix} \exp[i(l-1)\theta] \quad (3)$$



**Fig. 1.** (A) Illustration of the device with angular grating patterned along the inner wall of a microring resonator that is coupled to an access waveguide for optical input. (B) Illustration of a linear waveguide with gratings and the tilted wavefront of the radiated light. (C) Illustration of an angular grating together with the helical wavefront of the radiated beam. (D and E) SEM images of a fabricated device ( $R = 3.9 \mu\text{m}$ ).



**Fig. 2.** (A) Measured near-field intensity distribution of the radiated beam with  $l = 0$ . (B to E) Measured intensity distributions after a polarizer in the directions indicated by the arrows. A two-lobe intensity pattern arranged orthogonal to the polarizer axis is obtained. When the polarizer is rotated, the two-lobe pattern rotates in the same manner, confirming that the radiated beam is a CV beam with azimuthal polarization.



which consists of a right-hand circularly polarized (RHCP) beam with topological charge of  $l + 1$  and a left-hand circularly polarized (LHCP) beam with  $l - 1$ . This indicates a new scheme of measuring the value of  $l$ : When the radiated beam interferes with a copropagating circularly polarized reference beam, spiral interference patterns should be produced, with the number of arms equal to either  $l - 1$  or  $l + 1$ , depending on the handedness of the reference beam SOP.

The measured interference patterns (Fig. 3, B and C) have spiral arms equal to  $l - 1$  (RHCP) or  $l + 1$  (LHCP) as predicted in the aforementioned scheme, and the sign of the topological charge is

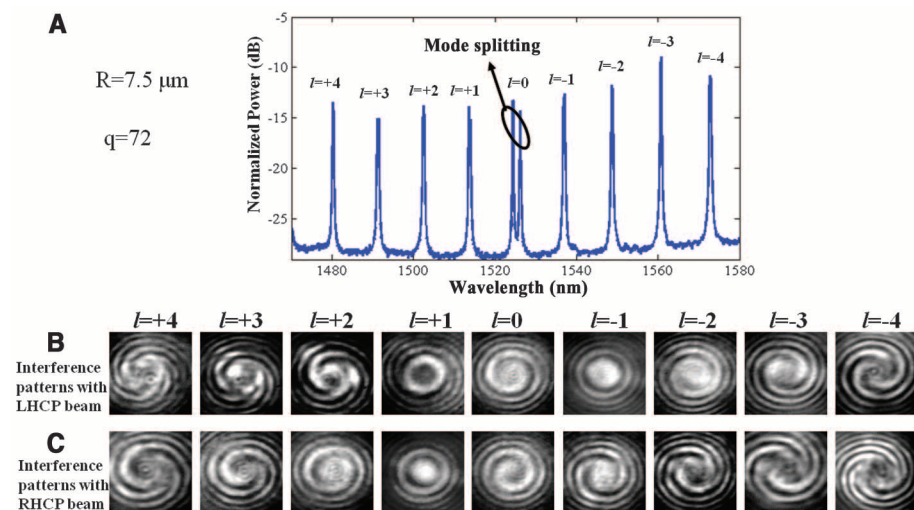
indicated by the chirality of the pattern. Thus, the nine resonances correspond to  $l = 0, \pm 1, \pm 2, \pm 3$ , and  $\pm 4$ . Similar results for the device with  $R = 3.9 \mu\text{m}$  are given in the supplementary materials (16). Moreover, the spiral patterns rotate when the phase of the reference beam is changed continuously (movies S1 and S2). These results show unambiguously that the wavefront of the radiated beams is helical with  $l = p - q$ . Beams with larger OAM quantum numbers  $l$  can be generated from the device. However, the observable  $l$  is limited by the tuning range of the tunable laser.

To demonstrate the potential of photonic integration, we fabricated OAM emitter arrays consisting of three identical emitters ( $R = 7.5 \mu\text{m}$ ,  $q = 72$ ) coupled to the same access waveguide (Fig. 4, A and B). Simultaneous emission of identical vortices has been verified, as shown in Fig. 4, C and D. The spiral patterns rotate synchronously when the phase of the reference beam is changed (movie S3).

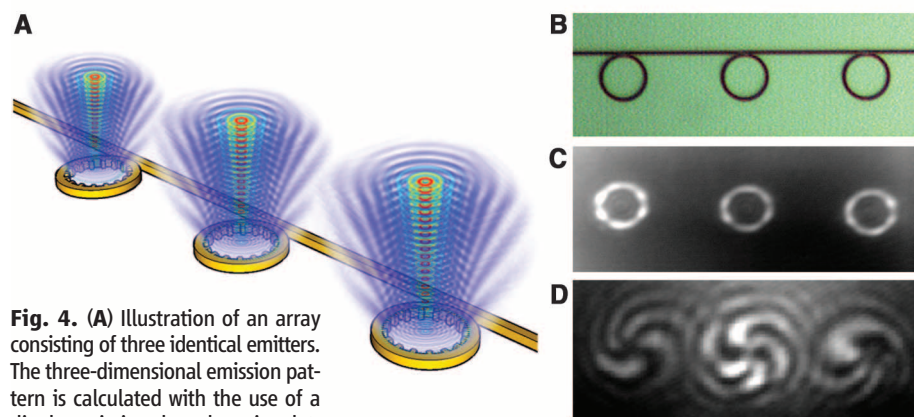
Our OAM emitters based on complementary metal oxide semiconductor compatible silicon PICs produce optical vortex beams with distinctive and variable OAM values from a very simple and small device, with no need for any fine adjustment of optical phase. While we have already achieved useful emission efficiency of up to 13%, efficiency can be maximized by engineering the coupling ratio between the resonator and the access waveguide to the critical coupling point (23), at which all of the input power enters the resonator. As demonstrated by the integrated arrays, integration of a large number of devices can be realized with the use of standard integrated circuit technology to form complicated formations on silicon wafers.

Such scalable integration could open up truly large-scale integrated applications opportunities. For example, it is possible to build OAM quantum communications channels between two chips, each containing the same integrated OAM PICs—one as the OAM transmitter and the other as the OAM receiver (according to the principle of reciprocity, the device emitting a specific vortex beam will selectively receive the same beam). Though it has been shown that OAM multiplexing and demultiplexing can be achieved with the use of PICs (14), our device enables rapid switching among OAM states, as semiconductor tunable lasers can already switch wavelengths in nanoseconds (24), and silicon microrings have been shown to tune at frequencies up to 40 GHz (25). Therefore, our device provides an approach for an integrated OAM switch or modulator. Another application area could be micromanipulation of particles (26). By selectively lighting groups of integrated emitter arrays, controllable and reconfigurable drivers can be configured for microfluidic and nanoparticle manipulation machines, such as lab-on-a-chip, optical tweezers, and optical spanners.

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**Fig. 3.** (A) Radiation spectrum for a device with  $R = 7.5 \mu\text{m}$  measured by scanning input laser wavelength. The  $l = 0$  wavelength is  $\sim 1525 \text{ nm}$ . The doublets in the spectrum result from the mode splitting caused by cross-coupling between the otherwise degenerate clockwise and counterclockwise WGMs. The strongest cross-coupling occurs at the wavelength with  $l = 0$  due to Bragg reflection, which is therefore associated with the largest split. (B and C) Interference patterns with LHCP and RHCP reference beams. Each pattern in (B) has  $l + 1$  spiral arms, whereas each pattern in (C) has  $l - 1$  spiral arms.



**Fig. 4.** (A) Illustration of an array consisting of three identical emitters. The three-dimensional emission pattern is calculated with the use of a dipole-emission-based semianalytical model (16). (B) Micrograph of a fabricated array. (C) Near-field intensity patterns emitted from the array. The difference in their brightness is attributed to slight differences in their resonance peaks due to fabrication variations. (D) Example of an interference pattern between the emitted beams from the array and the copropagating RHCP Gaussian beam. All beams have four arms and, therefore, the same OAM order  $l = -3$ . The uneven brightness is due to the Gaussian distribution of the reference beam, which is only coaxial with the middle vortex. The two side vortices are somewhat deformed due to lens aberration causing phase-front distortion.

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### Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S7

References (27–31)

Movies S1 to S4

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# Lethally Hot Temperatures During the Early Triassic Greenhouse

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Global warming is widely regarded to have played a contributing role in numerous past biotic crises. Here, we show that the end-Permian mass extinction coincided with a rapid temperature rise to exceptionally high values in the Early Triassic that were inimical to life in equatorial latitudes and suppressed ecosystem recovery. This was manifested in the loss of calcareous algae, the near-absence of fish in equatorial Tethys, and the dominance of small taxa of invertebrates during the thermal maxima. High temperatures drove most Early Triassic plants and animals out of equatorial terrestrial ecosystems and probably were a major cause of the end-Smithian crisis.

**A**nthropogenic global warming likely is contributing to the rapid loss of biological diversity currently occurring (1). Climate warming also has been implicated in severe biotic crises in the geological past, but only as a corollary to more direct causes of death such as

the spread of marine anoxia (2). Here, we show that lethally hot temperatures exerted a direct control on extinction and recovery during and in the aftermath of the end-Permian mass extinction. As well as the scale of the losses, the aftermath of this event is remarkable for several

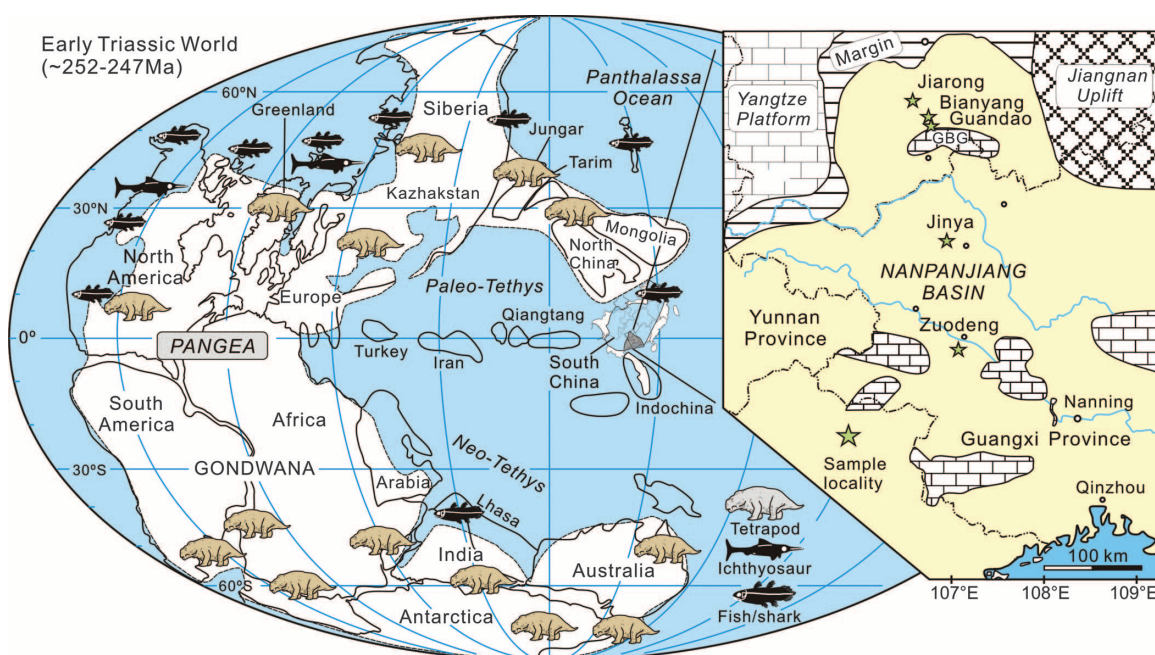
reasons, such as the prolonged delay in recovery (3), the prevalence of small taxa (4), and the absence of coal deposits throughout the Early Triassic (5). These and several facets of low-latitude fossil records shown below, including fish, marine reptile, and tetrapod distributions, can be related to extreme temperatures in excess of tolerable thermal thresholds.

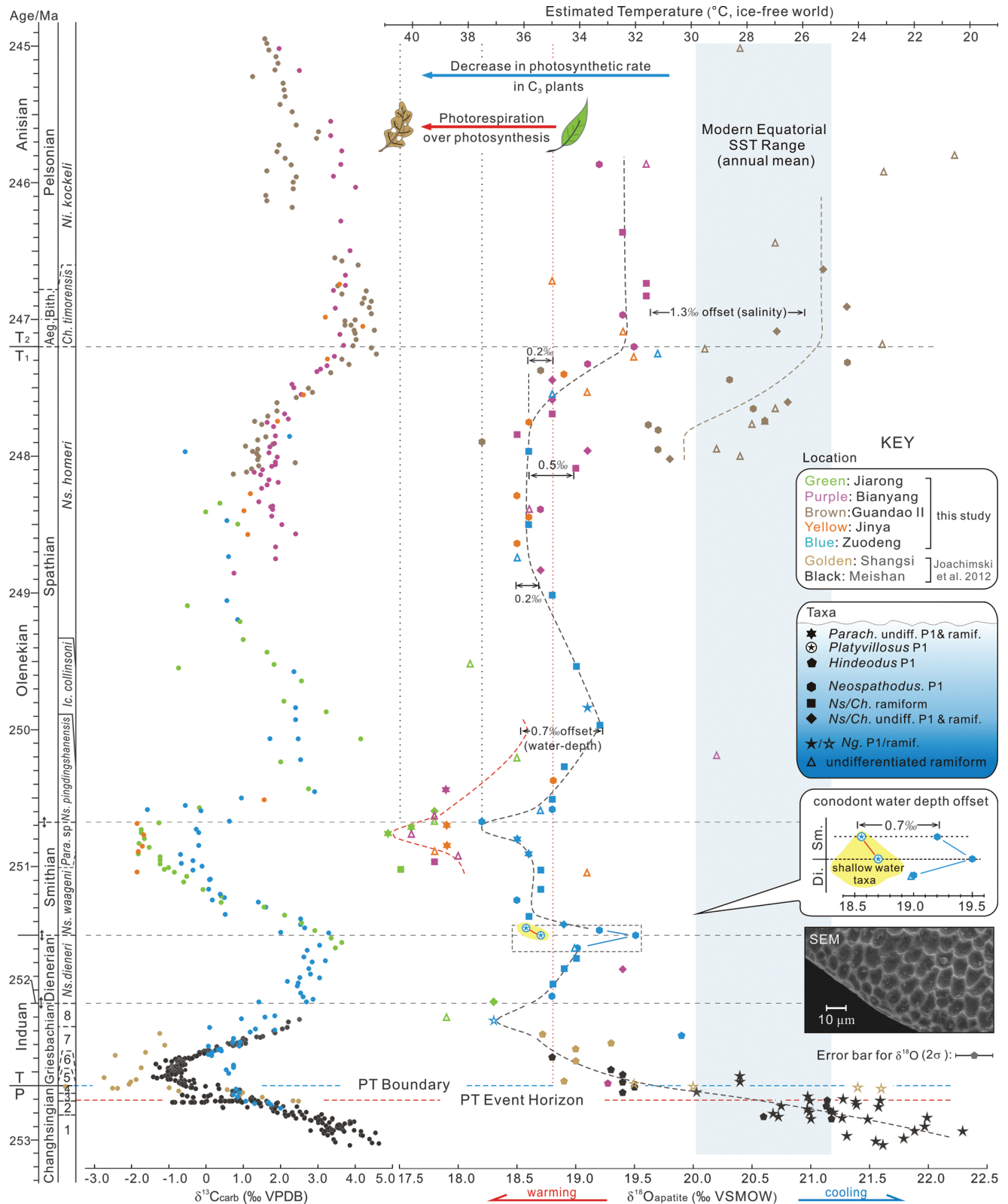
Climate warming long has been implicated as one cause of the end-Permian crisis (2, 6), with carbon dioxide release from Siberian eruptions and related processes providing a potential trigger for it (7, 8). Conodont apatite oxygen isotope

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**Fig. 1.** Early Triassic paleogeography showing reported occurrences of fish and marine reptiles in the Smithian. Note rare equatorial occurrence of both groups when ichthyosaurs had evolved in northern climes. The global distribution of tetrapods (25) indicates occurrences almost exclusively in higher latitudes (>30°N and >40°S) throughout the Early Triassic, with rare exceptions in Utah (*Parotosuchus* sp., paleolatitude ~10°N) and Poland (paleolatitude ~20°N), both probably of middle-late Spathian age (25, 26). (**Inset**) Paleogeography of Pangea and Nanpanjiang Basin after (45–47). Fish and ichthyosaurs occurrences, see table S2. GBG, Great Bank of Guizhou.





**Fig. 2.** Oxygen isotopes of conodont apatite and carbon isotopes of carbonates from the Nanpanjiang Basin. Oxygen isotopes show two thermal maxima in the late Griesbachian and late Smithian. Scanning electron microscope investigation of conodont surfaces shows microreticulation and no sign of recrystallization (supplementary text 3). Absolute age constraints are given in supplementary text 9; data for Meishan and Shangsi sections compiled from (9); leaf icons represent marine and terrestrial C<sub>3</sub> plants (14). Modern equatorial SST ranges (annual mean) from (48).

The error bar stands for external reproducibility of δ<sup>18</sup>O<sub>apatite</sub> measurements (2σ). The black trendline represents smoothed δ<sup>18</sup>O<sub>apatite</sub> fluctuations estimated from the upper water column taxa. Note uncertainty of correlating conodont zones with absolute ages. Aeg., Aegean; Bith., Bithynian. Conodont zonations: 1, *Ng. changxingensis*; 2, *Ng. yini*; 3, *Ng. meishanensis*; 4, *H. changxingensis*; 5, *H. parvus*; 6, *Is. staeschei*; 7, *Is. isarcica*; 8, *Ng. planata*; for genera abbreviations, see table S4.VSMOW, Vienna Standard Mean Ocean Water; VPDB, Vienna Pee Dee Belemnite.

ratio ( $\delta^{18}\text{O}$ ) is a reliable proxy for paleoseawater temperatures (9), and conodonts suffered few genus-level losses at the end of the Permian (10), allowing continuous sampling of the same genera over multimillion-year intervals (11). We used  $\delta^{18}\text{O}_{\text{apatite}}$  of conodonts from sections in the Nanpanjiang Basin, South China, to reconstruct Late Permian to Middle Triassic equatorial seawater temperatures (Fig. 1 and supplementary text 1). Our main record, measured on the genus *Neospathodus*, is a monitor of upper water column temperatures (estimated ~70 m water depth, supplementary text 2), whereas data from extremely shallow water taxa (*Pachycladina* or *Parachirognathus* spp., *Platyvillosus* spp.) provide sea surface temperatures (SSTs).

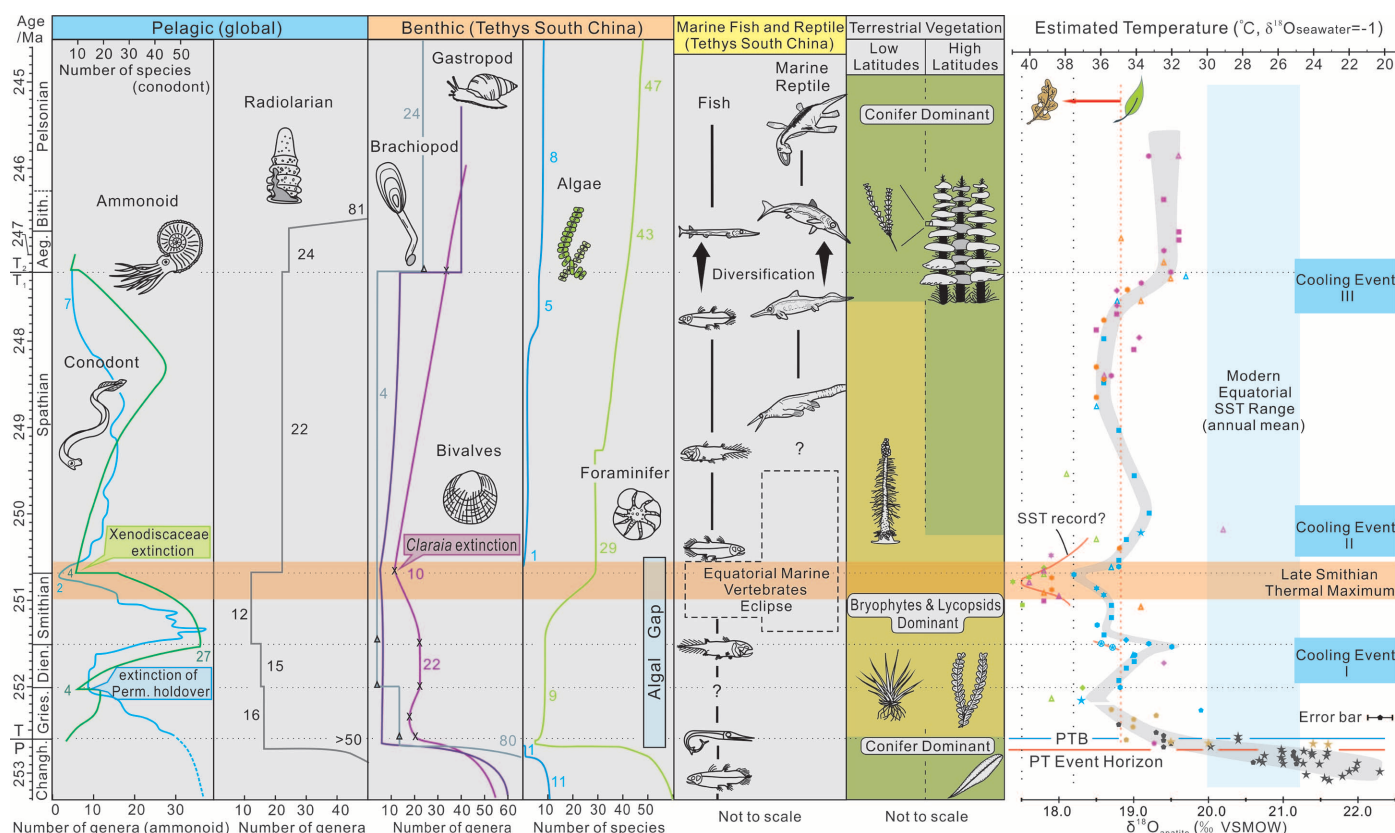
Our results show large, near-synchronous perturbations in both carbon isotope ratios ( $\delta^{13}\text{C}_{\text{carb}}$ ) and  $\delta^{18}\text{O}_{\text{apatite}}$  with three positive excursions observed in the Dienerian (~251.5 million years ago (Ma)), early Spathian (~250.5 Ma), and at the Spathian-Anisian (Early-Middle Triassic) transition (~247.5 Ma). The minima in  $\delta^{13}\text{C}_{\text{carb}}$  and  $\delta^{18}\text{O}_{\text{apatite}}$  are measured in the Griesbachian (~252.1 Ma) and the Smithian-Spathian transition (~250.7 Ma) (Fig. 2). The  $\delta^{18}\text{O}_{\text{apatite}}$  values

of the analyzed conodonts taxa accord with their habitats in different water depth: *Neospathodus* spp. shows ~0.7 per mil (‰) heavier values than those from shallow-water *Pachycladina/Parachirognathus* spp. and *Platyvillosus* spp. Deeper-water gondolellids show even heavier  $\delta^{18}\text{O}_{\text{apatite}}$  (~0.4‰) than *Neospathodus* spp. (supplementary text 2 and table S1). Latest Spathian–early Anisian oxygen isotope data from Bianyang and Guandao are more scattered and up to 1.3‰ heavier compared with samples from other sections. These two locations are close to the Great Bank of Guizhou (Fig. 1), and such  $^{18}\text{O}$  enrichment toward platform interior is interpreted to be due to evaporation as seen on the modern Bahama Bank (12). However, most of the presented data are from distal, open-water environments and therefore present a faithful paleotemperature record (supplementary texts 3 and 4).

Calculation of seawater temperatures from  $\delta^{18}\text{O}$  values (supplementary text 5) reveals rapid warming across the Permian-Triassic boundary [21° to 36°C, over ~0.8 million years (My); (9)], reaching a temperature maximum within the Griesbachian (~252.1 Ma) followed by cooling in the Dienerian. A second rise to high temperatures

is seen in the late Smithian (~250.7 Ma), followed by relatively stable temperatures in the Spathian, cooling at the end of this stage and stabilization in the early Middle Triassic (Fig. 2). The late Smithian Thermal Maximum (LSTM) marks the hottest interval of entire Early Triassic, when upper water column temperatures approached 38°C with SSTs possibly exceeding 40°C (Fig. 3).

The entire Early Triassic record shows temperatures consistently in excess of modern equatorial annual SSTs. These results suggest that equatorial temperatures may have exceeded a tolerable threshold both in the oceans and on land. For  $\text{C}_3$  plants, photorespiration predominates over photosynthesis at temperatures in excess of 35°C (13), and few plants can survive temperatures persistently above 40°C (14). Similarly, for animals, temperatures in excess of 45°C cause protein damage that are only temporarily alleviated by heat-shock protein production (15). However, for most marine animals, the critical temperature is much lower, because metabolic oxygen demand increases with temperature while dissolved oxygen decreases (16). This causes hypoxaemia and the onset of anaerobic mitochondrial metabolism that is only



**Fig. 3.** Early Triassic diversity of major marine groups and temperature trends showing inverse relationship: Peak diversity corresponds to cool climate conditions around the Dienerian-Smithian boundary, early Spathian, and early Anisian (named cooling events I to III), whereas low diversity in Griesbachian and Smithian correlates with peak temperatures. Diversity of marine groups from (37–39, 49–52); fish and marine reptile only show the general presence of taxa; no quantitative diversity data are available (sup-

plementary text 6). Floral data (28–30, 42) show the loss of equatorial conifer-dominated forests above the Permian-Triassic (PT) boundary, with the earlier reappearance of this forest type at high latitudes. Gray band represents the first-order seawater temperatures trend (upper water column, ~70-m water depth) estimated by this study; red trend line represents possible SST derived from shallow water taxa. Same stratigraphic scheme as Fig. 2.



sustainable for short periods (17). As a consequence, marine animals cannot long survive temperatures above 35°C, particularly those with a high performance and high oxygen demand, such as cephalopods (16).

Extreme equatorial warmth should have left a distinct signature in the Early Triassic fossil records, a proposition that we examine here. The fossil fish record is exceptionally good in the Early Triassic, with many well-preserved faunas known from locations such as Madagascar, Greenland, and British Columbia (supplementary text 6). This is related to the widespread distribution of anoxic facies (18) that provide excellent preservational conditions for such fossils. However, our compilation of fish occurrences reveals that they are very rare in equatorial locales, especially during the late Griesbachian and the Smithian, despite being common at higher latitudes at these times (fig. S1 and table S2). This rarity is extraordinary because Early Triassic units, such as the dysoxic-anoxic Daye Formation of South China, are widespread (supplementary text 7) and yet do not yield a fossil fish fauna. The general absence of ichthyofauna in equatorial regions coincides with the temperature maxima reconstructed from the  $\delta^{18}\text{O}_{\text{apatite}}$  record, and we interpret this coincidence as recording equatorial exclusion because of inhospitably high temperatures. In contrast, invertebrates remain common in these intervals (19), especially sessile mollusks with their better adapted oxyconforming metabolism allowing them to cope with synergistic stresses of high temperature and low oxygen (17, 20). Like fish, marine reptiles also exhibit high aerobic activity and are likely to have had a relatively low oxygen-limited thermal tolerance. Examining Early Triassic marine reptile (ichthyosaur) occurrences reveals that they too are not found in equatorial waters until the middle-late Spathian (supplementary text 6), ~1 to 2 My after their first appearance in higher latitudes during the Smithian (21, 22). Other notable absences from equatorial oceans are calcareous algae, whose outage spans the entire end-Permian–early Spathian interval although they are present in higher latitudes [e.g., Spitsbergen, (23)]. Their equatorial absence (supplementary text 8) likely reflects inhibiting temperatures, whereas the abundance of calcimicrobial carbonates in shelf waters, one of the stand-out features of the Early Triassic (24), was possible because of the much higher temperature tolerance of cyanobacterial photosynthesis (16).

Critically high temperatures may also have excluded terrestrial animal life from equatorial Pangea, and with SSTs approaching 40°C the land temperatures are likely to have fluctuated to even higher levels. Our compilation of tetrapod fossil occurrences reveals them to be generally absent between 30°N and 40°S in the Early Triassic (Fig. 1), with rare exceptions (25, 26); this is a stark contrast to Middle and Late Triassic occurrences, when they occur at all latitudes (fig. S1). This equatorial “tetrapod gap” does

not reflect an absence of suitable strata for their preservation. For example, the Buntsandstein of Europe is one of the best known and most intensively investigated terrestrial formations of the Early Triassic; tetrapods are exceptionally rare in the lower part (Induan) and only become common in middle and upper units (late Early Triassic to Middle Triassic) (27). The tetrapod gap of equatorial Pangea coincides with an end-Permian to Middle Triassic global “coal gap” that indicates the loss of peat swamps (5). Peat formation, a product of high plant productivity, was only reestablished in the Anisian and then only in high southern latitudes (5), although gymnosperm forests appeared earlier (in the Early Spathian), but again only in northern and southern higher latitudes (28, 29). In equatorial Pangea, the establishment of conifer-dominated forests was not until the end of the Spathian (30), and the first coals at these latitudes did not appear until the Carnian ~15 My after their end-Permian disappearance (5). These signals suggest equatorial temperatures exceeded the thermal tolerance for many marine vertebrates at least during two thermal maxima, whereas terrestrial equatorial temperatures were sufficiently severe to suppress plant and animal abundance during most of the Early Triassic.

Thermal tolerance is likely to decrease for organisms with larger body sizes (31). Nonlethal effects of temperature increase include smaller adult size, which, in conjunction with increased juvenile mortality at higher temperatures (32, 33), will produce a fossil record dominated by small individuals. This is a well-known phenomenon in the Early Triassic marine fossil record and has been termed the Lilliput effect (4). We suggest that this effect is a response to high temperatures and that it should be most clearly seen in equatorial assemblages, especially during the Griesbachian and Smithian thermal maxima. This prediction is confirmed by data from equatorial marine fossils where small body and trace fossil assemblages are confined to these intervals (34, 35). Low oxygen levels also are known to cause small size in marine invertebrates (36), but, although marine dysoxia was a global phenomenon in the Early Triassic (18), the restriction of the Lilliput effect to equatorial latitudes indicates that this was primarily a temperature-controlled phenomenon.

The relation between global warming and extinction can be examined in the Early Triassic. The rapid temperature rise across the Permian–Triassic boundary coincides with mass extinction, although absolute temperatures at the time of crisis were only modest [ $< 30^\circ\text{C}$  (9)]. Together with temperature rise, synergistic factors, such as spread of anoxia, may also play important roles in marine extinction (2, 18). However, the subsequent loss of many Permian holdover taxa later in the Griesbachian (conodonts, radiolarian, and brachiopods) may reflect lethal temperatures followed by temporary recovery and radiation in the cooler Dienerian (Fig. 3). The clearest temperature-extinction link is with the LSTM and the end-

Smithian event that saw major losses among many marine groups, including bivalves, conodonts, and ammonoids (37–39). Contemporaneous losses among tetrapods on land (25) suggest that this was a crisis that affected a broad diversity of ecosystems.

The ultimate driving factor behind the end-Permian warming long has been attributed to greenhouse gas emissions, either from volcanogenic (8) or thermogenic sources (40). Both are expected to leave a negative excursion in the  $\delta^{13}\text{C}$  record, and this is the case for both the end Permian–Griesbachian and Smithian intervals (Fig. 2), although it has yet to be demonstrated that a second pulse of Siberian volcanism occurred in the Smithian. However, to maintain high temperatures for the ~5 My of the Early Triassic requires strong, persistent greenhouse conditions. High temperatures also could greatly enhance the activity of decomposers (e.g., fungi and bacteria), resulting in the release of large amounts of terrestrial light carbon into the atmosphere (41) and consequently forming oligotrophic, humus-poor soils as observed in modern Amazon rainforests and in Early Triassic soils of Australia and Antarctica (42). Together with global suspension of peat formation, elevated decomposition rates may have led to a significant reduction in organic carbon burial on land further contributing to higher atmospheric  $\text{CO}_2$  levels (43).

High and oscillating temperatures in the Early Triassic likely controlled the pace and nature of recovery in the aftermath of the end-Permian mass extinction as shown by an inverse relationship between the temperature and biodiversity changes, the temporary loss of both marine and terrestrial vertebrates, and the reduced size of the remaining invertebrates. SSTs derived from  $\delta^{18}\text{O}$  data offer no evidence that a climate thermostat may ameliorate tropical warming by redistributing warmth to the poles (44). Rather, extreme global warming may progressively force taxa to vacate the tropics and move to higher latitudes or become extinct. Marine organisms exhibiting low oxygen-dependent thermal tolerance, such as vertebrates, are the first to leave.

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#### Supplementary Materials

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Materials and Methods

Supplementary Text

Fig. S1

Tables S1 to S4

References (53–150)

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# A Complete Terrestrial Radiocarbon Record for 11.2 to 52.8 kyr B.P.

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Radiocarbon (<sup>14</sup>C) provides a way to date material that contains carbon with an age up to ~50,000 years and is also an important tracer of the global carbon cycle. However, the lack of a comprehensive record reflecting atmospheric <sup>14</sup>C prior to 12.5 thousand years before the present (kyr B.P.) has limited the application of radiocarbon dating of samples from the Last Glacial period. Here, we report <sup>14</sup>C results from Lake Suigetsu, Japan (35°35'N, 135°53'E), which provide a comprehensive record of terrestrial radiocarbon to the present limit of the <sup>14</sup>C method. The time scale we present in this work allows direct comparison of Lake Suigetsu paleoclimatic data with other terrestrial climatic records and gives information on the connection between global atmospheric and regional marine radiocarbon levels.

Lake Suigetsu contains annually laminated sediments that preserve both paleoclimate proxies and terrestrial plant macrofossils that are suitable for radiocarbon dating. The lake's

potential to provide an important archive of atmospheric radiocarbon (<sup>14</sup>C) was realized in 1993 (1). However, the single SG93 sediment core then recovered included missing intervals between successive sections (2). This, together with the difficulty of visual varve counting, resulted in inconsistency between the SG93 and other <sup>14</sup>C calibration records (3). The SG06 core-set recovered in 2006 consists of four parallel cores that together avoid any such sedimentary gaps (4). Here, we report 651 <sup>14</sup>C measurements covering the period between 11.2 and 52.8 thousand years before the present (kyr B.P.) tied to a time scale derived from varve counting and temporal constraints from other records. Using visual markers, we applied a composite depth (CD) scale to all cores, including SG93. We also define an event-free depth (EFD), which is the CD with substan-

tial macroscopic event layers (such as turbidites and tephras) removed.

Accelerator mass spectrometry radiocarbon dating (5) has been conducted on terrestrial plant macrofossils selected from the SG06 cores to cover the full <sup>14</sup>C time range, from the present to the detection limit of the <sup>14</sup>C method (0 to 41 m CD) (table S1). The results already reported from the control period (0 to 12.2 kyr B.P.) (6), covered by the tree-ring-derived calibration curve (7), act to demonstrate the integrity of the sediments and to anchor the floating SG06 varve chronology, because varves do not extend into the Holocene.

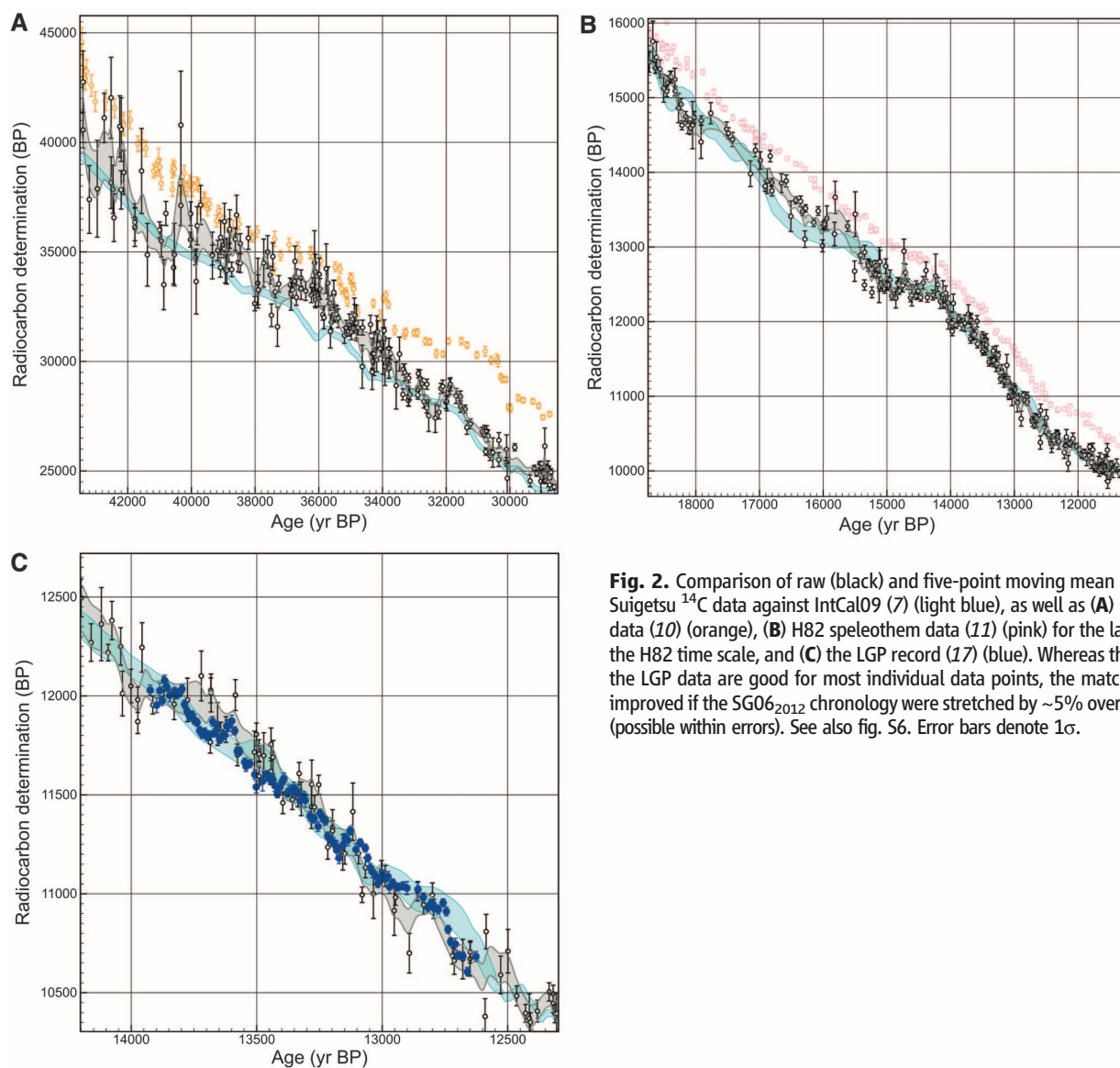
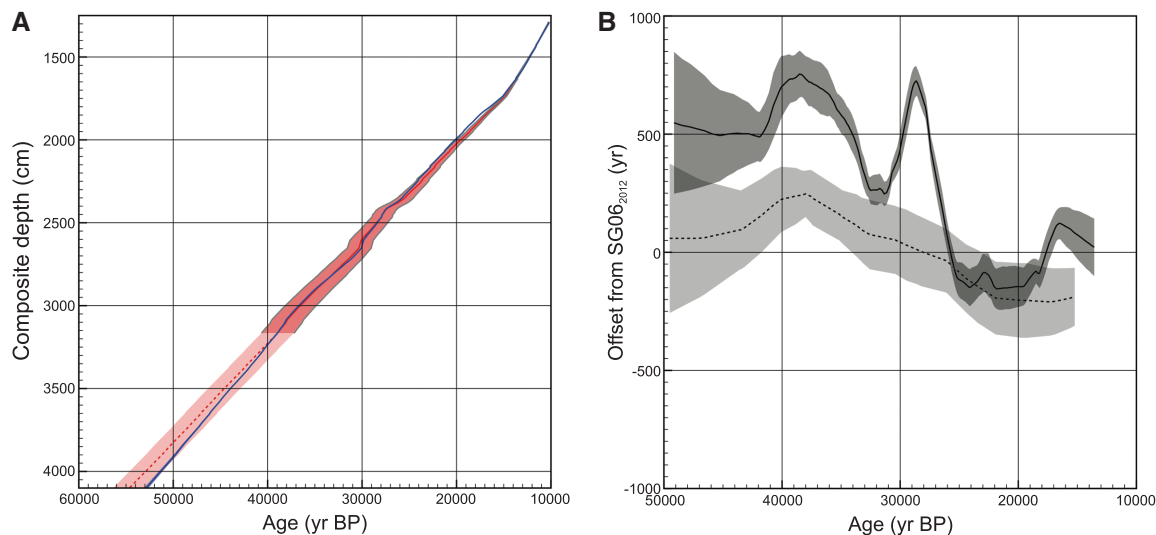
The varve-based chronology for SG06 (5, 8, 9) provides our best estimate of the true age of the cores for the period ~10.2 to 40.0 kyr B.P., based only on information from the site. It provides good relative chronological precision and has the advantage of being independent of other dating techniques. However, the cumulative counting uncertainty inevitably increases with age (~6% at 40 kyr B.P.). The full varve chronology (Fig. 1A and table S1) has been extrapolated on the basis of EFD to cover the period 40 to 53 kyr B.P.

To better constrain the uncertainties in the varve chronology, we can directly compare the Suigetsu data set and other archives that provide information on atmospheric <sup>14</sup>C and associated independent ages. The two most useful records for this purpose are the Bahamas speleothem GB89-25-3 (10) and the Hulu Cave speleothem H82 (11), both of which have extensive <sup>14</sup>C- and U-Th-based chronologies. In both cases, we would expect the radiocarbon in the speleothems to respond to changes in atmospheric <sup>14</sup>C content, despite the groundwater containing a dead-carbon fraction (DCF) from dissolved carbonates. Estimated DCF for these speleothems was 2075 ± 270 radiocarbon

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**Fig. 1. (A)** Age-depth model for SG06, based on varves (red), extrapolated by EFD (pink), and constrained by speleothems H82 and GB89-25-3 (blue). **(B)** Inferred differences in the age models for the Cariaco Basin (22) (solid curves) and Iberian Margin (23) (dotted curves) data sets in IntCal09 (7) compared to the SG06<sub>2012</sub> modeled chronology (higher offset implies that the time scale is older); see figs. S9 and S10.



**Fig. 2.** Comparison of raw (black) and five-point moving mean (gray) Lake Suigetsu <sup>14</sup>C data against IntCal09 (7) (light blue), as well as **(A)** GB89-25-3 data (10) (orange), **(B)** H82 speleothem data (11) (pink) for the latter part of the H82 time scale, and **(C)** the LGP record (17) (blue). Whereas the match to the LGP data are good for most individual data points, the match would be improved if the SG06<sub>2012</sub> chronology were stretched by ~5% over this period (possible within errors). See also fig. S6. Error bars denote 1 $\sigma$ .



years ( $^{14}\text{C}$  yr) for GB89-25-3 (10) and  $450 \pm 70$   $^{14}\text{C}$  yr for H82 (11), each at  $1\sigma$ . We have modeled these two records onto the SG06<sub>2012</sub> varve chronology using a Poisson process model (12), which allows for nonlinear random deviation between the SG06<sub>2012</sub> varve chronology and the U-Th time scale underlying the speleothem records (5). The model provides independent estimates for the mean DCF of the speleothems of  $2500 \pm 90$  for GB89-25-3 and  $440 \pm 25$  for H82, in agreement with the initial estimates.

The model results can also be used to refine the SG06 chronology by including the constraints provided by the speleothem U-Th dates to 44 kyr B.P. This greatly reduces the uncertainty in the absolute chronology of the age-depth profile (Fig. 1A and table S1) and ensures that the SG06 data are on a U-Th-moderated time scale. There are some significant differences between the varve chronology and the model (notably in the period around 12.6 kyr B.P.), possibly associated with changes in the sedimentation rate and sedimentary processes at Suigetsu through that period; however, for most of the core, the agreement between the chronologies is good (Fig. 1A), with the ratio between the varve-only and model-inferred deposition rates being  $1.01 \pm 0.10$ .

The modeled chronology (SG06<sub>2012</sub> yr B.P.) is based on all of the available information from both Suigetsu and the other long records of atmospheric radiocarbon. This precise time scale (sub-centennial  $1\sigma$  uncertainties to  $\sim 25$  kyr B.P.) allows us to place the detailed paleoclimate data from Suigetsu in a global context, facilitating the identification of potential leads and lags in climate change recorded in key paleoenvironmental archives.

The 651 terrestrial radiocarbon dates from the period 11,241 to 52,820 SG06<sub>2012</sub> yr B.P. provide a quasi-continuous record of atmospheric  $^{14}\text{C}$  for this period (Fig. 2), for which the current calibration curve (7) is largely based on reservoir-affected marine data. As such, the Suigetsu calibration data set provides the first “backbone” upon which a comprehensive atmospheric calibration curve can be built. It will allow, for example, the archeology related to the extinction of Neanderthals and spread of anatomically modern humans into Europe to be calibrated against terrestrial reference data. With its high density of  $^{14}\text{C}$  measurements, this data set also allows direct linking between SG06 and any other paleoenvironmental record with terrestrial radiocarbon data.

In addition to its importance for radiocarbon calibration and correlation between different climate records using radiocarbon, a full record of atmospheric  $^{14}\text{C}$  has important implications for our understanding of the carbon cycle. The links between atmospheric  $^{14}\text{C}$  and primary production are key, as is the connection between the atmosphere and the ocean. Figure 3 shows the inferred level of radiocarbon in the atmosphere ( $\Delta^{14}\text{C}$ ) at Lake Suigetsu, compared to the  $^{10}\text{Be}$  record from Greenland (13, 14). The peak in  $^{10}\text{Be}$  production around 41 kyr B.P., assumed to be enhanced by the Laschamp geomagnetic excursion

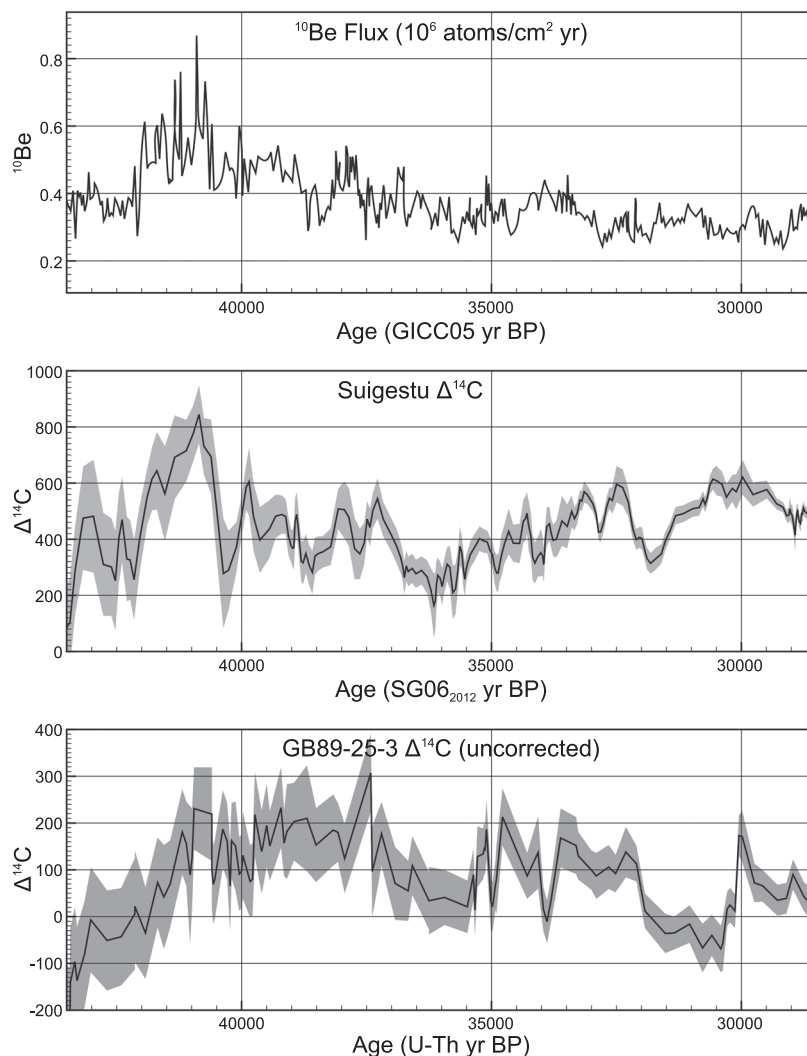
(15), is clearly visible in the Suigetsu data; the similar timing indicates congruence between the SG06<sub>2012</sub> and North Greenland Ice Core Project (NGRIP) GICC05 (16) time scales at this point.

Agreement between the Suigetsu radiocarbon record and those of the speleothems (GB89-25-3 and H82) is generally good (Fig. 2, A and B), though for the period 28–32 SG06<sub>2012</sub> kyr B.P., the implied reservoir offset for GB89-25-3 seems higher than for the older sections (Fig. 2A). Another  $^{14}\text{C}$  record of great importance is the European Late Glacial Pine (LGP) record (17). Figure 2C shows the match of this record to that of Suigetsu. The match puts the younger end of the LGP sequence at  $12627 \pm 35$  yr B.P. and gives the average radiocarbon offset of Suigetsu to the LGP data set as  $8 \pm 6$   $^{14}\text{C}$  yr (older). Our modeling supports the fit provided by the linkage of the LGP to Tasmanian Huon Pine (18), rather than the suggested  $^{10}\text{Be}$ -based fit to NGRIP (19).

We have used the radiocarbon sequence from Lake Soppensee, Switzerland, as an example of direct comparison with European varved lake sed-

iments (20). This enables us to place the climate proxy signal associated with the Younger Dryas onset at Soppensee at  $12607 \pm 85$  SG06<sub>2012</sub> yr B.P., some  $156 \pm 88$  years later than cooling in Suigetsu, which, assuming congruence with GICC05, probably lags the rapid cooling in Greenland (but only by 83 years, which could be synchronous within error margins). The match to Soppensee also enables us to place the European tephra marker of the Laacher See at  $12842 \pm 53$  SG06<sub>2012</sub> yr B.P., which agrees well with the independent age estimate of  $12880 \pm 40$  varve years (vyr) B.P. in Meerfelder Maar, Germany (21).

Direct comparison of the terrestrial radiocarbon signal from Suigetsu can also be made to that recorded in marine archives. The long records from the Cariaco Basin, west Atlantic (22), and the Iberian Margin record, northeast Atlantic (23), are climatically tuned to the Hulu Cave U-Th chronology. By constraining the total marine reservoir age for these two locations within reasonable limits (5), we are able to quantify offsets between the tuned chronologies and that of SG06

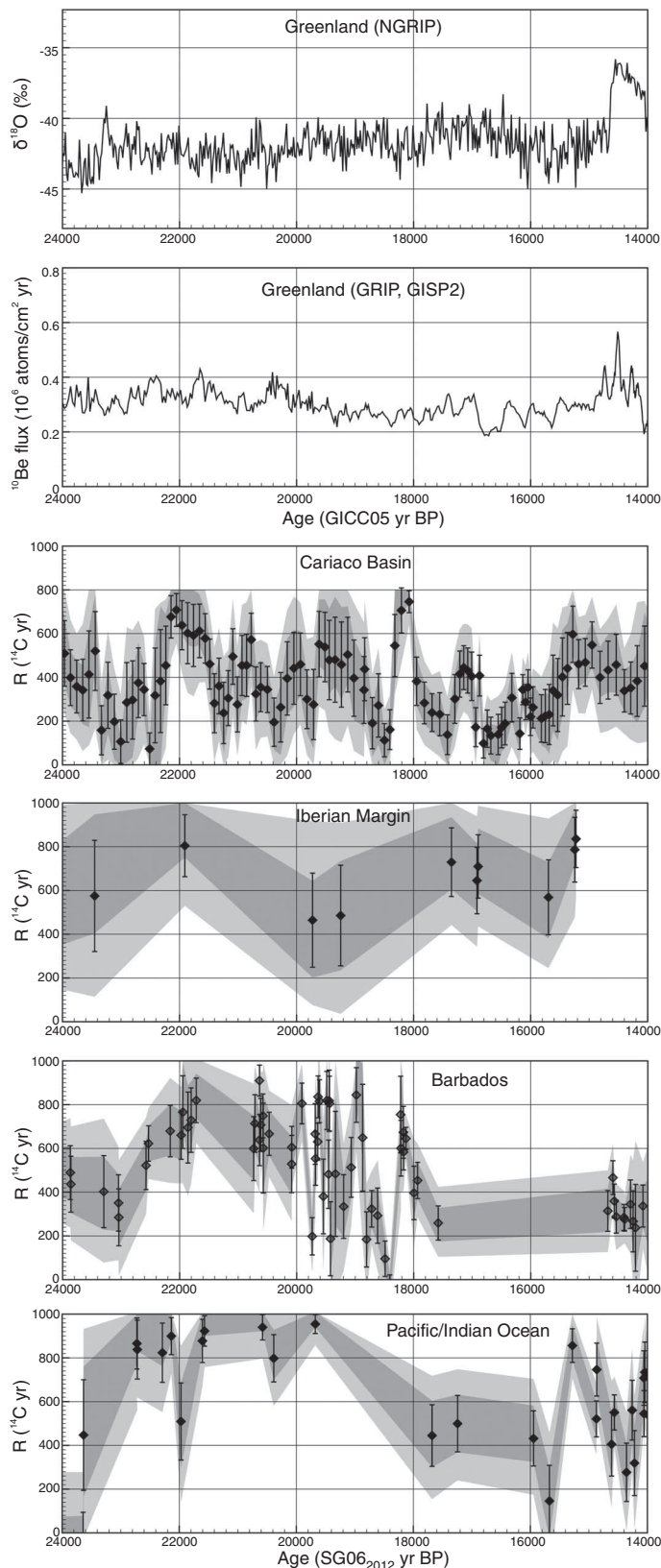


**Fig. 3.** Inferred  $\Delta^{14}\text{C}$  values from Lake Suigetsu compared to data from GB89-25-3 (10) uncorrected for DCF. The  $^{10}\text{Be}$  flux in Greenland (13, 14) is shown for comparison.

(Fig. 1B). The Iberian Margin time scale is most consistent with SG06 (within uncertainties), except that it is ~200 years older at ~40 kyr B.P. and ~200 years younger at ~15 kyr B.P. The latter could be due to greater reservoir ages in the north-

east Atlantic at the Last Glacial Maximum (LGM) than have previously been allowed. The Cariaco Basin time scale seems consistent with that for SG06 back to ~26 kyr B.P., at which point there is a discrete peak (amplitude ~500 years), perhaps

**Fig. 4.** Marine reservoirs relative to atmosphere ( $R$ ), as deduced from the Suigetsu data on the modeled SG06<sub>2012</sub> chronology. For comparison, we show the  $^{10}\text{Be}$  flux (13, 14) and the  $\delta^{18}\text{O}$  signal from Greenland on the GICC05 time scale (16).



due to specific choices in the climate-tuning. At ~40 kyr B.P., the Cariaco time scale, like that of the Iberian Margin, is older than that of SG06<sub>2012</sub>, but by a much greater extent (~700 years). Given that both records are tuned to the same (Hulu) chronology, these differences are more likely to be due to lags in different regional environmental responses to global climate rather than offsets between the fundamental time scales of Hulu and GB89-25-3 (and, hence, SG06<sub>2012</sub>) over this period.

We can also infer total marine reservoir age ( $R$ ) from these models (Fig. 4 and fig. S11). In general, most of the signal we see for the Cariaco Basin is due to greater fluctuations in the  $\Delta^{14}\text{C}$  of the atmosphere from that recorded in marine sediments. Some of the features mirror those of the  $^{10}\text{Be}$  production rate in Greenland and might be related to  $^{14}\text{C}$  production, others may reflect changes in local ocean dynamics. Although there are fewer data from the Iberian Margin, the rise in  $R$  between 16 and 15 kyr B.P. is still apparent, and there is a higher reservoir age inferred at ~22 kyr B.P., during the LGM.

For the marine coral data from IntCal09 (7), we additionally have direct U-Th dates; thus,  $R$  can be calculated more directly. Coral data, however, are not available at regular intervals, so we do not have a continuous record. Pacific coral data (7) in the range 30 to 39 kyr B.P. are consistently higher in  $\Delta^{14}\text{C}$  than the terrestrial data from Suigetsu (fig. S12). We have to deduce that either the SG06<sub>2012</sub> time scale is substantially too young (a hypothesis not supported by the Iberian Margin data, identification of the Laschamp event as compared to Greenland, or the speleothem data used to correct the modeled SG06<sub>2012</sub> time scale) or some of the coral data have elevated U-Th ages or overestimated  $^{14}\text{C}$  measurements (more likely, given the range of  $^{14}\text{C}$  measurements on some coeval samples). It is clear that  $R$  for the Pacific and Barbados corals (7), as for the Cariaco Basin and the Iberian Margin foraminifera, is greater at ~22 kyr B.P. (Fig. 4)—possibly a consequence of lower global  $\text{CO}_2$  during the LGM. In the period around 16 to 14 kyr B.P., there is a similar pattern in the Pacific/Indian Ocean as in Cariaco: a rapid rise in  $R$ , followed by a gradual fall.

The terrestrial radiocarbon record from Lake Suigetsu presented here, together with Holocene measurements (6), comprises 808 radiocarbon determinations from two core sites in the center of the lake, measured by three laboratories. Together, these give us a single, quasi-continuous record of purely atmospheric  $^{14}\text{C}$  covering the full range of the radiocarbon technique. This will greatly benefit calibration of terrestrial radiocarbon samples in the period 12.5 to 52.8 kyr B.P. and will enable direct correlation between other key climate records and the Lake Suigetsu record itself, without any assumptions of climatic synchrony. An atmospheric record of  $^{14}\text{C}$  over this whole time scale also facilitates a more comprehensive understanding of the long marine records in their oceanic context, rather than simply assuming that they represent atmospheric  $^{14}\text{C}$ .

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## Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6105/370/DC1  
Materials and Methods

Figs. S1 to S12  
Tables S1 to S3  
References (24–29)

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# Genomic Variation in Seven Khoe-San Groups Reveals Adaptation and Complex African History

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The history of click-speaking Khoe-San, and African populations in general, remains poorly understood. We genotyped ~2.3 million single-nucleotide polymorphisms in 220 southern Africans and found that the Khoe-San diverged from other populations ≥100,000 years ago, but population structure within the Khoe-San dated back to about 35,000 years ago. Genetic variation in various sub-Saharan populations did not localize the origin of modern humans to a single geographic region within Africa; instead, it indicated a history of admixture and stratification. We found evidence of adaptation targeting muscle function and immune response; potential adaptive introgression of protection from ultraviolet light; and selection predating modern human diversification, involving skeletal and neurological development. These new findings illustrate the importance of African genomic diversity in understanding human evolutionary history.

Genetic, anthropological, and archaeological studies provide substantial support for an African origin of modern humans, but the process by which modern humans arose has been vigorously debated (1, 2). African populations show the greatest genetic diversity, with genetic variation in Eurasia, Oceania and the Americas largely being a subset of the African diversity (3–6), with limited contribution from archaic humans (7). Within Africa, click-speaking southern African San and Khoe populations [“Khoe-San” from here on, following the San

Council recommendations] harbor the deepest mitochondrial DNA lineages (5), have great genomic diversity (8–10), and probably represent the deepest historical population divergences among extant human populations (11, 12). However, African populations have been underrepresented in genome-wide studies of genetic diversity, including assessment of the ethnic diversity within the Khoe-San in southern Africa, where previous studies have focused either on single-locus markers (13) or a few individuals from one or two populations (3, 4, 8–10).

We genotyped, quality-filtered, and phased ~2.3 million single-nucleotide polymorphisms (SNPs) in 220 individuals representing 11 populations from southern Africa: Ju/hoansi, !Xun, /Gui and //Gana, Karretjie People (hereafter “Karretjie”), #Khomani, Nama, Khwe, “Coloured” (Colesberg), “Coloured” (Wellington), Herero, and Bantu-speakers (South Africa) [Fig. 1A, (14), and table S4]. These data were analyzed together with published data (4, 9, 10, 15) after the removal of related and recently admixed individuals (14). To minimize the potential effect of ascertainment bias on results, we used several approaches that have previously been shown to be robust to these biases, including analyzing haplotypes, using minor allele frequency filtering within populations,

and comparing results to available sequence data (14). In a principal components analysis (PCA), the first two PCs closely recapitulate many aspects of a geographic map of Africa [Fig. 1B, Procrustes correlation: 0.585,  $P < 10^{-5}$  (14)], with the first PC representing a north-south axis that separates southern African Khoe-San populations from other populations, and the second PC representing an east-west axis that separates east African populations (including Hadza and Sandawe hunter-gatherers) from central African hunter-gatherers (Mbuti and Biaka Pygmies) and Niger-Kordofanian speakers (Fig. 1B). In this two-dimensional representation of sub-Saharan genetic diversity, hunter-gatherer populations from southern, central, and eastern Africa constitute three extremes, respectively, of a scaffold, where the fourth extreme is represented by all Niger-Kordofanian-speaking groups from across the African continent. Although Niger-Kordofanian-speaking populations have been sampled from southern, eastern, and western Africa, they all cluster closely in the vicinity of West African populations (Fig. 1B), a consequence of the recent “Bantu expansion.” If Bantu-speaking populations are removed from the analysis, the correlation between the first two PCs and geography increases to 0.715 ( $P < 10^{-5}$ ). In addition to geography, genetic structure can also be correlated with language and subsistence strategies, and we assessed the capacity of these factors to predict genetic components in sub-Saharan Africa (14). Geography predicted genetic components better than either language or subsistence, but combining geographic information with subsistence and especially linguistic information improved the prediction (Fig. 1E), suggesting that all of these factors contribute to genetic structure in sub-Saharan Africa.

Genetic cluster analysis (16) showed substantial structure among sub-Saharan individuals and reiterated the substructure among Khoe-San populations, Niger-Kordofanian speakers, east African populations, and central African hunter-gatherers (Fig. 2B) (14). Increasing the number of allowed clusters distinguishes finer levels of population substructure (Fig. 2B), including distinct non-African ancestry components for individuals who self-identify as “Coloured” (figs. S16, S18, and S21). Within the Khoe-San group, there was a distinct separation of Northern San populations (Ju speakers: !Xun and Ju/hoansi) and

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Southern Khoe-San populations [Tuu and Khoe speakers: Karretjie, #Khomani, and Nama; Figs. 1C and 2, B and C (14)]. Genetic differentiation (measured by Wright's  $F_{ST}$ ) between Northern San and Southern Khoe-San groups was  $\sim 0.015$  to  $0.025$  (Fig. 1D and fig. S25), similar to that between Nilo-Saharan (Maasai) and Niger-Kordofanian (Yoruba) groups.

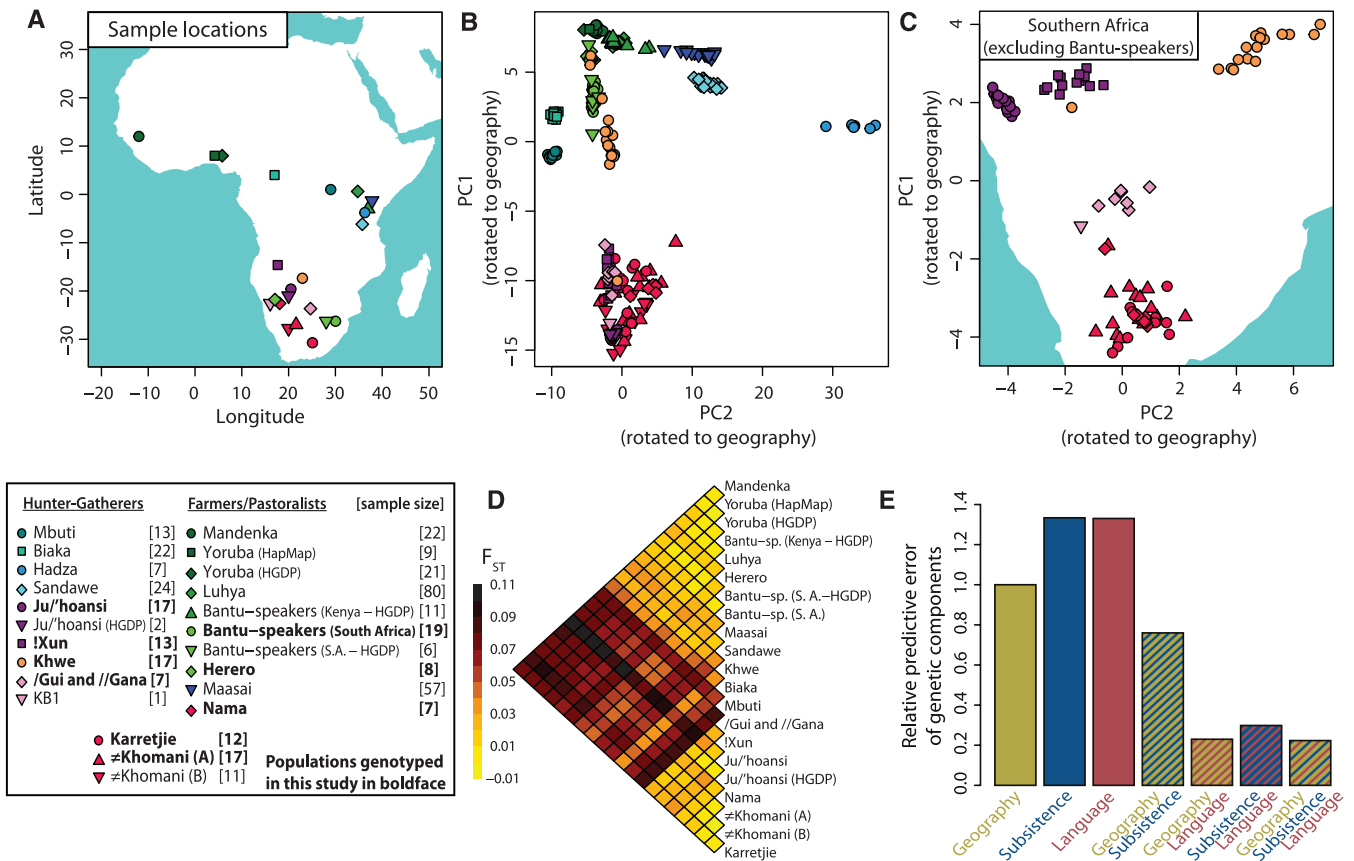
Assuming a population divergence model, we reconstructed the demographic history of sub-Saharan populations using genealogical concordance (17), which is robust to substantial levels of recent admixture and genetic drift (14). The inferred population history resembled the population structure results (Figs. 1B and 2, B and C), and six of seven Khoe-San groups shared a common history that was separate from that of all other extant populations. This division forms the deepest divergence among extant humans (Fig. 2A and fig. S32), and assuming an effective population size ( $N_e$ ) of 21,000 individuals (11, 12), the maximum likelihood divergence time is  $T_s = 0.083 \times 2 N_e$  generations (95% maximum likelihood confidence interval: 0.075 to 0.091), corresponding to  $\sim 100,000$  years ago (14), which is in agreement with previous estimates of 110,000 to 160,000 years ago (11, 12). The second deepest divergence involved central African pygmies and

was estimated to be less than half of the deepest divergence time ( $0.45 T_s$ ), and the subsequent population split involving East African hunter-gatherers and Maasai was even younger [compare with (6)]. The deep divergence between Northern and Southern Khoe-San groups corresponded to 25,000 to 43,000 years (14), which is similar to estimates between West Africans and Eurasians (11). Strict divergence models are unlikely to capture all features of human history; for instance, gene flow, which has probably been weak given the observed level of population structure but which was inferred even between isolated hunter-gatherer groups (12, 14), could affect these divergence estimates (11, 12, 14).

The origin and ancestry of the Khwe, who speak the "Central Khoisan" language Khoe-Kwadi, is uncertain (14). The genetic makeup of the Khwe was distinct from that of other Khoe-San groups [Fig. 2, (14), and fig. S5] but could be explained by high levels of (nonrecent) admixture between Bantu-speaking and Khoe-San groups. In contrast to the Khwe, the /Gui and //Gana, who also speak "Central Khoisan" languages, clustered with other Khoe-San groups but also formed a distinct group [Fig. 2 and fig. S5 (PC5)]. They had the third greatest level of private haplotypes among all sub-Saharan pop-

ulations (figs. S42 and S43), despite the fact that the dense sampling of Khoe-San groups decreases private haplotypes in these groups. These observations show that the /Gui and //Gana represent a distinct San group. Furthermore, the only San individual (KB1) whose complete genome has been sequenced (9) was most closely related to the /Gui and //Gana (Figs. 1C and 2, B and C), despite the fact that this individual speaks a Southern San language (Tuu).

The Nama also speak a "Central Khoisan" language and are a Khoe group that traditionally had a pastoralist lifestyle, in contrast to the hunter-gatherer lifestyle of the San groups. The Nama showed great genetic similarity to the Southern San groups, such as the #Khomani and Karretjie (Figs. 1 and 2), and shared a small, but distinct, genetic ancestry component with East African groups, specifically the Maasai (Fig. 2B), and direct tests showed gene flow from the Maasai to the Nama (14). This "East African" component was also present at lower levels in the two #Khomani groups but was basically absent ( $<1\%$ ) from the !Xun, the Ju/'hoansi, and the /Gui and //Gana. The Nama also had a high frequency of a haplotype putatively associated with lactase persistence in the Maasai (14), which was rare in southern African Bantu-speakers, suggesting that



**Fig. 1. (A)** Sampling locations. **(B)** PCA of African individuals showing PC1 and PC2 rotated to fit geography. **(C)** PCA for Khoe-San populations ( $\sim 2.3$  million SNPs). **(D)** Pairwise  $F_{ST}$  for sub-Saharan populations (excluding the Hadza; see fig. S24 for comparison). **(E)** Prediction of the genetic

components from geographic, linguistic, and subsistence covariates. The predictive error relative to geography is given for each combination of covariates (values  $<1$  show improved predictive capacity as compared to that of geography).

lactase persistence in the Nama [50% in adults as compared to <10% in San groups (18)] has an East African origin (table S24). These observations support an East African connection for the Nama (14) and suggest that they originate from a Southern San group that adopted pastoralism with some introgression from an East African group that potentially brought pastoralist practices.

Greater levels of genetic diversity and lower levels of linkage disequilibrium (LD) have pinpointed the origin of modern humans to sub-Saharan Africa (3, 19), and these patterns of African genetic variation have also been used to suggest a southern African origin (5, 10), although the fossil record suggests an East African origin (2). We characterized and contrasted four patterns of African genetic variation (Fig. 3) (14): haplotype heterozygosity, haplotype richness, genomic runs of homozygosity (RoHs), and LD measured by the squared correlation of allele frequencies ( $r^2$ ). Consistent with previous observations (3, 19), sub-Saharan populations have greater genetic diversity, lower levels of LD, and shorter RoHs than non-African populations [except for the Hadza, a population that is known to have decreased drastically in size (10) (figs. S40, S46, and S48)]. However, within sub-Saharan Africa, these summary statistics pointed to different regions or groups within regions (Fig. 3). Although the descendants of the Bantu expansion in eastern and southern Africa sometimes had greater levels of genetic diversity than populations closer to their West African origin, illustrating the effect of recent admixture, inclusion or exclusion of these groups did not affect the overall pattern. Thus, these patterns of genetic variation do not localize the origin of modern humans to a single geographic region in Africa; instead they suggest a complex (potentially both recent and ancient) population history within Africa.

We searched for signs of selective sweeps across the genomes of San, Khoe, and Bantu-speaking populations in the set of ~2.3 million SNPs using the integrated haplotype statistic iHS (14, 20). Several of the strongest and previously unknown signals of selection coincide with regions of the genome that have been associated with distinct phenotypes. A particularly interesting region was found on chromosome 10 in the Ju/hoansi (Fig. 4A and fig. S73) and overlapped the *MYPN* (myopalladin) gene, which is associated with muscle growth and function (21). Although the signal for a selective sweep was strongest in the Ju/hoansi, it was also found in other groups, including non-African populations, suggesting that the sweep was either old or reoccurring. A particular variant found in another muscle gene (*ACTN3*) associated with “fast-twitching” muscles and elite athletic performance (22) has greater frequencies (>90%) in all the investigated Khoe-San groups than in other African populations (fig. S81).

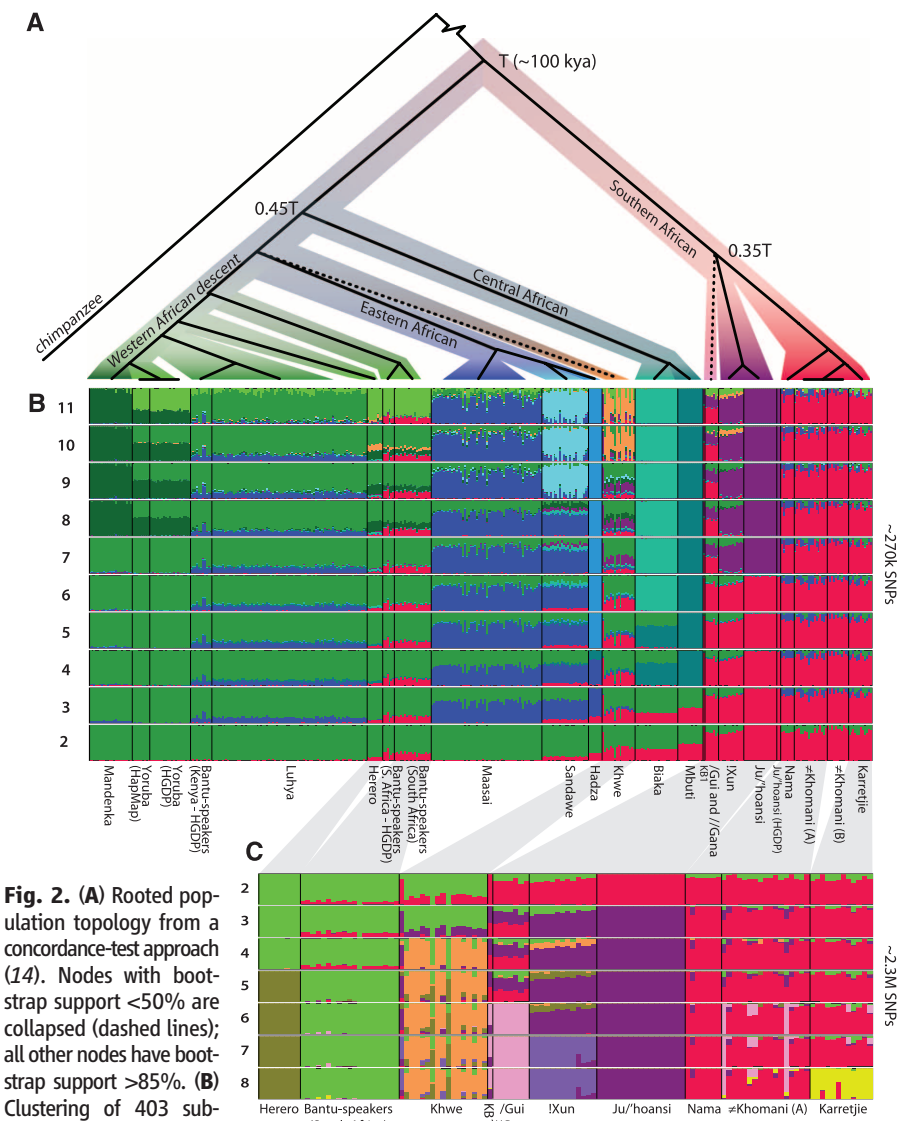
The most prominent peak across the genome and among all populations was found on chromosome 6 near the major histocompatibility complex in the #Khomani and the Karretjie (Fig. 4B

and fig. S76) (14). Several genes that are suggested to protect against infectious diseases surround the peak, including *PRSS16* and *POM121L2* (Fig. 4B). The fact that the strong signal was unique to the Southern Khoe-San could be related to their early and extensive contact with European colonists and novel (to the Khoe-San) infectious diseases such as smallpox leading to drastic population reduction (18).

To search for genome regions with unusually differentiated SNP variants in pairs of populations, we contrasted genome-wide estimates of  $F_{ST}$  with the single greatest  $F_{ST}$  value observed among the ~2.3 million SNPs (14). Although genome-wide  $F_{ST}$  between the pastoralist Nama and other Khoe-San groups was moderate (0.012 to 0.034), the top  $F_{ST}$  values in such comparisons (Fig. 4C) were all >0.88 and located in the same region on chromosome 16. The region overlaps an active binding site of transcription enhancers that prob-

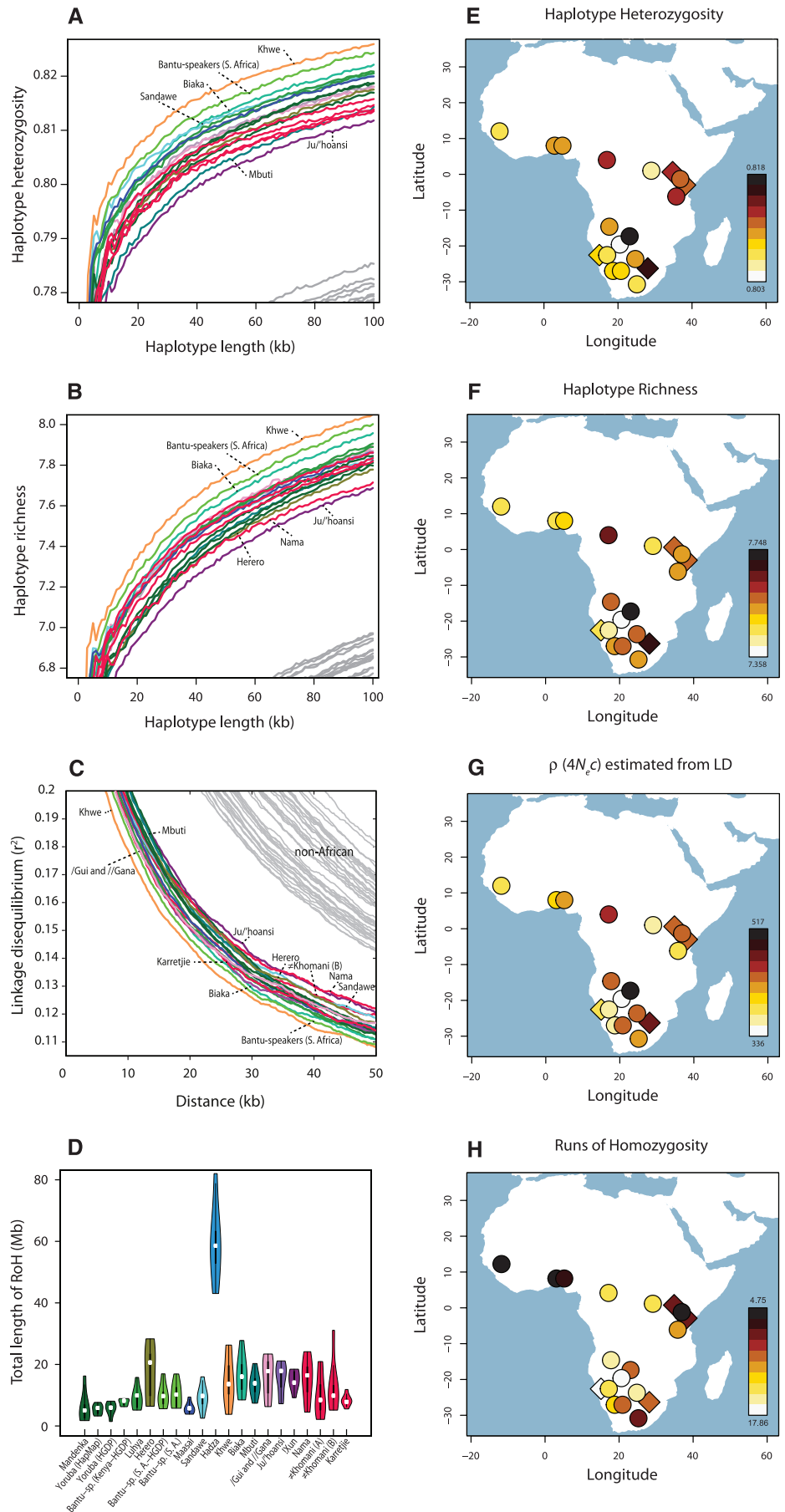
ably regulate the *ERCC4* gene (some 200 kb further downstream), which is linked to pigmentation and sensitivity to ultraviolet light (xeroderma pigmentosum). Individuals with mutations in the *ERCC4* gene display pigmented freckles, mild skin lesions, and an elevated risk of skin cancer (23). When a supervised genome-local clustering strategy was used (24), this region showed an extraordinary fraction of ancestry from Bantu-speakers (South Africa) in the Nama (Fig. 4D and figs. S68 to S71), which is probably the result of introgression and, potentially, ensuing selection.

Because of their early divergence, signals of selection shared between Khoe-San and other populations offer a window into the evolutionary processes that occurred >100,000 years ago—the critical period for the origin of anatomically modern humans (1, 2). We devised a novel approach to search for unusual stretches of high-frequency derived variants shared among extant

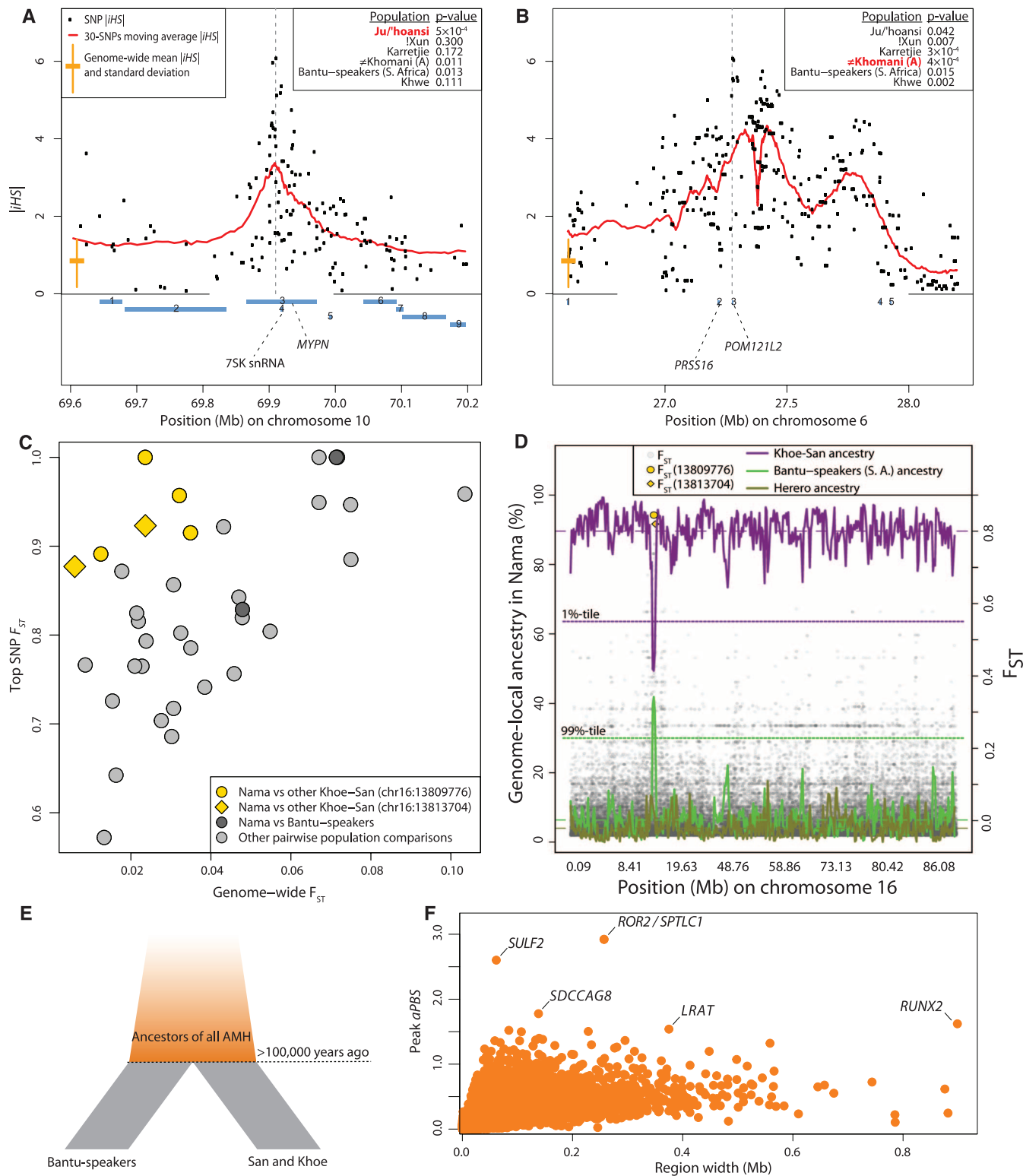


**Fig. 2. (A)** Rooted population topology from a concordance-test approach (14). Nodes with bootstrap support <50% are collapsed (dashed lines); all other nodes have bootstrap support >85%. **(B)** Clustering of 403 sub-Saharan African individuals (~270,000 SNPs), assuming 2 to 11 clusters. **(C)** Clustering of 118 southern African individuals (~2.3 million SNPs), assuming 2 to 8 clusters. Compare with fig. S16, which includes recently admixed individuals.

**Fig. 3.** (A) Expected heterozygosity of 5 SNP haplotypes as a function of haplotype length. (B) Haplotype richness for 5 SNP haplotypes as a function of haplotype length. (C) LD, represented as  $r^2$ , as a function of distance. (D) Cumulative RoHs for each population (0.5- to 1-Mb runs and averaged across individuals). (E to H) Heat maps of the summary statistics indicated in (A) to (D). (E) and (F) show the results at 50-kb windows; (G) shows  $\rho = 4 N_e \times c$ , where  $\rho$  is estimated from fitting  $r^2$ -decay curves to simulated data from a constant-size model (14) and  $c$  is the unscaled recombination rate; and (H) shows the population cRoHs (0.5- to 1-Mb class) averaged across 50 replicates of subsampling. For (A) to (C), the colors of the African populations are as in Fig. 1, and gray lines represent various non-African groups. For (A) to (H), all populations were randomly downsampled to seven individuals (without replacement), and SNPs with minor allele frequency < 10% were excluded.







**Fig. 4. (A)** *iHS* values for each SNP on chromosome 10 in Ju/'hoansi, surrounding the muscle gene *MYPN*, and **(B)** on chromosome 6 in ≠Khomani, surrounding the immune system genes *PRSS16* and *POM121L2*. The empirical *P* values (14) for 200-kb regions centered on the peak are given for each population. Locations of genes are shown by blue rectangles. **(C)** The greatest  $F_{ST}$  values for particular SNPs and pairwise population comparisons versus genome-wide  $F_{ST}$  estimates for the same population comparison. The top pairwise comparisons involving the Nama and another Khoe-San population (yellow) are found in the same region, separated by less than 4000 bp. **(D)** Proportion of genome-local ancestry (14, 24)

for chromosome 16 in the Nama assigned to Khoe-San, Herero, or Bantu-speakers (South Africa). The population-specific chromosome-wide means are shown as dashed horizontal lines. The 99 percentile for Bantu-speakers (South Africa) ancestry, and the 1 percentile for the Khoe-San ancestry are shown as dotted horizontal lines. The two top SNP  $F_{ST}$  values are highlighted in yellow in **(C)** and **(D)**. **(E)** Illustration of the aPBS approach for detecting selective sweeps in early modern humans. AMH, anatomically modern humans. **(F)** Stretches of consecutive positive aPBS values, with the top aPBS value plotted against the size of the stretch.

populations: the ancestral population branch statistic (aPBS) (Fig. 4E) (14). The top candidate for selection in early modern humans was located in a region immediately upstream of the *ROR2* gene (Fig. 4F and fig. S84), which is involved in regulating bone and cartilage development, and the *SPTLC1* gene, which is involved in hereditary sensory neuropathy (14). Mutations in *ROR2* cause recessive brachydactyly (shortening of digits) and Robinow syndrome (skeletal abnormalities). The second greatest aPBS value (fig. S81) was observed immediately upstream of *SULF2*, which regulates cartilage development, and phenotypes associated with mutations in *SULF2* include skeletal malformations and distorted brain development (14, 25). The largest of all regions (~900 kb), containing the fourth-highest aPBS value (Fig. 4F), comprises the *RUNX2* gene (fig. S87), which is implicated in craniocladial dysplasia. Thus, three of the top five regions contain genes involved in skeletal development, and syndromes associated with mutations in these genes display similar morphological features. *RUNX2* variation has been associated with phenotypic differences between anatomically modern and archaic humans, such as frontal bossing, clival morphology, a bell-shaped rib cage (26), and regulating the closure of the fontanel, which is crucial for brain expansion (27). The region spanning *RUNX2* was also identified in a scan for selected regions with the draft Neandertal genome (7). Because gracile modern human morphology appeared abruptly as compared to previous rates of morphological change in the human lineage (2), it is possible that selection on a few morphology genes, perhaps including these candidates, was involved in the emergence of anatomical modernity.

The remaining two of the top five regions for putative selection in early modern humans comprise *SDCCAG8* (fig. S86), involved in microcephaly (28), and *LRAT* (fig. S88), associated with Alzheimer's disease (29). Including *SULF2*, three of the top five candidate regions are thus associated with neuronal function.

Our study demonstrates substantial stratification among sub-Saharan populations, including among Khoe-San, and both population structure and the geographic distribution of genetic variation suggest a complex human population history within Africa. It remains unclear whether modern humans originated from a single randomly mating population or emerged from a geographically structured population (2, 30), potentially exchanging genetic material with archaic humans (6). The finding of several genes involved in skeletal development as candidates for selection in the ancestral human population of Khoe-San and Bantu-speakers, and the fact that no currently studied population diverged from the ancestral human population before the ancestors of the Khoe-San, suggest that anatomical modernity appeared before this first modern human diversification event. However, the complex patterns of genetic diversity, admixture, and selection; deep

population structure; historically large effective population size; and ancient divergence of Khoe-San populations described in this study highlight the complexity of human evolutionary history in Africa and suggest that genomic studies in Africa hold some of the keys to the main questions surrounding modern human origins.

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#### Supplementary Materials

[www.sciencemag.org/cgi/content/full/science.1227721/DC1](http://www.sciencemag.org/cgi/content/full/science.1227721/DC1)  
Materials and Methods  
Supplementary Text  
Figs. S5 to S89  
Tables S1 to S25  
References (31–171)

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## Cold-Inducible RNA-Binding Protein Modulates Circadian Gene Expression Posttranscriptionally

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In mammalian tissues, circadian gene expression can be driven by local oscillators or systemic signals controlled by the master pacemaker in the suprachiasmatic nucleus. We show that simulated body temperature cycles, but not peripheral oscillators, controlled the rhythmic expression of cold-inducible RNA-binding protein (CIRP) in cultured fibroblasts. In turn, loss-of-function experiments indicated that CIRP was required for high-amplitude circadian gene expression. The transcriptome-wide identification of CIRP-bound RNAs by a biotin-streptavidin-based cross-linking and immunoprecipitation (CLIP) procedure revealed several transcripts encoding circadian oscillator proteins, including CLOCK. Moreover, CLOCK accumulation was strongly reduced in CIRP-depleted fibroblasts. Because ectopic expression of CLOCK improved circadian gene expression in these cells, we surmise that CIRP confers robustness to circadian oscillators through regulation of CLOCK expression.

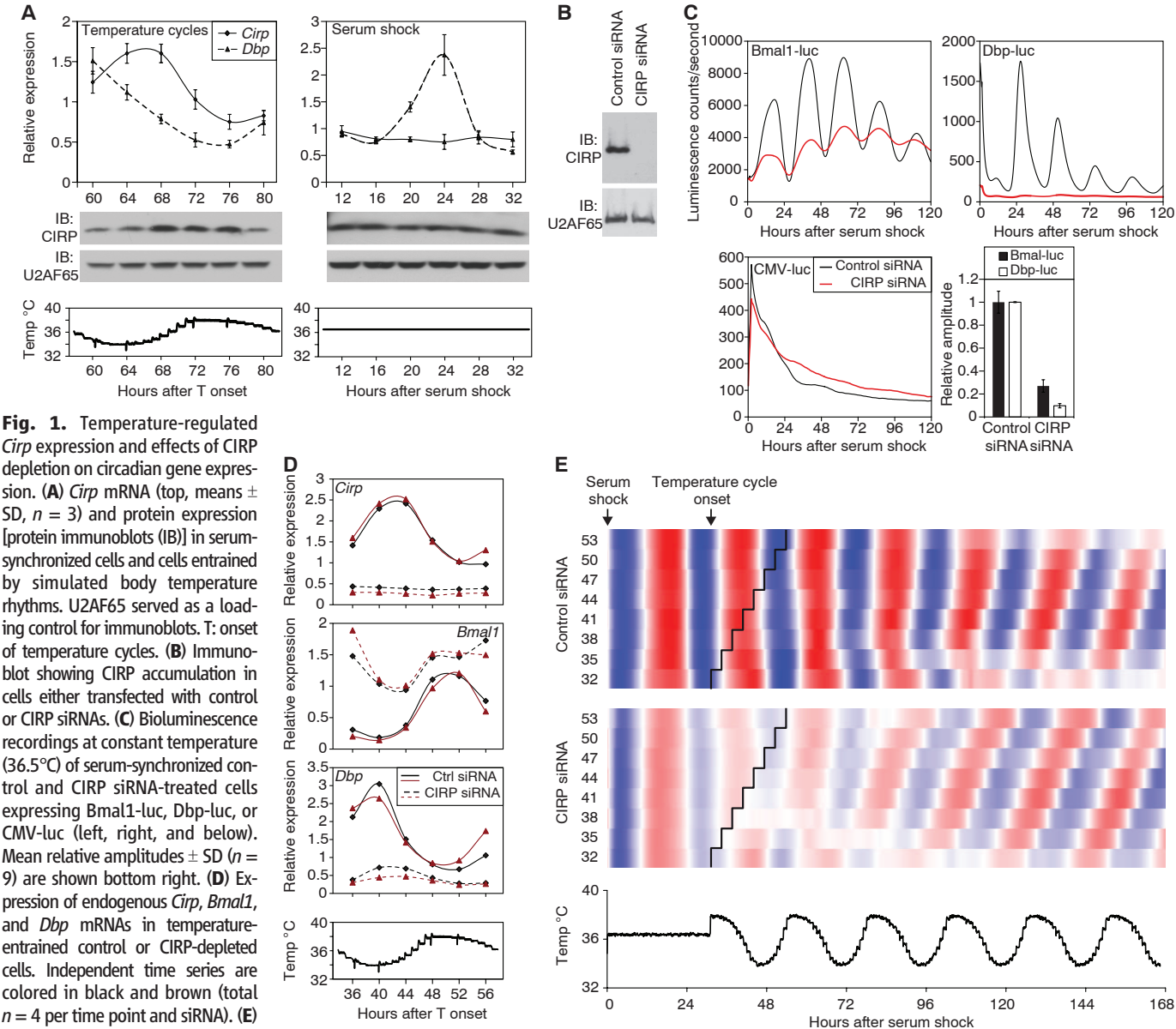
The mammalian circadian timing system is composed of a central pacemaker located in the brain's suprachiasmatic nu-

clei (SCN) that synchronizes subsidiary clocks present in most peripheral tissues (1, 2). The various phase-resetting signals controlled by

the SCN include body temperature rhythms (3–6).  
In mouse liver, the expression of the cold-inducible RNA-binding protein (CIRP) (7) fol-

lows a diurnal rhythm that is controlled by systemic cues rather than local oscillators (fig. S1) (8). Because a moderate reduction in temperature increases *Cirp* expression in cultured cells (7), we assessed the impact of simulated body temperature cycles on the temporal expression of *Cirp* in NIH3T3 fibroblast cells. *Cirp* mRNA and protein expression cycled over the course of 24 hours in cells synchronized by physiological temperature rhythms (38° to 34°C) (9), but not in cells synchronized by a serum shock and kept at 36.5°C (Fig. 1A). In contrast, the expression of *Dbp*, which is controlled by core clock transcription factors (10, 11), oscillated under both conditions (Fig. 1A). Hence, *Dbp* expression appeared to be regulated by the core oscillator, whereas CIRP oscillations required ongoing temperature cycles.

To examine whether CIRP affected core clock function, we depleted cells of CIRP using different RNA interference approaches (Fig. 1B and fig. S2) and monitored the expression of various luciferase reporters (Fig. 1C) and circadian genes (Fig. 1D). After serum synchronization of CIRP-depleted cells that had been transfected with small interfering RNAs (siRNAs), oscillations of Bmal1-luciferase (Bmal1-luc) exhibited an amplitude one-fourth that observed in cells transfected with control siRNAs, despite the constant expression of CIRP in control cells over time (Fig. 1C). Likewise, CIRP depletion strongly decreased the magnitude and amplitude of Dbp-luciferase (Dbp-luc) expression, whereas it had no effect on control cytomegalovirus-luciferase (CMV-luc) expression (Fig. 1C). In



represent high and low luminescence signals, respectively. Black lines indicate onsets of temperature cycles for each culture. An example of a simulated body temperature cycle (starting after 32 hours) is shown at the bottom.



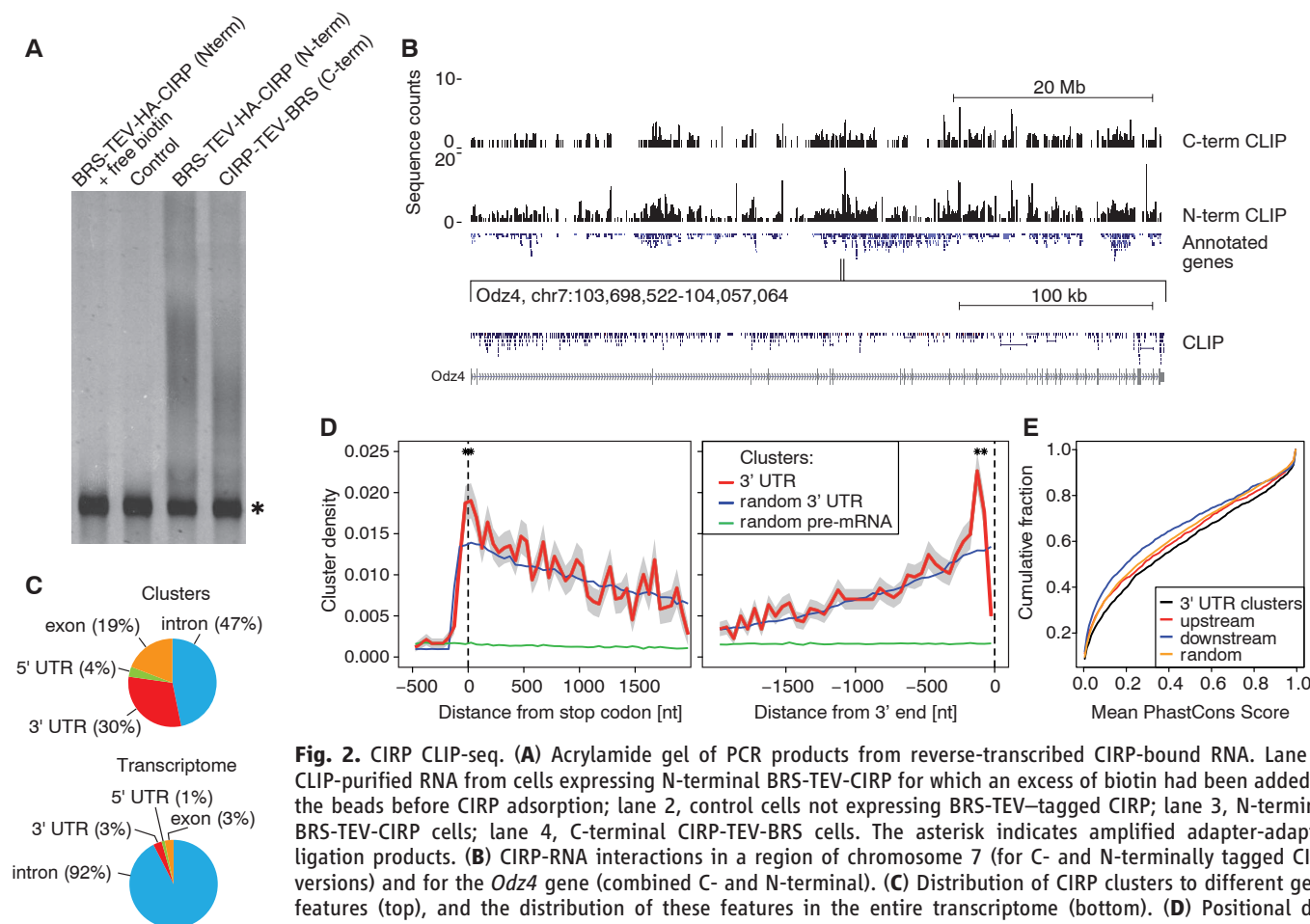
cells subjected to temperature cycles, amounts of endogenous *Dbp* and *Bmal1* mRNA oscillated as well with dampened amplitudes after CIRP depletion (Fig. 1D). Loss of CIRP furthermore affected the phase-entrainment kinetics of oscillators to body temperature rhythms (Fig. 1E and fig. S2). Thus, serum-synchronized cells lacking CIRP adapted considerably faster to temperature cycles with phases in conflict to the serum shock than did control cells. In accordance with these results, mathematical modeling of low- and high-amplitude oscillators predicted fast and slow kinetics, respectively, in phase entrainment (fig. S3) (12–14).

In order to identify CIRP-bound transcripts in a transcriptome-wide fashion, we incorporated the capture of biotinylated proteins on streptavidin beads into sequencing after RNA isolation by cross-linking and immunoprecipitation (CLIP-seq) (or high-throughput sequencing of RNA isolated by cross-linking and immunoprecipitation, HITS-CLIP) procedure (15, 16). A CIRP protein modified to contain a biotin-ligase recognition site (BRS) followed by a Tobacco

Etch Virus endopeptidase cleavage site (TEV) was stably coexpressed in NIH3T3 cells with the bacterial BirA biotin ligase (17), which biotinylated BRS-CIRP in vivo. Ultraviolet light-cross-linked CIRP-interacting RNAs were purified and processed for sequencing on streptavidin beads (Fig. 2A and figs. S4 and S5) (9). Two independent CLIP-seq experiments with cell lines stably expressing either N- or C-terminally BRS-tagged CIRP yielded highly similar sequencing data sets (Fig. 2B and fig. S6) that we combined for further analyses. To identify CIRP interaction sites in transcripts, we extended an algorithm (16) detecting locally enriched CLIP sequences (clusters) by incorporating transcript position-specific minimal thresholds for CLIP signals. For this purpose, we used expression densities determined by RNA sequencing (RNA-seq) from control siRNA-treated cells. The distribution of a total of 4420 clusters in 2079 annotated protein-coding genes revealed 10-fold and 6-fold enrichments of clusters in 3' untranslated regions (3' UTRs) and exons, respectively, when compared with the representation of these features in

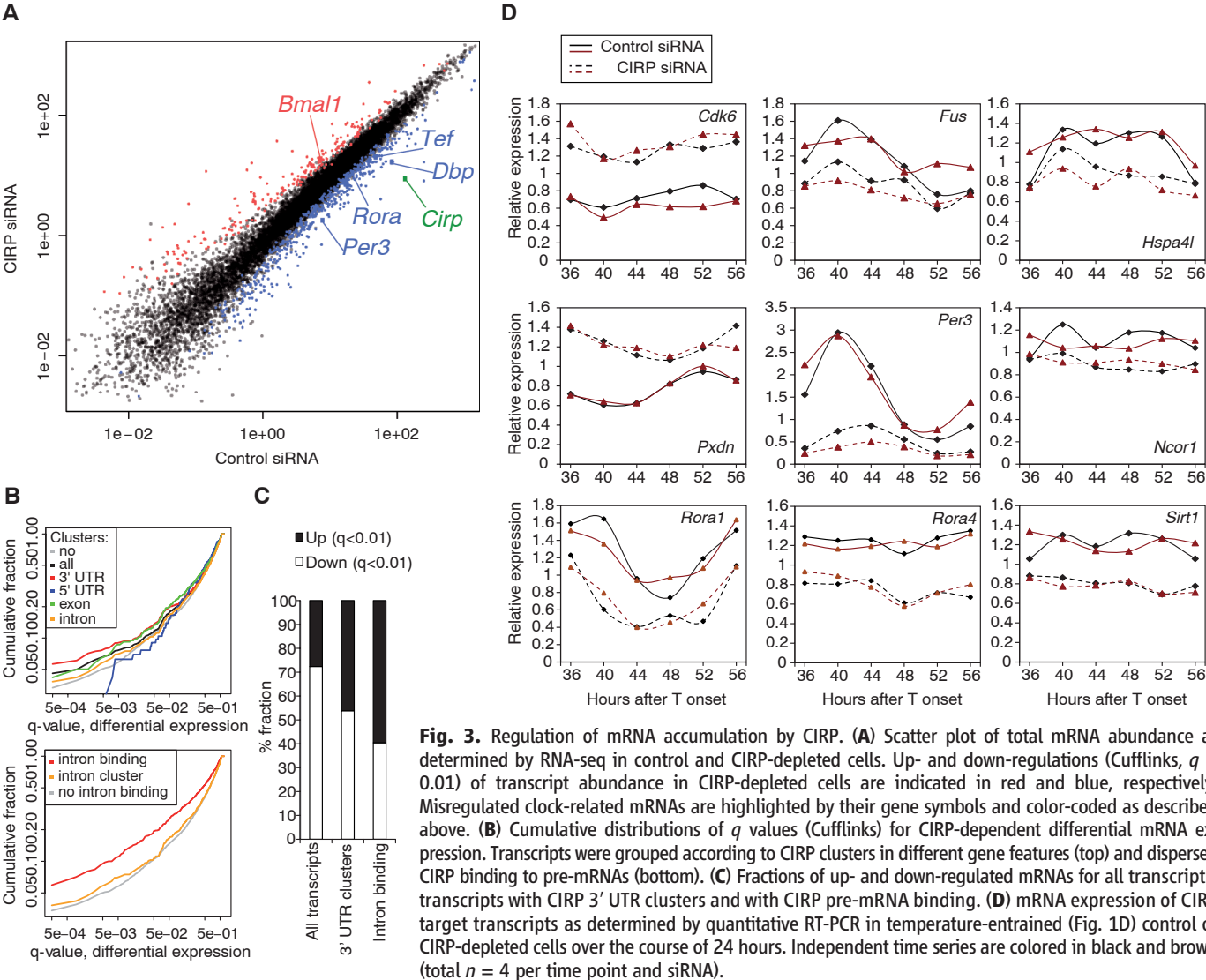
the transcriptome (Fig. 2C). Clusters in 3' UTRs were particularly enriched close to stop codons and ~150 nucleotides upstream of poly(A) sites (Fig. 2D). Phylogenetic analysis revealed that CIRP binding sites in 3' UTRs were more conserved than simulated control clusters (Fig. 2E) (Wilcoxon rank-sum test, 3' UTR clusters versus upstream, downstream, and random clusters,  $P < 0.01$ ,  $P < 10^{-8}$ , and  $P < 10^{-5}$ , respectively). However, we did not identify short sequence motifs enriched in CIRP clusters when compared with randomly selected regions in all annotated 3' UTRs (fig. S7). Hence, either structural motifs or other positional determinants must have conferred the binding specificity.

Comparison of the accumulation of transcripts with and without CIRP clusters in control and CIRP-depleted cells by RNA-seq (Fig. 3, A and B; fig. S8; and tables S1 and S2) showed that the former were more frequently misregulated [Cufflinks (18),  $q < 0.01$ ] than the latter (chi-squared test,  $P < 0.01$ ). This effect was more pronounced for the subset of transcripts with CIRP clusters in their 3' UTR (Fig. 3B, top), ( $P < 5 \times 10^{-4}$ ).



**Fig. 2.** CIRP CLIP-seq. (A) Acrylamide gel of PCR products from reverse-transcribed CIRP-bound RNA. Lane 1, CLIP-purified RNA from cells expressing N-terminal BRS-TEV-CIRP for which an excess of biotin had been added to the beads before CIRP adsorption; lane 2, control cells not expressing BRS-TEV-tagged CIRP; lane 3, N-terminal BRS-TEV-CIRP cells; lane 4, C-terminal CIRP-TEV-BRS cells. The asterisk indicates amplified adapter-adaptor ligation products. (B) CIRP-RNA interactions in a region of chromosome 7 (for C- and N-terminally tagged CIRP versions) and for the *Odz4* gene (combined C- and N-terminal). (C) Distribution of CIRP clusters to different gene features (top), and the distribution of these features in the entire transcriptome (bottom). (D) Positional distributions of CIRP clusters and of random control clusters in pre-mRNAs and 3' UTRs, respectively, relative to stop

codons (left) and 3' ends (right) of transcripts. The standard deviation (gray) was estimated by assuming a binomial distribution. Asterisks indicate significant differences between distributions of CIRP clusters and random 3' UTR clusters (binomial test,  $P < 0.01$ ). (E) Cumulative distributions show the fractions of 3' UTR clusters and of control up- and downstream and randomly located clusters with mean PhastCons conservation scores less than or equal to a given score.



**Fig. 3.** Regulation of mRNA accumulation by CIRP. **(A)** Scatter plot of total mRNA abundance as determined by RNA-seq in control and CIRP-depleted cells. Up- and down-regulations (Cufflinks,  $q < 0.01$ ) of transcript abundance in CIRP-depleted cells are indicated in red and blue, respectively. Misregulated clock-related mRNAs are highlighted by their gene symbols and color-coded as described above. **(B)** Cumulative distributions of  $q$  values (Cufflinks) for CIRP-dependent differential mRNA expression. Transcripts were grouped according to CIRP clusters in different gene features (top) and dispersed CIRP binding to pre-mRNAs (bottom). **(C)** Fractions of up- and down-regulated mRNAs for all transcripts, transcripts with CIRP 3' UTR clusters and with CIRP pre-mRNA binding. **(D)** mRNA expression of CIRP target transcripts as determined by quantitative RT-PCR in temperature-entrained (Fig. 1D) control or CIRP-depleted cells over the course of 24 hours. Independent time series are colored in black and brown (total  $n = 4$  per time point and siRNA).

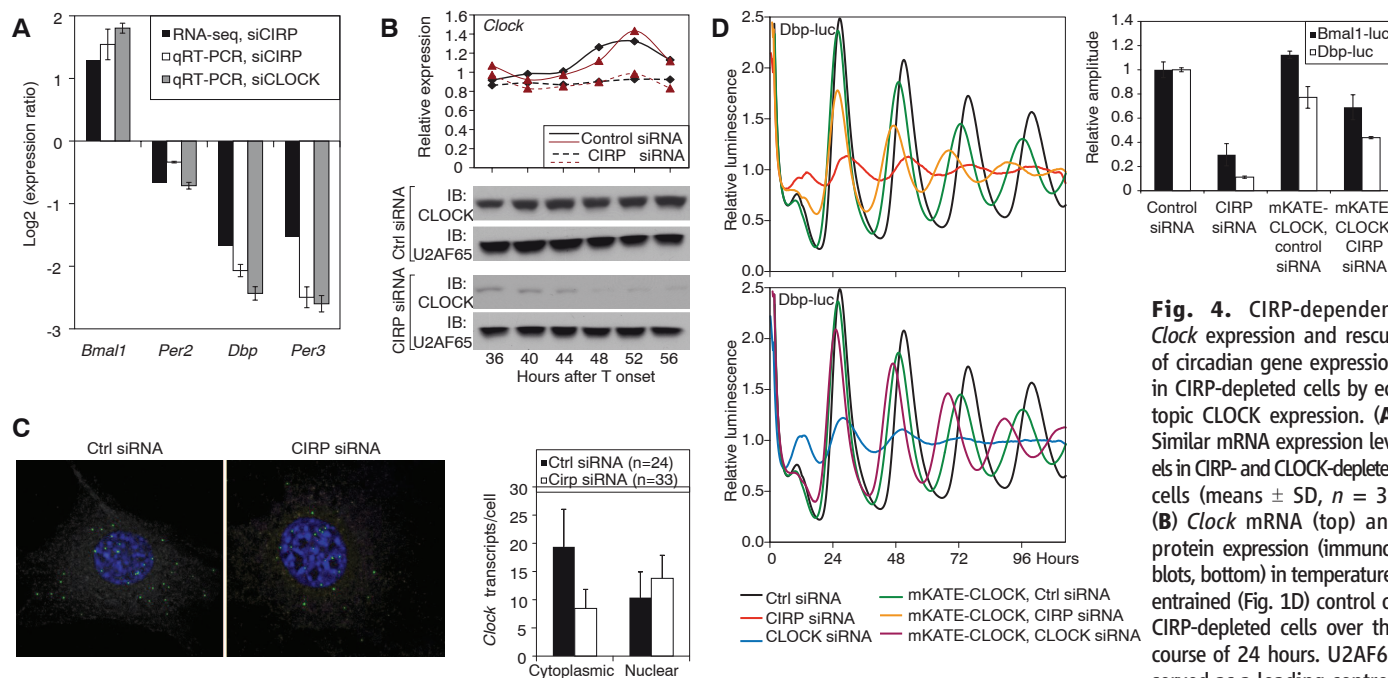
RNAs with CIRP cluster binding sites were found both up- and down-regulated in CIRP-depleted cells (Fig. 3C).

CLIP sequences were not only present as clusters, but in some cases dispersed over the entire length of pre-mRNAs. We used CLIP sequences in introns, normalized for intron abundance (9), as a proxy for CIRP-precursor interactions. Amounts of mRNAs of transcripts with dispersed CIRP binding in introns were more likely to be misregulated in CIRP-deficient cells than those of pre-mRNAs devoid of CIRP binding (Fig. 3B, bottom) (chi-squared test,  $P < 10^{-8}$ ). Moreover, ~32% of pre-mRNAs with dispersed CIRP binding also contained CIRP clusters (table S1). Conceivably, the site-specific clusters and dispersed arrays of CIRP cooperated in the regulation of mRNA abundance. Previous reports on CIRP function have suggested a role of CIRP in the control of cellular proliferation (19–21). In keeping with these studies, pathway analyses of CIRP-interacting transcripts and transcripts expressed differentially in control and CIRP-

depleted cells revealed an enrichment for cell cycle-related RNAs (table S3). Here, the consequence of CIRP depletion on circadian gene expression has been analyzed primarily in confluent cells, but we observed qualitatively similar results in proliferating cells (fig. S10).

We found two CIRP-bound and regulated transcripts, *Fus* and *Hspa4l* (Fig. 3D), for which diurnal accumulation—like that of *Cirp* mRNA itself—also persists in mouse liver devoid of functional hepatocyte clocks (22). Hence, CIRP may contribute to the systemic control of *Fus* and *Hspa4l* expression. In keeping with the role of CIRP in circadian gene expression (Fig. 1), we also identified CIRP-interacting transcripts associated with circadian clock function. These included the RNAs encoding Sirtuin 1 (SIRT1), PERIOD 3 (PER3), NCOR1, ROR $\alpha$  (23–29), and CLOCK (see below) whose amounts were decreased throughout the day in CIRP-depleted cells (Fig. 3D). Comparison of changes in mRNA abundance of clock genes in CIRP-depleted cells with those in cells in which various core clock

components were progressively depleted (30) revealed that the phenotypes caused by CIRP deficiency resembled most closely that observed in cells depleted of CLOCK (Fig. 4A). Thus, in both CIRP- and CLOCK-depleted cells, amounts of *Bmal1* mRNA were increased, whereas *Per3*, *Per2*, and *Dbp* mRNAs were decreased (Fig. 4A). Amounts of total *Clock* mRNA were only moderately affected in CIRP-depleted cells (Fig. 4B). But abundance of CLOCK protein was strongly attenuated at all time points throughout the circadian cycle (Fig. 4B). Single-molecule RNA-fluorescence in situ hybridization (RNA-FISH) (Fig. 4C) and subcellular fractionations (fig. S11) unveiled a decrease in cytoplasmic (and hence potentially translatable) *Clock* transcript numbers in CIRP-depleted cells (RNA-FISH, two-sample  $t$  test,  $P < 10^{-7}$ ). In contrast, nuclear RNA counts remained largely unchanged in CIRP-deficient cells (Fig. 4C), which suggested the regulation of *Clock* RNA export and/or compartment-specific RNA stability through CIRP. Expression of a fluorescently tagged



**Fig. 4.** CIRP-dependent *Clock* expression and rescue of circadian gene expression in CIRP-depleted cells by ectopic CLOCK expression. **(A)** Similar mRNA expression levels in CIRP- and CLOCK-depleted cells (means  $\pm$  SD,  $n = 3$ ). **(B)** *Clock* mRNA (top) and protein expression (immunoblots, bottom) in temperature-entrained (Fig. 1D) control or CIRP-depleted cells over the course of 24 hours. U2AF65 served as a loading control.

**(C)** Single-molecule *Clock* RNA-FISH in control (left) and CIRP siRNA (middle) treated cells. *Clock* transcripts are shown as green spots; nuclei are in blue. Mean *Clock* transcript number per cell  $\pm$  SD in the cytoplasm and nuclei are summarized (right). **(D)** Expression of an mKATE-CLOCK fusion protein using lentiviral transduction rescues circadian amplitudes in CIRP-depleted cells stably expressing *Dbp-luc* (top). Cells treated with siRNAs targeting only the *Clock* 3' UTR were used as control for mKATE-CLOCK rescue (bottom). Mean relative amplitudes  $\pm$  SD ( $n = 3$ ) of *Dbp-luc* and *Bmal1-luc* cells (fig. S12) are shown (right).

CLOCK protein (mKATE-CLOCK) in CIRP-depleted cells increased the magnitude and amplitude of *Dbp-luc* oscillations (Fig. 4D and fig. S12) and the amplitude of *Bmal1-luc* oscillations (fig. S12) to an extent similar to that in cells depleted of endogenous CLOCK. This indicated that CLOCK became a limiting factor in CIRP-depleted cells. Ectopic CLOCK expression also elicited a shortening of the period length in control cells and CIRP- or CLOCK-depleted cells (Fig. 4C), similar to that in transgenic mice harboring extra copies of *Clock* genes (31).

In conclusion, we identified transcripts bound to cold-inducible RNA-binding protein CIRP in a transcriptome-wide fashion in NIH3T3 cells and established a role for CIRP in circadian clock function in these cells. The dampened amplitude and accelerated phase-adaptation kinetics to temperature rhythms observed in CIRP-deficient cells are in keeping with the theoretical prediction that low-amplitude oscillators are more sensitive to Zeitgeber cues than high-amplitude oscillators in both cells (12–14) and animals, such as mice heterozygous for a dominant-negative *Clock* mutation and *Clock*-deficient mice (13, 32). Indeed, our findings support a scenario in which CIRP enhances the amplitude of circadian oscillators by increasing the cytoplasmic accumulation of mRNAs encoding CLOCK and, perhaps, other regulators relevant for the circadian timing system, such as ROR $\alpha$ , NCOR1, SIRT1, and PER3.

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## Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1217726/DC1  
Materials and Methods  
Figs. S1 to S12  
Tables S1 to S4  
References (33–49)

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# Real-Time Evolution of New Genes by Innovation, Amplification, and Divergence

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Gene duplications allow evolution of genes with new functions. Here, we describe the innovation-amplification-divergence (IAD) model in which the new function appears before duplication and functionally distinct new genes evolve under continuous selection. One example fitting this model is a preexisting parental gene in *Salmonella enterica* that has low levels of two distinct activities. This gene is amplified to a high copy number, and the amplified gene copies accumulate mutations that provide enzymatic specialization of different copies and faster growth. Selection maintains the initial amplification and beneficial mutant alleles but is relaxed for other less improved gene copies, allowing their loss. This rapid process, completed in fewer than 3000 generations, shows the efficacy of the IAD model and allows the study of gene evolution in real time.

The origin of new genetic functions poses a fundamental biological question. In many bacteria, most new genes are acquired by horizontal gene transfer (HGT) from related organisms (1), but new genes with novel functions can also evolve from extra copies of duplicated genes in both bacteria and eukaryotes (2). New genes can evolve from a redundant copy of a duplicated parental gene, by removing one copy from selection. This extra copy is then able to acquire one or more mutations providing a new beneficial function. At this point, natural selection would maintain the duplication and drive further evolution. However, this model requires that the extra gene be maintained without selection long enough for rare improvements to occur; because tandem duplications are generally very unstable and short-lived, they are unlikely to remain long enough to acquire mutations (3, 4). Furthermore, duplications typically have fitness costs (3, 4), and deleterious mutations outnumber beneficial mutations, making inactivation of the gene (pseudo-gene formation) the most likely fate for any redundant gene copies.

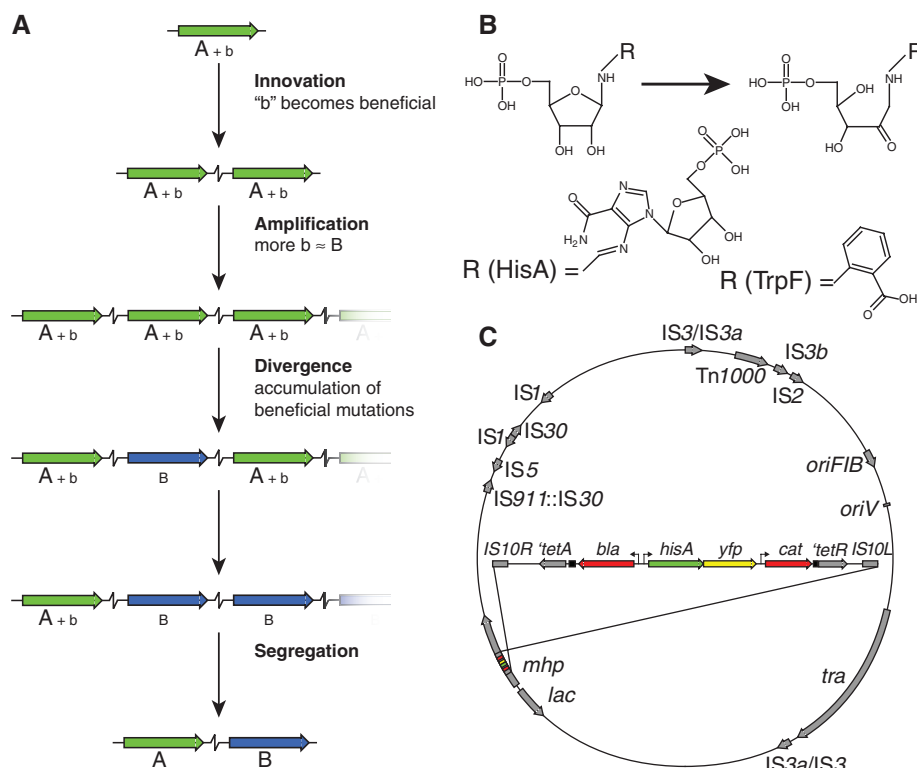
We propose the innovation-amplification-divergence (IAD) model (Fig. 1A), which allows the evolution of new genes to be completed under continuous selection that favors maintenance of the functional duplicate copies and divergence of the extra copy from the parental allele (5). The IAD model proposes that the ancestral gene has a weak secondary activity (innovation) (6, 7), and when a change in conditions makes this activity useful, selection favors increased gene dosage (amplification), resulting in two or more copies of the parent allele. The increased copy number provides multiple targets for beneficial mutations and buffers any negative effects a new mutation may have on the original activity. During continuous growth under conditions that select for both the

original activity and the new activity, beneficial mutations will accumulate (divergence) in the copies. Any improved copy can be further amplified, whereas less functional copies, including the parental gene, can be lost. Ultimately, this results in a gene duplication in which one gene copy en-

codes the parent activity and another copy provides an improved, new activity.

To experimentally test the IAD model, we examined a histidine biosynthetic enzyme (HisA), and through continuous selection we created, by duplication and divergence, a new gene that catalyzes a step in tryptophan synthesis. The original HisA and TrpF enzymes both catalyze isomerization of a phosphoribosyl compound, but each acts on different substrates in the biosynthesis of the amino acids histidine and tryptophan (Fig. 1B). HisA and TrpF enzyme activities are selectable by growth in minimal media lacking histidine and tryptophan. In addition, the enzymes are structurally related and evolved from a common ancestor (8). Furthermore, *Streptomyces* and *Mycobacteria* lack TrpF but instead have one enzyme, PriA, that is a HisA ortholog and catalyzes both reactions (9).

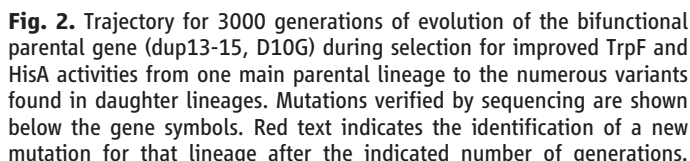
In a strain lacking *trpF*, we selected a spontaneous *hisA* mutant of *Salmonella enterica* that maintained its original function (HisA) but acquired a low level of TrpF activity sufficient to support slow growth on a medium lacking histidine and tryptophan, representing the innovation of the IAD model (see table S1 for



**Fig. 1.** (A) The IAD model. Innovation occurs when the ancestral gene (green) encodes a protein with the main function “A” and a minor activity “b.” Amplification occurs when an environmental change makes the b activity beneficial and selection favors variants with increased b activity. Divergence may occur in any one of the amplified gene copies that acquires a beneficial mutation that increases “B” activity (blue gene copy). After a B mutation, selection for the amplified array is relaxed, and segregation occurs to leave alleles with original A activity and the evolved B activity. (B) The isomerization reaction catalyzed by HisA and TrpF. The respective substrates and products differ in which chemical group (R) is attached to the 1'-amino group of the phosphoribosylamine. (C) Structure of the T-his element (linear insert) and its location on F'128 (circle) with the relative genetic elements on the F' as shown (transposons; IS elements; replication origins; and the *tra*, *lac*, and *mhp* operons).

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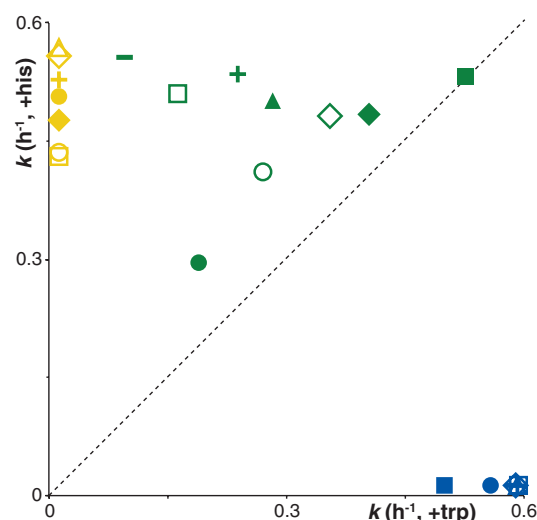
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Additional lineages are shown in fig. S1, A to C. Asterisks next to a mutation indicate the presence of more than one subpopulation, differing in which of the indicated mutations they contain. Two asterisks indicate that only a subpopulation of the cells in the culture contained the indicated gene copy. A, Ala; Q, Gln; L, Leu; S, Ser; N, Asn; V, Val; M, Met; E, Glu; I, Ile.

We placed this bifunctional parental gene (dup13-15, D10G) under the control of a constitutive promoter that cotranscribed a yellow fluorescent protein (*yfp*) gene. We also placed the T-*his* operon in a transposition-inactive transposable element Tn10d Tet close to the *lac* operon on the low-copy number (about two copies per chromosome) (11) F'128 plasmid (Fig. 1C). Duplications and amplifications of this region are frequent and have low fitness cost (3), allowing experimental study of the process within a reasonable time frame. An F' plasmid with the bifunctional gene inside T-*his* was introduced into a *S. enterica* strain with deleted *hisA* and *trpF* genes, dependent on the bifunctional gene for synthesis of both histidine and tryptophan. In the absence of both amino acids, the bifunctional gene supported a generation time

**Fig. 3.** Characteristics of 22 different evolved mutant gene variants. Each point represents the fitness of one specific mutant gene for its HisA activity on the x axis [assayed as growth rate ( $k$ ) in minimal glycerol medium with added tryptophan] and TrpF activity on the y axis (assayed as growth rate in minimal glycerol medium with added histidine). Mutant genes fall into three main classes as indicated by the colors: Blue, HisA specialists [open diamond, *hisA*(wt); open triangle, D10G G102A; dash, D10G G102A V106M; cross, D10G; open square, D10G G102A S143N; solid circle, D10G R83C; solid square, D10G G102A V106M V88I]. Yellow, TrpF specialists (open triangle, dup13-15 D10G G102A Q24L V106L; open diamond, dup13-15 D10G G102A V106M V88I; cross, dup13-15 D10G G102A V106M Q24L; solid circle, dup13-15 D10G G102A V106M; open circle, dup13-15 D10G G102A V106M V88I). Red, generalist enzymes [solid circle, dup13-15 D10G G102A V106M V45M; cross, dup13-15 D10G G102A V106M V45M G11D G44E; open square, dup13-15 D10G G102A V106M V45M G11D G44E; open diamond, dup13-15 D10G G102A V106M V45M G11D G44E; solid circle, dup13-15 D10G G102A V106M V45M G11D G44E].



**Fig. 3.** Characteristics of 22 different evolved mutant gene variants. Each point represents the fitness of one specific mutant gene for its HisA activity on the  $x$  axis [assayed as growth rate ( $k$ ) in minimal glycerol medium with added tryptophan] and TrpF activity on the  $y$  axis (assayed as growth rate in minimal glycerol medium with added histidine). Mutant genes fall into three main classes as indicated by the colors: Blue, HisA specialists [open diamond, *hisA*(wt); open triangle, D10G G102A; dash, D10G G102A V106M; cross, D10G; open square, D10G G102A S143N; solid circle, D10G R83C; solid square, D10G G102A V106M V88I]. Yellow, TrpF specialists [open triangle, dup13-15 D10G G102A Q24L V106I; open diamond, dup13-15 D10G G102A V106M V88I; cross, dup13-15 D10G G102A V106M Q24L; solid circle, dup13-15 D10G G102A Q24L V14:2M; solid diamond, dup13-15 D10G G102A V106M; open circle, dup13-15 D10G R83C; open square, dup13-15 D10G G81D]. Green, generalist enzymes [solid circle, dup13-15 D10G (ancestral bifunctional gene); dash, dup13-15 D10G G102A V106M V45M; cross, dup13-15 D10G G102A Q24L G44E; solid square, dup13-15 D10G G102A G11D G44E; open square, dup13-15 D10G G102A Q24L; solid triangle, dup13-15 D10G G102A V88I; open diamond, dup13-15 D10G G102A S143N; solid diamond, dup13-15 D10G G102A G11D; open circle, dup13-15 D10G G102A].

of ~5.1 hours in minimal medium with doubling times of ~2.8 hours in the presence of tryptophan alone, ~2.6 hours in the presence of histidine alone, and ~1.5 hours in presence of both amino acids.

Several independent lineages of this strain evolved under continuous selection for improved growth and increased HisA and TrpF activities by serial passages in minimal glycerol medium lacking both amino acids (10). Within a few hundred generations, the growth rate increased from 5 hours per division to 1.9 to 2.5 hours, depending on the lineage. Associated with the increased growth rate, expression of the parental bifunctional gene (fig. S2 and table S2) increased stepwise (up to 20-fold) in most cultures due to amplifications of a region of the plasmid that includes the bifunctional parental gene and *yfp* (see fig. S3 and table S3 for structures of amplified units).

After evolution for up to 3000 generations, all lineages acquired mutations resulting in faster growth. In many of the lineages, different gene copies in the amplified array diverged by mutations that allowed enzymatic specialization (Fig. 2 and fig. S1, A to C). As predicted from the IAD model, we observed the appearance of a diverged gene copy with improved activity, relaxed selec-

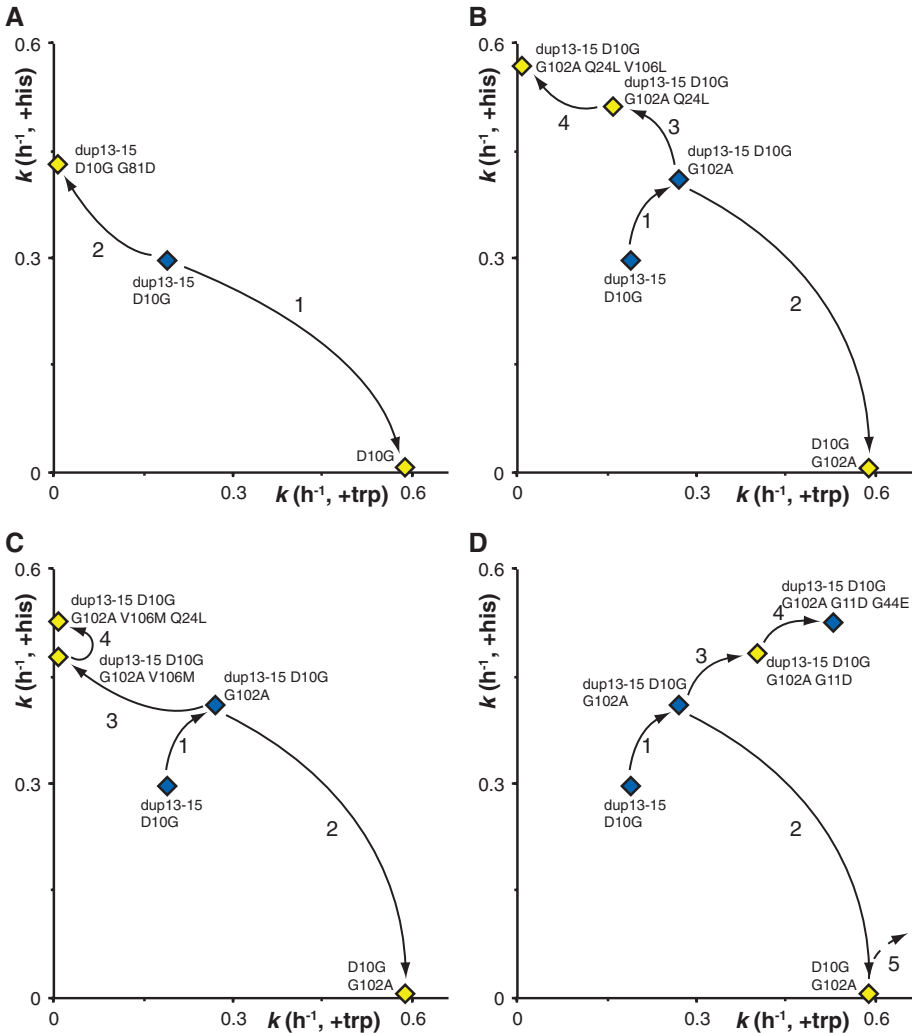
tion for maintenance of the unimproved copies in the amplified array, loss of the unimproved copies, and, in some cases, reduction in the total gene copy number. (fig. S2 and table S2).

To test the HisA and TrpF activities of the evolved enzymes, 22 different genes from the evolved strain were individually cloned into the chromosomal *cobA* gene of a strain (lacking both the *hisA* and *trpF* genes) that had never been subjected to a histidine-tryptophan selection (10). This assured that the strain had only one copy of the gene to be tested, and no outside mutations contributed to activity. Strains with these single-copy evolved genes were tested for their ability to grow on a minimal medium with single amino acids (Trp or His), both, or neither. In every case, the evolved mutated gene increased the growth rate in the absence of either histidine or tryptophan or when both amino acids were absent. The evolved genes fell into three classes: (i) specialized genes with strongly improved HisA activity and loss of TrpF activity, (ii) specialized genes with strongly improved TrpF activity and loss of HisA activity, and (iii) generalist genes whose encoded enzyme showed a moderate increase in both activities (Fig. 3; Fig. 4, A to D; and table S4). In several

cases, specialized mutant genes of both types (i) and (ii) were found in single bacterial clones, demonstrating that gene copies within a single amplified array had diverged to become specialized to perform either the HisA- or TrpF-specific reactions (Fig. 4, A to C). In other cases, the ancestral gene evolved into an individual gene with an improved level of both HisA and TrpF activities (Fig. 4D). In some strains an improved generalist enzyme evolved first and then duplicated with copies, subsequently diverging and becoming specialized (Fig. 4, B and C). Figure S4 shows the locations of the identified mutations on the HisA structure from *Thermotoga maritima*.

Thus, under suitable selective conditions, the IAD process rapidly generates genes with distinct enzymatic activities. In *Salmonella*, duplications of any particular gene form at a rate of roughly  $10^{-5}$  per cell per division and reach a high steady-state frequency in the population, providing a reservoir of standing copy number variation upon which selection can act (12). Amplification to higher copy numbers occurs at  $10^{-2}$  per cell per division (3), several orders of magnitude more frequent than point mutations. Thus, whenever a limiting gene product restricts cell growth, initial escape from

**Fig. 4.** Multiple evolutionary trajectories recovered through IAD. The *x* axis indicates the HisA activity (assayed as growth rate in minimal glycerol medium with added tryptophan); the *y* axis indicates the TrpF activity (assayed as growth rate in minimal glycerol medium with added histidine). (A) Evolution of specialist enzymes in which one activity is improved at the expense of the other. (B and C) Evolution of specialist enzymes after initial evolution of a generalist enzyme. (D) Evolution of a generalist enzyme with improvement of both activities. Arrows and numbers indicate the sequential order of appearance of the various mutations in the population. Yellow symbols denote gene variants that were always accompanied by another gene variant (generalist or with the complementary activity) in the same amplified array. Blue symbols denote gene variants that, at some point during the evolution, were the only variants found in the population.





this restriction may initially occur by duplication events and higher amplification, rather than rare point mutations that alter catalytic activity (13). The delayed appearance of point mutations suggests that the accumulation of a point mutation is the rate-limiting step in the IAD process.

Other sequence-based evidence supports the predictions of the IAD model, particularly in eukaryotes where new genes often evolve by amplification-divergence processes. For example, the evolution of a new gene may be accompanied by the appearance of paralogs and pseudogenes in the genome (14, 15), new genes may show evidence of continuous selection (16, 17), and new genes and pseudogenes may be tandemly clustered with the parent gene (18, 19). On the contrary, in bacteria duplicate genes most commonly arise via HGT (20), but the IAD process could still generate new genes that can be distributed to other organisms by HGT. Conversely, horizontally acquired genes have a higher likelihood of possessing a new side-activity upon which selection and the IAD process could act, suggesting a potential coupling between the IAD process and HGT (21).

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## Supplementary Materials

[www.sciencemag.org/cgi/content/full/338/6105/384/DC1](http://www.sciencemag.org/cgi/content/full/338/6105/384/DC1)  
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# Metagenome Mining Reveals Polytheonamides as Posttranslationally Modified Ribosomal Peptides

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It is held as a paradigm that ribosomally synthesized peptides and proteins contain only L-amino acids. We demonstrate a ribosomal origin of the marine sponge-derived polytheonamides, exceptionally potent, giant natural-product toxins. Isolation of the biosynthetic genes from the sponge metagenome revealed a bacterial gene architecture. Only six candidate enzymes were identified for 48 posttranslational modifications, including 18 epimerizations and 17 methylations of nonactivated carbon centers. Three enzymes were functionally validated, which showed that a radical S-adenosylmethionine enzyme is responsible for the unidirectional epimerization of multiple and different amino acids. Collectively, these complex alterations create toxins that function as unimolecular minimalistic ion channels with near-femtomolar activity. This study broadens the biosynthetic scope of ribosomal systems and creates new opportunities for peptide and protein bioengineering.

The marine sponge *Theonella swinhoei*, a composite organism containing numerous uncultivated bacterial symbionts, is a rich source of bioactive metabolites (1). Among

these, polytheonamides A and B (Fig. 1A) are particularly noteworthy for their structural complexity (2). Of the 19 different amino acids that constitute these unusual 48-residue peptides, 13 are nonproteinogenic. The compounds were therefore assumed to be products of a nonribosomal peptide synthetase (NRPS)—a large multifunctional protein complex that can generate peptides with unusual residues (3, 4). However, polytheonamides are larger than other known NRPS-synthesized secondary metabolites, and the size of an NRPS biosynthetic machinery required to assemble 48 residues prompted us to speculate whether, alternatively, a ribosomal pathway (5, 6) could be involved. This would require a ribosomal pathway that could introduce multi-

ple D-configured and C-methylated residues (6–8). To test the ribosomal hypothesis, a seminested polymerase chain reaction (PCR) protocol (fig. S1) was used with primers designed on the basis of a hypothetical precursor peptide consisting of proteinogenic L-configured amino acids (Fig. 1A). Sequencing revealed a succession of codons that precisely corresponded to an unprocessed polytheonamide precursor; this supports a ribosomal origin. To identify the surrounding DNA region, 920,000 clones of a library of *T. swinhoei* total DNA (9) were screened in a pool-dilution strategy (10), yielding a single cosmid pTSMAC1. The few other clones detected were repeatedly lost during isolation. To expand the upstream sequence, we amplified a 7-kilobase portion directly from the partially enriched pool by long-range PCR, using primers based on sequences of the cosmid vector and the pTSMAC1 insert. The authenticity of the amplified region was subsequently confirmed by repeated PCR and sequencing with metagenomic DNA.

The assembled DNA region contained 11 additional genes, clustered around the initially identified open reading frame (ORF) (Fig. 1B). Nine ORFs, which we termed *poy* genes (*poyA-I*), form an operon, as apparent from the short or often absent intergenic regions. This polycistronic architecture, as well as the presence of Shine-Dalgarno motifs and lack of detectable introns, suggests a bacterial endosymbiont as the origin of the cloned region. Beyond the gene cluster, the presence of an upstream prokaryotic *hicAB*-type toxin-antitoxin system, numerous genes and gene fragments resembling bacterial transposition elements, and two downstream genes encoding a polyketide synthase of as-yet-unknown function further support this hypothesis (table S1). The 3' terminus of *poyA* consists of 48 codons that match a

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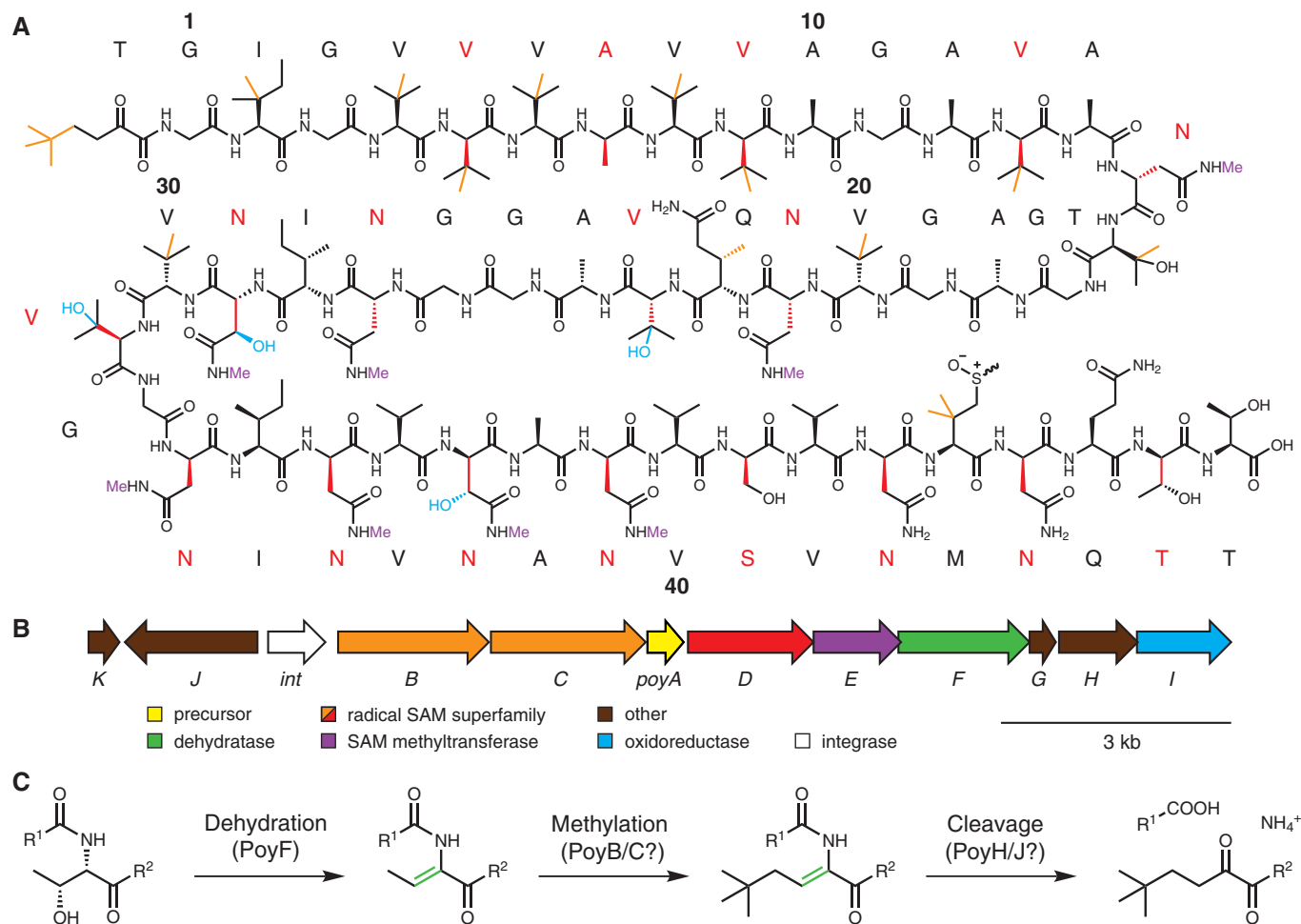
complete polytheonamide precursor. It is noteworthy that the encoded sequence suggests that the three 3-hydroxyvaline units originate from two different residues: residue 16 from threonine (Thr) by C-methylation and residues 23 and 31 from valine (Val) by hydroxylation (Fig. 1A). In addition to the propeptide-encoding core region, we identified an unusually long 5' leader sequence in *poyA* that exhibits homology to nitrile hydratases. This region does not resemble any component of characterized ribosomal pathways. However, Haft and co-workers (11) recently discovered similar leaders in several taxonomically diverse bacteria by in silico genome analysis and postulated the existence of a new natural-product family. Polytheonamides are likely the first characterized members of such a family for which we propose the name proteusins (from Proteus, a Greek shape-shifting sea god).

To generate polytheonamides from proteino-genic residues, four hydroxylations, 18 epimerizations, and at least 21 methylations are necessary (Fig. 1A). Considering the large number of post-

translational modifications, astonishingly few enzyme candidates were identified for these steps. These are PoyB, PoyC, and PoyD, homologous to members of the radical *S*-adenosylmethionine (rSAM) superfamily (12, 13); PoyE, homologous to SAM-dependent methyltransferases; PoyI, homologous to Fe(II)/ $\alpha$ -ketoglutarate oxidoreductases; and PoyF, homologous to the dehydratase domain (14) of LanM-type lantibiotic synthetases. Besides these six enzymes, the cluster encodes other proteins likely involved in regulation, transport, and proteolytic removal of the leader region (PoyJGH) on the basis of homologies. No homology was found for PoyK, and it is unclear whether it belongs to the pathway. The limited number of maturation factors suggests that individual enzymes convert positionally and structurally diverse residues. For example, C-methylation occurs on at least five different units, whereas at least four types of residues are epimerized. Conversely, identical residues are processed in different ways, such as Val and asparagine (Asn), which each appear as three structural variants.

Thus, the intriguing question arises of how this biosynthetic machinery reconciles substrate promiscuity with regioselectivity.

To obtain initial insights into *poy* gene functions and to test whether enzymes indeed act iteratively, individual ORFs were expressed in *Escherichia coli*. Numerous attempts to produce the precursor PoyA resulted in, at best, minute amounts of insoluble protein. However, after codon-optimization and coexpression trials using various gene combinations, we found that protein yields and solubility of PoyA dramatically improved in the presence of the rSAM protein PoyD (fig. S2). PoyD exhibits close similarity to only a small number of uncharacterized proteins, mostly from hypothetical proteusins gene clusters. Mass spectrometric (MS) analysis of purified PoyA did not reveal a mass shift or apparent modification of the protein sequence. Further analysis of PoyA involving acid hydrolysis, derivatization, and chromatographic separation of MS-verified amino acids revealed the presence of epimerized asparagines and valines within the PoyA core



**Fig. 1.** Polytheonamides: structures, genes, and biosynthetic model. **(A)** Polytheonamides A and B (shown) differ in the configuration of the sulfoxide moiety in residue 44. The sulfoxide arises from spontaneous oxidation during polytheonamide isolation. Residues are numbered on the basis of the typical notation for polytheonamides (2). The core peptide sequence is indicated by bold letters,

with the color red denoting posttranslational epimerization. All other biosynthetic transformations during maturation of the core peptide are colored as follows: orange, C-methylation; purple, N-methylation; blue, hydroxylation; and green, dehydration (C). **(B)** Map of the polytheonamide (*poy*) biosynthetic gene cluster. **(C)** Model for the formation of the N-acyl terminus from threonine.

sequence, confirming that PoyD is capable of epimerizing most, and perhaps all, of observed D-amino acids present in polytheonamides A and B (figs. S3 to S5). The unidirectional L- to D-amino acid epimerization observed with PoyD is in contrast to known nonradical amino acid racemases, which generate an equilibrium mixture of epimers (6). Next, we constructed five triple expression strains by adding either *poyB*, *C*, *E*, *F*, or *I* to *poyA* and *poyD*. Unlike all other triple expressions, the strain coexpressing *poyF* produced PoyA with a mass of 18 D less than expected (observed 17,090 D, calculated 17,108 D) (Fig. 2A and figs. S6 to S7), which suggested a loss of water and supported the dehydratase function of PoyF. The modified residue was subsequently identified as Thr<sup>97</sup> by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Fig. 2B and figs. S8 to S9).

The identification of the Thr residue as part of the PoyA core peptide sequence is corroborated by the presence of an N-terminally adjacent, highly conserved GG motif that was previously proposed as the cleavage site in homologous precursors (11). Comparison of the polytheonamide structure with the peptide core suggests that the Thr residue is converted to the unusual N-terminal acyl unit by a remarkable biosynthetic sequence involving PoyF-catalyzed dehydration, formal *t*-butylation, and spontaneous formation of the 2-oxo moiety (15) after hydrolytic cleavage of the enamide (Fig. 1C). We are not aware of precedent for the introduction of a *t*-butyl group at nonactivated carbon positions in biology or synthetic chemistry. This transformation may be accomplished by four successive methylations

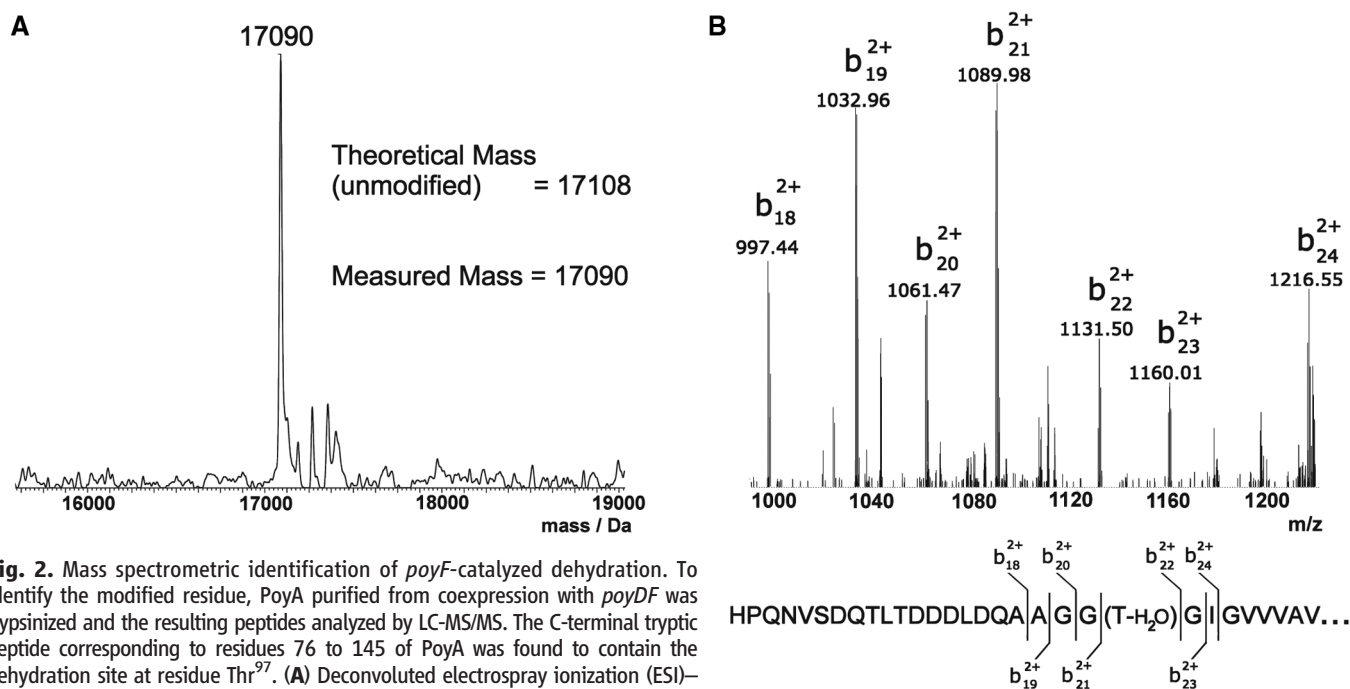
catalyzed by one or more of the rSAM candidates PoyB and PoyC, both of which contain a cobalamin-binding motif sometimes found within rSAM methyltransferases (12). Although radical methylation has been previously observed (16), this use for extensive modification of peptide structures is notable. In addition, close homologs of *poyB* and *poyC* occur in several other ribosomal peptide gene clusters of unknown function (17, 18), which suggests similar modifications in other natural products.

The activity of a third gene encoded in the polytheonamide gene cluster, *poyE*, was detected only after codon optimization and coexpression harboring two copies of the gene in either 3 or 7-day inductions at 16°C. In-depth MS analysis of the coexpressed PoyA revealed a suite of peptides increasing by 14 mass units (0 to 8 modifications) correlating perfectly with all expected positions for asparagine N-methylation, indicative of iterative N-methyltransferase activity (figs. S10 to S11). Unlike with NRPS-derived peptides, N-methylation of ribosomal natural products is rare—only N-terminal methylation of the cytotoxin cypemycin has been reported with genetic and biochemical verification (6, 19). N-methylation of a single Asn has also been observed in cyanobacterial phycobiliproteins; however, PoyE bears little sequence homology to these enzymes (20). These data highlight the iterative activities of tailoring enzymes to generate a complex natural-product architecture. Convincing gene candidates for the remaining two transformation types are present in the *poy* cluster, which suggests that C-methylation and hydroxylation are also iterative. Determining whether

the three remaining clustered biosynthetic genes are sufficient to complete the structural maturation of polytheonamides will require further study.

Mature polytheonamides form minimalistic unimolecular ion channels in cell membranes (21). This mode of action motivated us to investigate possible antibacterial effects in more detail. We found polytheonamide B to be active against Gram-positive bacteria with minimal inhibitory concentrations in the microgram per milliliter range of concentrations (table S3). The peptide rapidly depolarized the bacterial cytoplasmic membrane, simultaneously decreasing the membrane potential and intracellular K<sup>+</sup> contents, which is consistent with the formation of transmembrane ion channels (fig. S12) (21, 22).

In conclusion, we provide evidence for a bacterial origin of a sponge-derived peptide natural product. This discovery supports the hypothesis that many, if not most, bioactive natural products from sponges are endosymbiont-derived (23) and highlights the value of symbiotic bacteria as a rich source of unusual biochemistry (24–28). Although polytheonamides are currently the only attributed proteusin members, a small number of compounds exhibit structures that suggest a close biosynthetic relation. These are the sponge-isolated yaku'amides (29) and discodermins (30), all of which contain residues with additional C-methyl groups and D-configured  $\alpha$ -carbon atoms. The use of ribosomal machinery to generate products containing D-amino acids and other modifications offers promise for the artificial engineering of peptides, peptidomimetics, and proteins with new structural and functional properties.



**Fig. 2.** Mass spectrometric identification of *poyF*-catalyzed dehydration. To identify the modified residue, PoyA purified from coexpression with *poyDF* was trypsinized and the resulting peptides analyzed by LC-MS/MS. The C-terminal tryptic peptide corresponding to residues 76 to 145 of PoyA was found to contain the dehydration site at residue Thr<sup>97</sup>. **(A)** Deconvoluted electrospray ionization (ESI)–MS spectrum of PoyA exhibiting a mass shift of –18 D. **(B)** ESI-MS/MS (ESI–tandem MS) spectrum of tryptic peptide 76–145 from PoyA [precursor ion  $[M+6H]^{6+}$  at mass/charge ratio ( $m/z$ ) 1130.560; calculated  $m/z$  1130.562 (monoisotopic)] showing a series of b-type fragment ions.



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## Supplementary Materials

[www.sciencemag.org/cgi/content/full/science.1226121/DC1](http://www.sciencemag.org/cgi/content/full/science.1226121/DC1)  
Materials and Methods  
Figs. S1 to S12  
Tables S1 to S3  
References (31–34)

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# Processing and Subcellular Trafficking of ER-Tethered EIN2 Control Response to Ethylene Gas

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Ethylene gas is essential for many developmental processes and stress responses in plants. ETHYLENE INSENSITIVE2 (EIN2), an NRAMP-like integral membrane protein, plays an essential role in ethylene signaling, but its function remains enigmatic. Here we report that phosphorylation-regulated proteolytic processing of EIN2 triggers its endoplasmic reticulum (ER)-to-nucleus translocation. ER-tethered EIN2 shows CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) kinase-dependent phosphorylation. Ethylene triggers dephosphorylation at several sites and proteolytic cleavage at one of these sites, resulting in nuclear translocation of a carboxyl-terminal EIN2 fragment (EIN2-C'). Mutations that mimic EIN2 dephosphorylation, or inactivate CTR1, show constitutive cleavage and nuclear localization of EIN2-C' and EIN3 and EIN3-LIKE1-dependent activation of ethylene responses. These findings uncover a mechanism of subcellular communication whereby ethylene stimulates phosphorylation-dependent cleavage and nuclear movement of the EIN2-C' peptide, linking hormone perception and signaling components in the ER with nuclear-localized transcriptional regulators.

The plant hormone ethylene (C<sub>2</sub>H<sub>4</sub>) is essential for a myriad of physiological and developmental processes. Molecular genetic dissection has revealed that ethylene is perceived by a family of the endoplasmic reticulum (ER)-membrane-bound receptors that are sim-

ilar in sequence and structure to bacterial two-component histidine kinases (1–4). Each receptor has an N-terminal transmembrane domain that binds ethylene via a copper cofactor, most likely provided by the copper transporter RESPONSIVE TO ANTAGONIST1 (5). Signaling from one of the receptors, ETR1 (ETHYLENE RESPONSE1), is promoted by interacting with another ER-localized protein REVERSION TO ETHYLENE SENSITIVITY1 (6). The ethylene receptors function redundantly to negatively regulate ethylene responses (2) via CTR1 (CONSTITUTIVE TRIPLE RESPONSE1), a downstream Raf-like protein kinase (7, 8). CTR1 is also associated with the ER membrane, where it directly interacts

with ETR1 (8, 9). Downstream of CTR1 is EIN2 (ETHYLENE INSENSITIVE2) (10, 11), an essential positive regulator of ethylene signaling, which shares sequence identity at its N terminus with the 12-transmembrane domain of the NRAMP family of metal transporters and contains a large ~800-amino acid C-terminal domain (CEND) (11). Previous studies using heterologous expression of *Arabidopsis* EIN2 in *Nicotiana benthamiana* suggested that EIN2 might be localized to the ER, where it can interact with ETR1 (12). Furthermore, EIN2 is targeted by F-box proteins EIN2-INTERACTING PROTEIN1 and EIN2-INTERACTING PROTEIN2, which mediates protein degradation of EIN2 via the ubiquitin-proteasome pathway in the absence of ethylene (13). In an unknown fashion, EIN2 transduces signals to the transcription factors EIN3/EIL1 (EIL1, ETHYLENE INSENSITIVE LIKE1), which are sufficient and necessary for activation of all ethylene-response genes (14). A model for hormone signaling has emerged in which the perception of ethylene by the receptors alters the activity of CTR1, which in turn, by an unknown mechanism, functions to relieve repression of EIN2, resulting in activation of EIN3/EIL1-dependent transcription and the activation of an ethylene response.

To explore the mechanism of EIN2 function, we identified and tested the requirement for a putative nuclear localization signal (NLS) (15) in the evolutionarily conserved EIN2 C terminus (fig. S1, A to E) and found that a wild-type EIN2-YFP (YFP, yellow fluorescent protein) fusion protein maintained its normal function(s), because its expression was able to rescue the *ein2-5* mutant phenotype (Fig. 1, A and B, and fig. S1F); whereas an NLS-mutated EIN2Fm-YFP protein was unable to complement the *ein2-5* mutant phenotype (Fig. 1, A and B). In the absence of the

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ethylene precursor ACC (1-aminocyclo-propane-1-carboxylate), the EIN2-YFP protein was localized in the ER (Fig. 1C) (12) and accumulated in the nucleus upon exposure to ethylene (Fig. 1C and fig. S1G). However, nuclear localization of the EIN2Fm-YFP protein was not observed in the presence of ACC (Fig. 1C and fig. S1H). Therefore, we conclude that the NLS is necessary for EIN2 to function in the ethylene response.

Plants respond to ethylene rapidly, and this process is dependent on the function of EIN2 (16). Time-lapsed imaging was used to monitor the dynamics of EIN2 protein movement upon ethylene treatment. In the absence of ethylene (at time 0), EIN2 protein was absent from the nucleus (Fig. 1D). Nuclear accumulation of EIN2 protein was observed within 10 min of exposure to ethylene, and protein levels further increased in the nuclei of the same cells during the subsequent 30 min (Fig. 1D). A greater amount of EIN2 protein in the nucleus was observed with longer ethylene treatments (fig. S1I), demonstrating that the ER-nucleus translocation of EIN2 is important in response to ethylene.

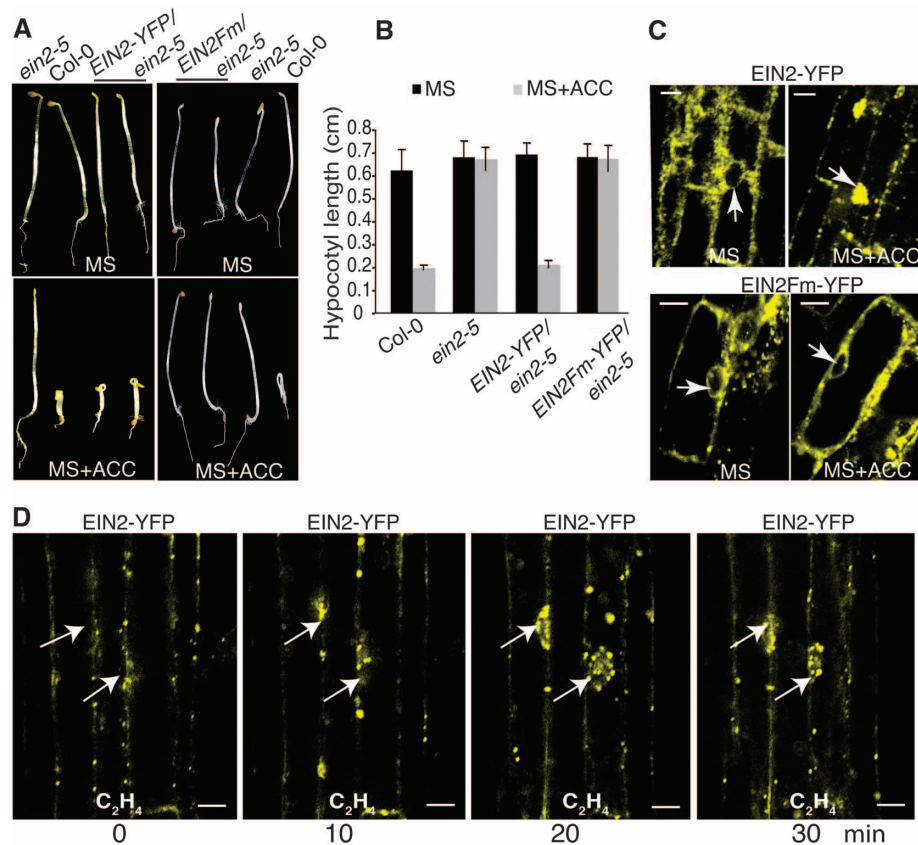
EIN2 protein specifically localized to the ER in the absence of ethylene (Fig. 2, A and B, and fig. S2, A to C) and amassed in the nucleus upon ethylene treatment (Fig. 2B), whereas a known ER-localized protein was unaffected by ethylene (Fig. 2C). Additionally, in wild-type Col-0, EIN2 protein was localized to the ER membrane in the absence of ACC, whereas upon growth on ACC, nuclear translocation of EIN2 protein was observed (Fig. 2D). However, in *etr1-1*, nuclear accumulation of EIN2 protein was abolished. In contrast, in *ctr1-1*, a constitutive nuclear accumulation of EIN2 protein was observed, even in the absence of ACC (Fig. 2D). An *ein3-1/eil1-1* double mutant had no effect on the nuclear translocation of EIN2 protein (Fig. 2D). Therefore, we conclude that ETR1 and CTR1 are important in the ER-nucleus translocation of EIN2, whereas EIN3/EIL1 are not required for this process.

EIN2 is a bifunctional protein (11), and positioning the EIN2-CEND polypeptide in the nucleus was sufficient to mimic both ethylene responses (fig. S3, A to E). We hypothesized that EIN2 may be proteolytically processed and its

C-terminal fragment translocated to the nucleus upon exposure to ethylene. To test this hypothesis, we first examined the presence of a cleaved form of the native EIN2 protein by Western blotting. Although full-length EIN2 was not detectable at 0 and 4 hours of ethylene treatment, it was detected in situations that mimic a constitutive ethylene exposure, such as in the *ctr1-1* mutant and in Col-0 plants treated with 16 hours of ethylene. Additionally, we observed the presence of an ~75-kD C-terminal EIN2 fragment (called EIN2-C') whose abundance correlated with the duration of ethylene treatment (Fig. 3A and fig. S4, A and B). Although full-length EIN2 was easily detected in the membrane protein fraction [Fig. 3B and (13)], EIN2-C' was barely detected in the nuclear fraction without ethylene treatment (Fig. 3B). In contrast, the EIN2-C' polypeptide was readily detected in the nuclear protein fraction treated with ethylene (Fig. 3B). We generated transgenic lines expressing EIN2-C1-YFP, which contained a YFP protein fused to a fragment of EIN2 estimated from the observed EIN2-C' polypeptide size (638 to 1294 amino acids), and found that EIN2-C1-YFP protein was localized to the nucleus exclusively, and its expression was sufficient to cause a severe constitutive ethylene response phenotype, reminiscent of *ctr1-1* mutants (fig. S4, C and D).

We next used mass spectrometry (17) to map the cleavage site of the EIN2 protein. Three EIN2-C' peptides [630 to 647 amino acids (630-647aa), 648-662aa, and 754-766aa; table S1A] were detected at similar levels in samples treated with air. In the presence of ethylene, the abundance of the peptide 630-647aa was ~20 times less, suggesting that cleavage of the EIN2 protein may occur within this peptide. Pseudo-multiple reaction monitoring (18) was used to identify the EIN2 protein cleavage site. Contiguous peptides designated 630-647aa and 648-662aa behaved differently after ethylene treatment; only the former decreased in abundance, suggesting that it contained an ethylene-dependent protease cleavage site (table S1A). We tested a set of overlapping peptides that differed by the progressive loss of single amino acids from the C terminus of the peptide 630-647aa. These represent 18 possible cleavage products derived from cleaving within residues 630 to 647 (plus the trypsin cleavage site corresponding to the N terminus of the peptide). After ethylene treatment, none of the 10 deletions from the N terminus corresponded to a detectable peptide (table S1B). However, among the eight deletions from the C terminus, one peptide was significantly enriched (AAPTSTNFTVGSDDGPPS, 630-645aa; Fig. 3C, fig. S4E, and table S1B), indicating that ethylene-induced cleavage occurs between 645aa and 646aa.

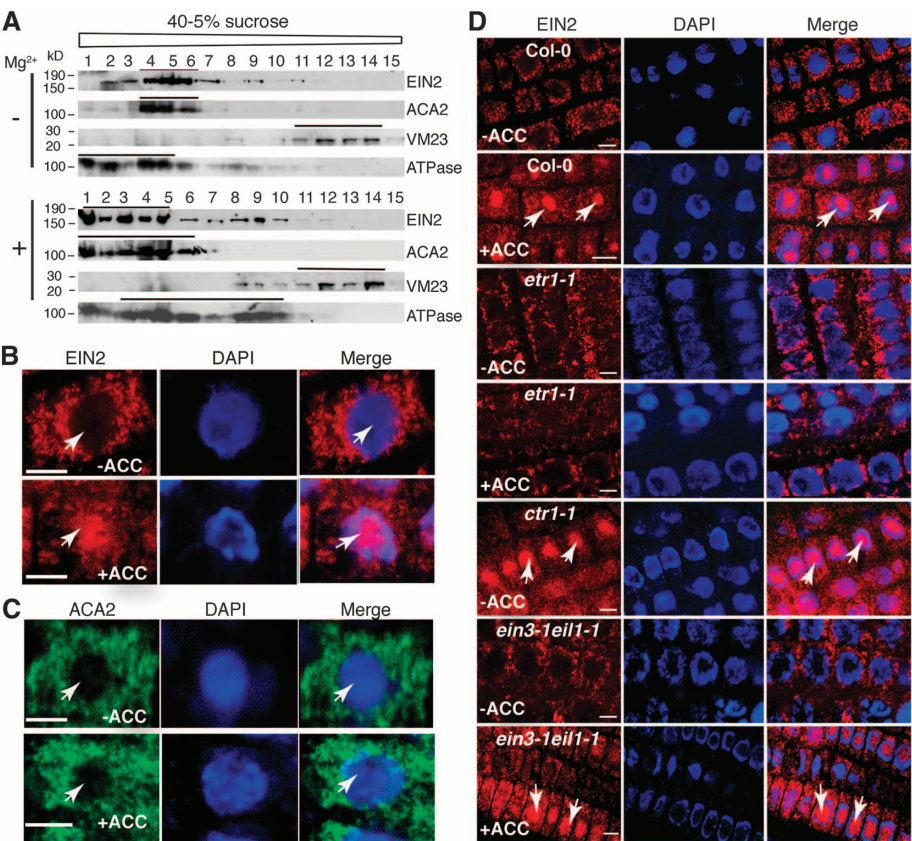
Although several phosphoproteomic studies have identified ethylene-responsive phosphopeptides (19, 20), the function of phosphorylation regulation in the ethylene signaling pathway is still unknown. We carried out a phosphoproteomic survey using proteins purified from etiolated seedlings treated with hydrocarbon-free air or ethylene



**Fig. 1.** The NLS in EIN2 is essential for nuclear localization and the response to ethylene. (A) Wild-type EIN2, but not EIN2 NLS mutations, fully rescue *ein2-5*. Seedlings were grown for 3 days in the dark with or without ACC. MS, Murashige and Skoog medium. (B) Hypocotyl measurements of 3-day-old etiolated seedlings. Each bar is the average length of at least 15 hypocotyls (error bars indicate mean  $\pm$ SD). (C) Confocal images of root cells from 3-day-old etiolated transgenic seedlings treated with or without ACC. (D) Time-lapsed confocal images of a series of root cells expressing EIN2-YFP in 3-day-old etiolated seedlings exposed to 10 ppm ethylene gas. Arrows track specific cell nuclei, showing the accumulation of EIN2-YFP in response to ethylene.



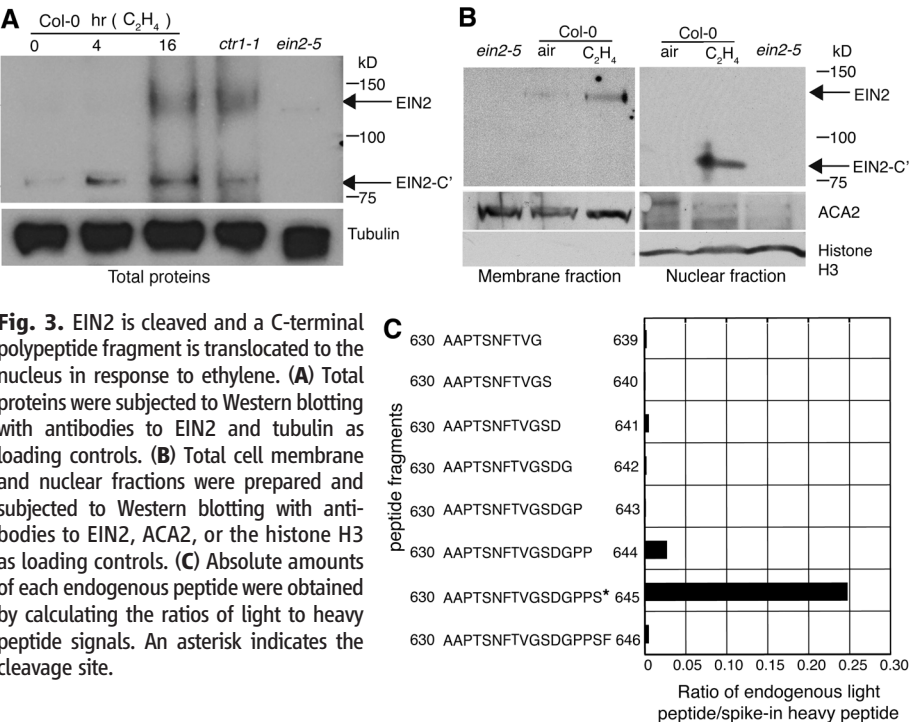
**Fig. 2.** Ethylene-stimulated nuclear accumulation of the ER-localized EIN2 requires ETR1 and CTR1 but not EIN3/EIL1. **(A)** Sucrose density-gradient centrifugation was performed by fractionation of microsomal membranes containing Mg<sup>2+</sup> or without Mg<sup>2+</sup>. ACA2 is an ER marker protein; VM23 is a vacuole membrane marker protein; adenosine triphosphatase (ATPase) is a plasma membrane marker protein. **(B)** In *Arabidopsis* root cells, ER localization of EIN2 in the absence of ACC contrasts with nuclear accumulation in the presence of ACC. **(C)** Immunofluorescence staining of the subcellular location of ACA2 in *Arabidopsis* root cells from 3-day-old etiolated seedlings grown with or without ACC. **(D)** Representative images were acquired from root cells using the same exposure times in all panels. Red staining indicates EIN2 immunofluorescence using a polyclonal antibody to the EIN2 C terminus. Green staining indicates ACA2 immunofluorescence. Blue staining indicates DAPI. Arrows indicate nuclei. Scale bar, 5 μm.



gas (10 ppm) and identified three sites in EIN2 where phosphorylation was highly enriched in air-treated samples (Fig. 4A, table S1A, and fig. S5A). Ser<sup>645</sup> (S645) is conserved in all plant species examined (fig. S5B) and this position coincided with the experimentally determined cleavage site. We examined the phosphorylation status of EIN2 S645 using *ctr1-1* and wild-type plants treated with or without ethylene. In wild-type plants treated with air, residue S645 of EIN2 was phosphorylated, whereas in wild-type plants treated with ethylene, S645 was not phosphorylated (Fig. 4B). In *ctr1-1* mutants, however, phosphorylation of S645 was undetectable (Fig. 4B) indicating that CTR1 is required for EIN2 S645 phosphorylation.

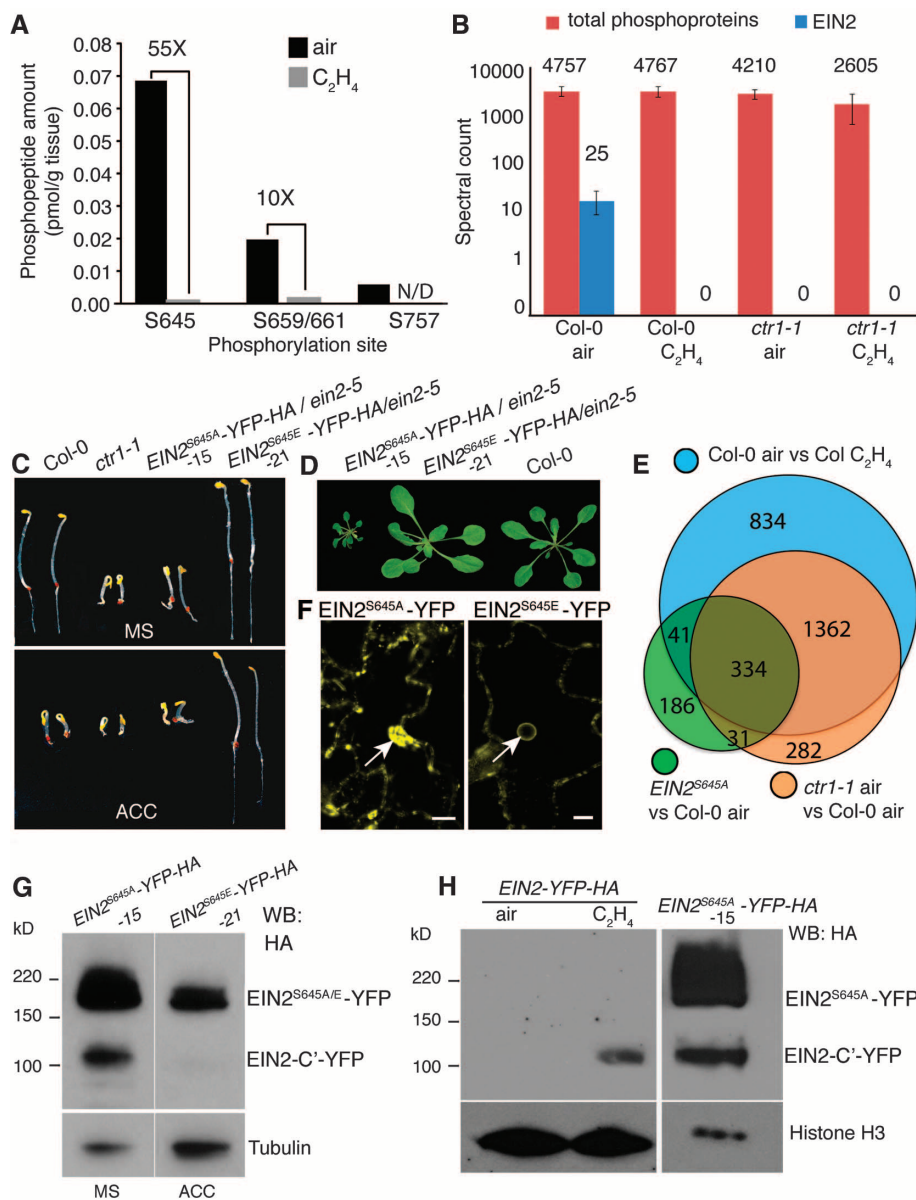
To test whether phosphorylation of S645 regulates ethylene-dependent EIN2 cleavage, constructs carrying point mutations in *EIN2* that convert serine to alanine (S645A) (*EIN2*<sup>S645A</sup>-YFP-HA) or serine to glutamic acid (S645E) (*EIN2*<sup>S645E</sup>-YFP-HA) were introduced into both wild-type and *ein2-5* plants. *EIN2*<sup>S645A</sup> did not alter the function of the EIN2 protein, because it fully rescued *ein2-5* in contrast to *EIN2*<sup>S645E</sup> (Fig. 4C). In fact, etiolated seedlings and adult *EIN2*<sup>S645A</sup> plants exhibited constitutive ethylene-response phenotypes in the absence of ethylene (Fig. 4, C and D, and fig. S5, C to E).

Transcriptome analysis revealed that >60% of genes with significant changes in expression in *EIN2*<sup>S645A</sup>-YFP-HA transgenic lines significantly overlapped with genes differentially expressed in wild-type ethylene-treated plants ( $P < 1 \times 10^{-200}$ , using Fisher's exact test) or with genes differen-



tially expressed in *ctr1-1* mutant plants treated with hydrocarbon-free air (Fig. 4E), suggesting that the *EIN2* S645A mutation affects numerous ethylene-responsive genes at the transcriptional level. Moreover, *EIN2*<sup>S645A</sup>-YFP-HA transgenic plants showed both constitutive cleavage of EIN2 at residue S645 and constitutive nuclear translocation of the EIN2-C' protein (Fig. 4, F and G). The predicted length of the EIN2-derived polypeptide released from *EIN2*<sup>S645A</sup>-YFP-HA matched





**Fig. 4.** CTR1-dependent ethylene-regulated phosphorylation of EIN2 S645 regulates proteolysis and nuclear translocation of EIN2-C'. (A) Absolute amounts of three EIN2-C' phosphopeptides before and after treatment with 10 ppm ethylene gas. N/D, not detectable. (B) Relative phosphorylation levels of EIN2 peptides in wild-type or *ctr1-1* plants treated for 4 hours of air or 10 ppm ethylene gas. Spectral counts were computed by averaging three biological replicates. The total spectral counts from all phosphorylated proteins in each sample are indicated as an internal control. (C) EIN2-C' S645A results in constitutive ethylene response phenotypes in dark-grown seedlings. (D) EIN2 S645A results in constitutive ethylene response phenotypes in 7-week-old plants. (E) EIN2 S645A plants show transcriptional activation of ethylene responses. (F) EIN2 S645A plants show constitutive nuclear localization without ethylene from leaf cells. Arrows indicate nuclei. (G) S645A leads to constitutive cleavage of EIN2. Total proteins from 3-day-old etiolated seedlings were subjected to Western blotting using antibodies to HA and to tubulin as loading controls. EIN2<sup>S645A/E</sup> represents either full-length EIN2<sup>S645A</sup> or EIN2<sup>S645E</sup>. (H) Purified nuclear proteins prepared from EIN2-YFP-HA plants treated with or without ethylene. Total protein from EIN2<sup>S645A</sup>-YFP-HA-overexpressing plants was prepared and subjected to Western blotting using antibodies to HA and to histone H3 as loading controls. Scale bar, 5  $\mu$ m.

with that observed in the nucleus of EIN2-YFP-HA transgenic plants after exposure to ethylene (Fig. 4H). In contrast, in transgenic plants containing the S645E mutant, both cleavage and nuclear translocation of EIN2-C' protein were abolished, even in the presence of ACC (Fig. 4, F and G).

We have uncovered a mechanism whereby EIN2 protein processing and subcellular nuclear translocation are required for response to ethylene (fig. S6). Recent studies in animals have also demonstrated the importance of dephosphorylation-dependent nuclear translocation of transcription

factor EB in regulating homeostasis of the lysosome (21), and of nuclear translocation of ATFS-1 (activating transcription factor associated with stress-1) in response to mitochondrial stress (22). Further studies to determine the biochemical mechanisms that are needed for the cleavage of EIN2 in response to ethylene will be of great importance. In addition, identification of the kinase(s) and phosphatase(s) that target the EIN2 protein directly, as well as the enzymes that promote processing of this key regulatory molecule, are future challenges that will further our understanding of this highly conserved and agriculturally important plant stress and growth-controlling signaling pathway.

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## Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S6

Table S1

References (23–27)

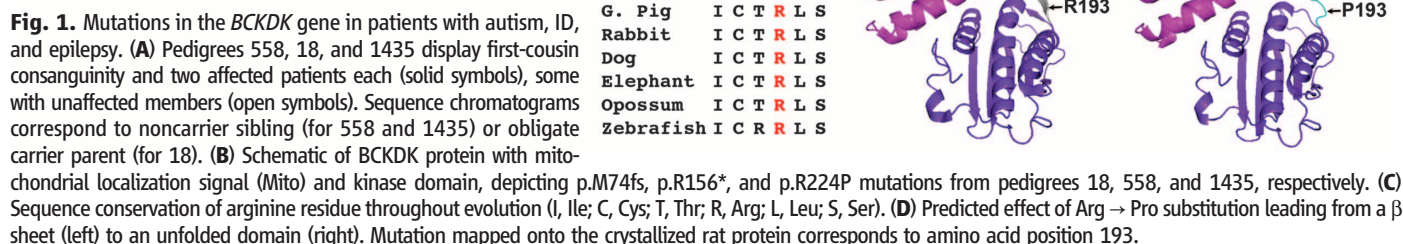
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text), carrying a missense (c.G671C) mutation (Fig. 1A) mapping within a linkage peak (lod score of 2.2) on chromosome 16 (fig. S1A) and leading to the substitution of a highly conserved arginine with a proline at position 224 in BCKDK (Fig. 1C). Modeling of the p.R224P mutation using the crystal structure of rat Bckdk (4) suggested disruption of the  $\beta$  sheet in a flexible linker domain (Fig. 1D).

The branched-chain ketoacid dehydrogenase (BCKDH) complex catalyzes the irreversible, rate-limiting step in the catabolism of branched-chain amino acids (BCAAs) (5), and BCKDK encodes a kinase that phosphorylates and thus inactivates the E1 $\alpha$  subunit of this complex (6) (Fig. 2E). Mutations in any of the three subunits of the BCKDH complex lead to toxic accumulation of BCAAs and their metabolites and are the cause of maple syrup urine disease (7), characterized by severe neurological complications and treated by dietary restriction of BCAAs, which are essential amino acids (8). BCKDK is expressed widely in mouse and human tissues (fig. S2) (9). Relative to controls, we found reduced mRNA levels in cell lines from the two affected patients in families 558 and 18, suggesting nonsense-mediated mRNA decay (Fig. 2A and fig. S3A), as well as undetectable levels of BCKDK protein on Western blot (Fig. 2B and fig. S3B). Supporting a role of BCKDK in negative regulation of the BCKDH complex, we tested a

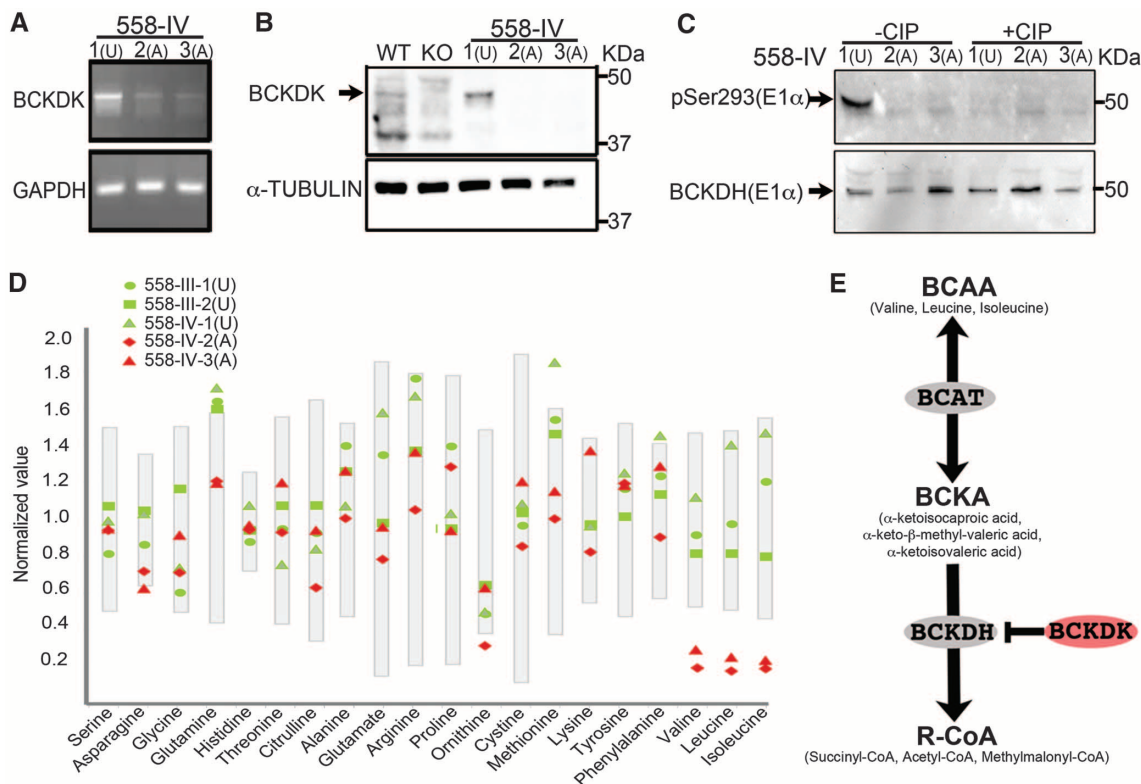
phospho-specific antibody to the BCKDK phosphorylation site at residue 293 of the E1 $\alpha$  BCKDH subunit (10) and found absence of reactivity in dermal fibroblasts of the affected individuals in family 558 (Fig. 2C) and in tissue of the *Bckdk*<sup>-/-</sup> mouse (fig. S4). These data suggest that patients with BCKDK mutations may lack basal, negative regulation of BCKDH activity.

Mice deficient for *Bckdk* display increased basal activity of the BCKDH complex as well as reduced BCAAs in various tissues (11). We measured plasma BCAAs in our patients and found that each patient with a homozygous mutation showed notably lower levels of plasma BCAAs than their healthy relatives and with respect to reference ranges (Fig. 2D and tables S3 to S5). Declines in BCAAs in affected patients with null mutations were more substantial (with respect to normal reference ranges) than those in patients with missense variants (tables S3 to S5) (12). There was no accumulation of BCAAs in the urine (table S6), excluding abnormally high BCAA urinary excretion. None of the affected individuals was known to eat a diet deficient in protein, as evidenced by their normal fasting plasma levels of the other essential amino acids (tables S3 to S5 and supplementary text). We did not observe accumulation of organic acids (Fig. 2E) in the blood of our patients (table S7), which suggests that these products were efficiently eliminated in the urine.

To uncover cellular phenotypes of human cells lacking BCKDK, we generated pluripotent stem cells (iPSCs) from the fibroblasts of the healthy brother and the two affected sisters from family 558 by means of episomal reprogramming (supplementary text) (13). All iPSC lines were positive for pluripotent markers (fig. S5B), showed normal karyotype, and demonstrated expected gene expression profiles as a function of these conditions (fig. S6A). We differentiated three iPSC lines per individual into embryoid bodies, then neural rosettes, and finally, neural progenitor cells (NPCs) (fig. S5A). To reduce variability in the differentiation efficiency, we used fluorescence-activated cell sorting (FACS) to generate homogeneous NPC populations. CD184<sup>+</sup>CD24<sup>+</sup>CD44<sup>+</sup>CD271<sup>-</sup> cells were sorted (14) and passaged for five cycles before analysis, resulting in 99.9% of the cells being positive for Nestin, a marker of neural stem cells. (fig. S7A). There were no notable differences in the morphology or in the proliferation of cells of wild-type and mutant genotypes (fig. S7, A and B).

To uncover potential cell-autonomous phenotypes, we exposed NPCs to reduced levels of BCAAs in the culture media. Typical culture medium is supplemented with a total concentration of ~1200  $\mu$ M BCAAs, so we reduced these concentrations over several orders of magnitude to nearly zero. We found no genotype-dependent differences in cell survival or proliferation (fig.

**Fig. 2.** Effect of BCKDK p.R156\* mutation in affected and unaffected individuals from family 558. (A) Reverse transcription polymerase chain reaction showing reduced levels of mRNA in affected patients; GAPDH levels are shown as a control. (B) Western blot probed with BCKDK-specific antibody (arrow). Fibroblast lysates from family 558 showing undetectable BCKDK protein in affected patients (A, IV-2, and IV-3) relative to unaffected patients (U, IV-1). Wild-type and *Bckdk* knockout (KO) mice lysates served as positive and negative controls. (C) Western blot probed with phospho-specific pSer293 antibody of E1 $\alpha$  subunit of BCKDH enzyme. In the absence of calf intestinal phosphatase (CIP), a distinct band is present in the unaffected individual (U), absent in the two affected individuals (A), and nearly abolished after CIP treatment. (D) Reduced levels of plasma BCAAs in affected patients (red symbols) compared with parents and unaffected sibling (green symbols). Gray bars represent normalized standard values. (E) Schematic diagram



of the BCAA catabolic pathway. BCAT, branched-chain aminotransferase; BCKA, branched-chain  $\alpha$ -keto acid; BCKDH, branched-chain  $\alpha$ -keto acid dehydrogenase; R-CoA, succinyl-CoA, acetyl-CoA, and methylmalonyl-CoA.



S7, B and C). We next differentiated the NPCs into mature neurons by basic fibroblast growth factor withdrawal, which resulted in nearly 100% of the cells becoming positive for neuron-specific  $\beta$ -tubulin Tuj1 and the cytoskeletal protein Map2 (fig. S8, A and B). We found no differences in numbers of differentiated cells per well, dendrites per cell, presynaptic punctae per length of dendrite, or area of the cell soma (fig. S8). There were no appreciable differences in gene expression or metabolic profiles of these cultured neurons, arguing against a major cell-autonomous role for *BCKDK* in the pathogenesis of the disease.

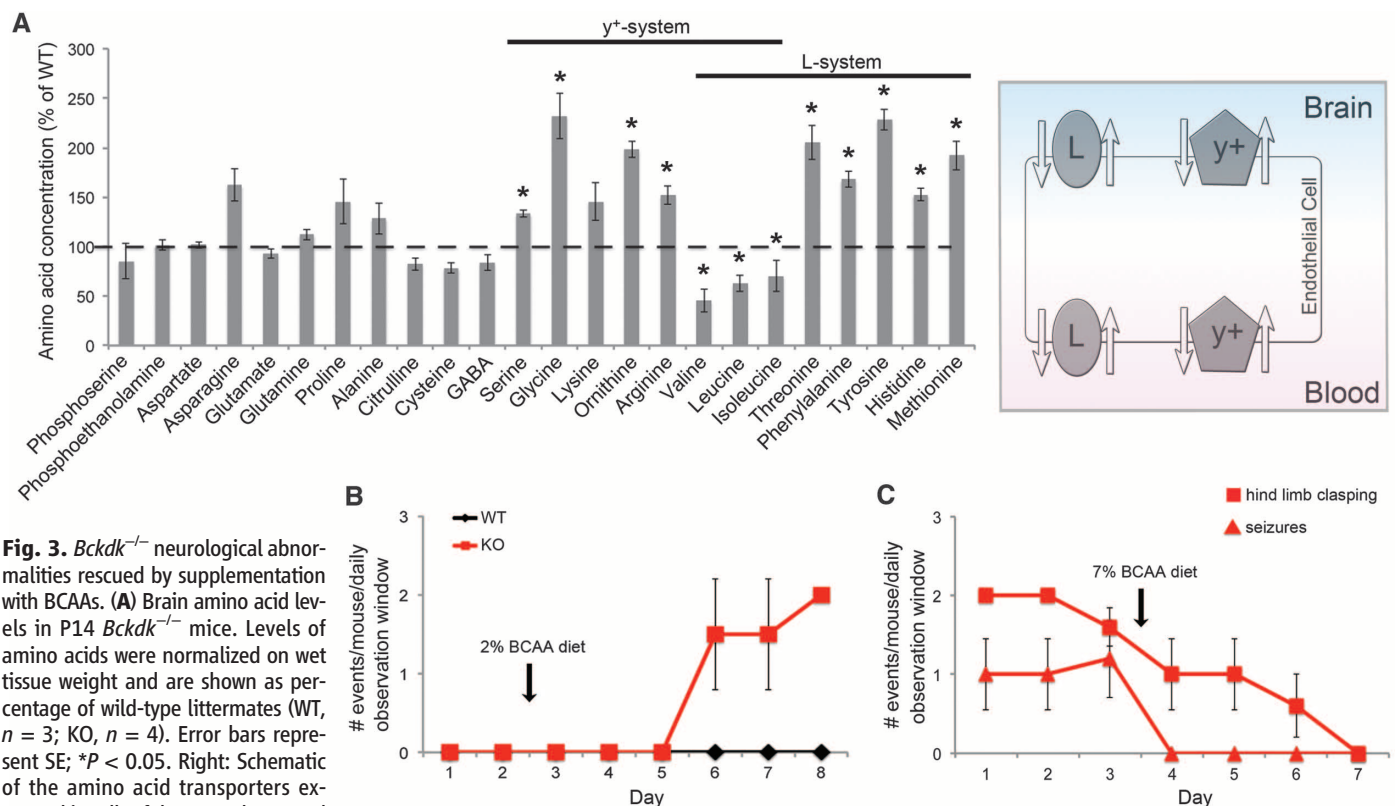
We next explored the possibility of cell-autonomous mechanisms by returning to the *Bckdk*<sup>−/−</sup> mice. Mutants were born at the expected Mendelian ratio and were healthy at birth but showed growth retardation that could be recovered by feeding a BCAA-rich diet (11). Adults developed neurological abnormalities, such as tremors, epileptic seizures, and hindlimb clasp- ing phenotypes observed in some other mouse models of autism spectrum disorders (15, 16). Gross brain histology, however, was normal (11) (fig. S9). To identify possible pathways contrib- uting to the neurological phenotype, we per- formed a gene expression profile of mouse brain cortex from wild-type and *Bckdk*<sup>−/−</sup> mice prior

to the onset of seizures (fig. S10A and supplemen- tary text) followed by comprehensive pathway analysis (fig. S10B). There were several relevant perturbed pathways, including the brain-expressed amino acid transporter network (fig. S10C).

BCAAs are transported across the blood-brain barrier (BBB) mainly by the heterodimeric amino acid transporter *SLC7A5/SLC3A2* (or LAT1), a member of the L-type transporter family and, to a lesser extent, by members of the y-type family and the Na<sup>+</sup>-dependent LNAA transporter (17) (Fig. 3A). In the brain, LAT1 is expressed almost exclusively in the basolateral and apical mem- branes of the BBB endothelial cells (18) (Fig. 3A). Expression levels of *SLC7A5/SLC3A2* change in response to amino acid availability (19, 20). We reasoned that the altered expression of the transporters might be a direct response to the low concentration of plasma BCAAs. At normal plasma amino acid concentrations, these amino acid transporters are fully saturated and shared by a number of large neutral amino acids (LNAAs). Therefore, plasma amino acids compete with each other for transport across the BBB. A decrease in plasma BCAAs could lead to a perturbation in brain concentrations of not only BCAAs but also other LNAAs. Thus, we quantified amino acid concentrations in brain homogenates from post-

natal day 14 (P14) wild-type and knockout mice. In addition to the expected reduced brain BCAAs, there were also significantly increased levels of threonine, phenylalanine, tyrosine, histidine, and methionine, the exact LNAAs that are carried by these transporters (Fig. 3A), which suggests im- balanced BBB transport. Because several of these amino acids are precursors for important neuro- transmitters, it remains a possibility that the re- duced BCAAs and/or the increased LNAAs contribute to the neurological phenotype. In fact, patients with uncontrolled MSUD display re- duced brain LNAAs, and the degree to which the reduced or increased amino acids contribute to the phenotype remains controversial (21).

The data suggest that the neurological pheno- type may be treated by dietary supplementation with BCAAs. To test this hypothesis, we studied the effect of a chow diet containing 2% BCAAs or a BCAA-enriched diet, consisting of 7% BCAAs, on the neurological phenotypes of the *Bckdk*<sup>−/−</sup> mice. Mice raised on the BCAA-enriched diet were phenotypically normal. On the 2% BCAA diet, however, *Bckdk*<sup>−/−</sup> mice had clear neurological abnormalities not seen in wild-type mice, such as seizures and hindlimb clasp- ing, that appeared within 4 days of instituting the 2% BCAA diet (Fig. 3B). These neurological deficits



**Fig. 3.** *Bckdk*<sup>−/−</sup> neurological abnormalities rescued by supplementation with BCAAs. **(A)** Brain amino acid levels in P14 *Bckdk*<sup>−/−</sup> mice. Levels of amino acids were normalized on wet tissue weight and are shown as percentage of wild-type littermates (WT, *n* = 3; KO, *n* = 4). Error bars represent SE; \**P* < 0.05. Right: Schematic of the amino acid transporters expressed in cells of the BBB. The L- and y<sup>+</sup>-type transporters are responsible for the transport of the BCAAs and other LNAAs. **(B)** Mice raised on a BCAA-enriched diet (7% BCAA) were assessed for 2 days for hindlimb claspings. Starting on the evening of the second day, the mice were fed a 2% BCAA diet until day 8. Mice were tested for hindlimb claspings twice per day. WT, *n* = 1; KO, *n* = 2. Error bars represent SD. The observer was

blinded to the genotype. **(C)** *Bckdk*<sup>−/−</sup> mice neurobehavioral tests for animals fed a 2% BCAA diet (day 1 to day 3) or a BCAA-enriched diet (day 3 to day 7). Mice were tested twice per day for hindlimb claspings (1 to 2 months old) and seizures (4 to 6 months old). Error bars represent SD; *n* = 5 for each test.

were completely abolished within a week of the *Bckdk*<sup>−/−</sup> mice starting the BCAA-enriched diet, which suggests that they have an inducible yet reversible phenotype (Fig. 3C).

Our experiments have identified a Mendelian form of autism with comorbid ID and epilepsy that is associated with low plasma BCAAs. Although the incidence of this disease among patients with autism and epilepsy remains to be determined, it is probably quite a rare cause of this condition. We have shown that murine *Bckdk*<sup>−/−</sup> brain has a disrupted amino acid profile, suggesting a role for the BBB in the pathophysiology of this disorder. The mechanism by which abnormal brain amino acid levels lead to autism, ID, and epilepsy remains to be investigated. We have shown that dietary supplementation with BCAAs reverses some of the neurological phenotypes in mice. Finally, by supplementing the diet of human cases with BCAAs, we have been able to normalize their plasma BCAA levels (table S10), which suggests that it may be possible to treat patients with mutations in *BCKDK* with BCAA supplementation.

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#### Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1224631/DC1  
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Supplementary Text  
Figs. S1 to S10  
Tables S1 to S10  
References (22–37)

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## Direct Observation of Cotranscriptional Folding in an Adenine Riboswitch

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Growing RNA chains fold cotranscriptionally as they are synthesized by RNA polymerase. Riboswitches, which regulate gene expression by adopting alternative RNA folds, are sensitive to cotranscriptional events. We developed an optical-trapping assay to follow the cotranscriptional folding of a nascent RNA and used it to monitor individual transcripts of the *pbuE* adenine riboswitch, visualizing distinct folding transitions. We report a particular folding signature for the riboswitch aptamer whose presence directs the gene-regulatory transcription outcome, and we measured the termination frequency as a function of adenine level and tension applied to the RNA. Our results demonstrate that the outcome is kinetically controlled. These experiments furnish a means to observe conformational switching in real time and enable the precise mapping of events during cotranscriptional folding.

Structured RNAs function during transcription or translation to influence a variety of processes. In vivo, nascent RNAs fold as they are transcribed, and this sequential process

affects the folding efficiency (1–4) and the predominant conformation (5–9). The study of cotranscriptional folding has heretofore been largely indirect, with most approaches monitoring the final RNA product (1–10). Using single-molecule spectroscopy, we developed a system to visualize RNA folding in an individual, nascent transcript directly and used it to record the functional switching of a riboswitch. The conformation of the riboswitch ligand-binding aptamer affects the structure

of the downstream RNA through changes in the availability of nucleotides shared between the aptamer and an “expression platform,” which lead to structure-dependent gene regulation (11, 12). The *pbuE* riboswitch from *Bacillus subtilis* regulates adenine levels, controlling transcription of downstream genes by forming an aptamer, which binds adenine and acts as an antiterminator, or an expression platform, consisting of a terminator hairpin that halts transcription (13) (Fig. 1A). Previous work has considered the posttranscriptional folding of this aptamer (13–16). Here, we consider the functional consequences of the aptamer and terminator domains acting in concert in real time. Following transcription of the full riboswitch, only the folded terminator has previously been observed (15–18), as expected, given the far greater energetic stability of this domain (19). Any switching behavior must therefore be studied in the context of active transcription.

In our assay (14, 20), a transcriptionally stalled molecule of *Escherichia coli* RNA polymerase (RNAP) carrying a short initial transcript was tethered in a dual-beam optical tweezers apparatus in a “dumbbell” configuration (19), with RNAP linked to one bead and the transcript linked to the other via hybridization to a complementary, cohesive end of a dsDNA “handle” (14, 21) (Fig. 1B). Transcription was restarted by the addition of nucleoside triphosphates (1 mM NTPs) in the presence or absence of saturating levels of adenine base

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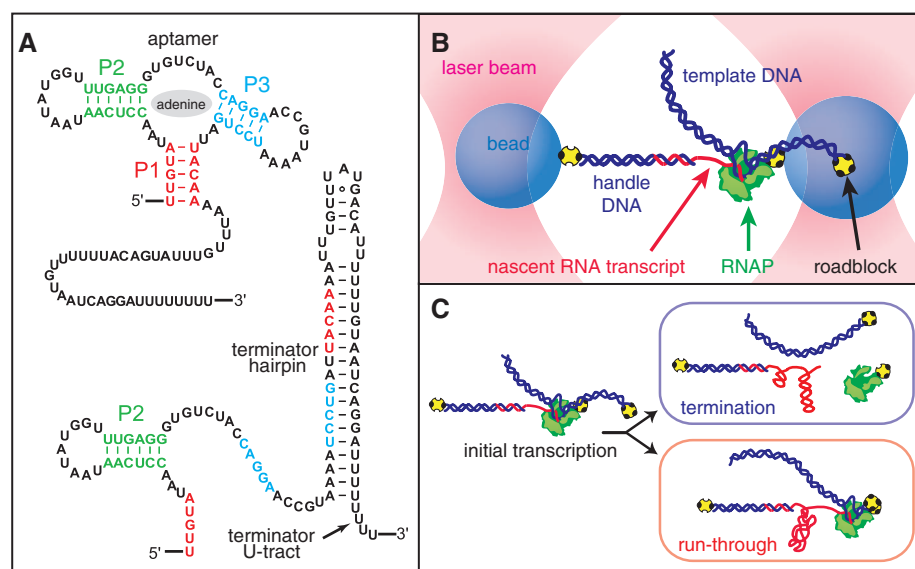
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(300  $\mu\text{M}$ ), and the separation between beads was measured. Transcription proceeded through the riboswitch, either finishing at the position of the terminator—where the dumbbell assembly dissociated as RNAP released the RNA—or continuing to the end of the template, where RNAP was arrested by a streptavidin roadblock—at which point the dumbbell tether remained intact (Fig. 1C).

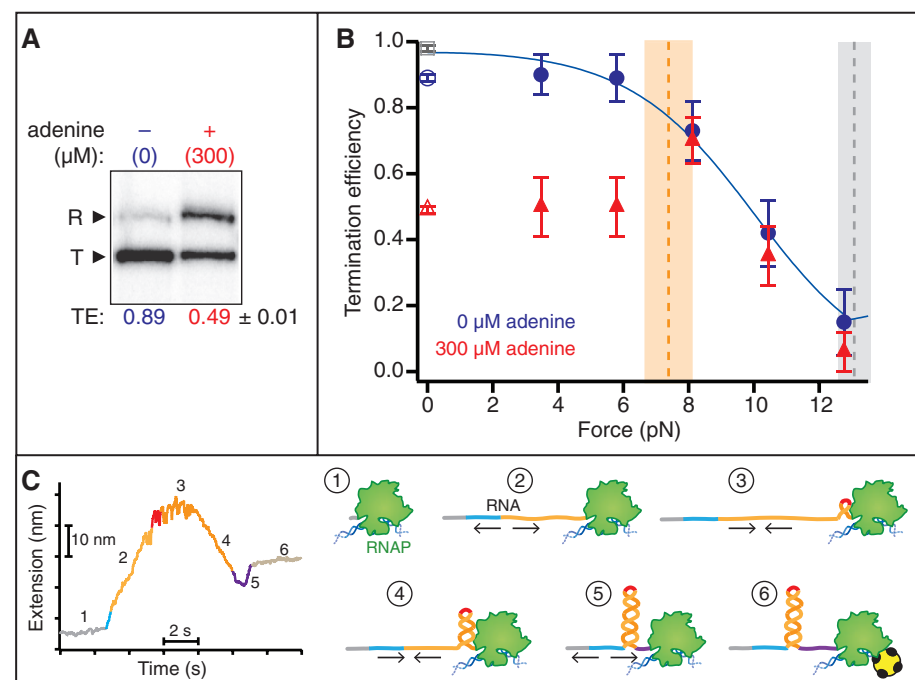
Adenine-dependent termination was measured both in bulk (no force) and for single molecules (Fig. 2, A and B). Termination was scored as the termination efficiency (TE), defined as the fraction of complexes dissociating at the terminator position. Bulk assays gave high levels of termination in the absence of adenine (89%) that decreased in its presence (49%, for saturating adenine). For single molecules, the TE depends force, because folded conformations of the aptamer and terminator are destabilized by increasing loads. At low forces, where these structures can form ( $<7$  pN), TEs were similar to those in bulk. Without adenine (Fig. 2B, blue), most transcripts terminated at low force, but TEs decreased under loads approaching the unfolding force of the terminator hairpin ( $\sim 13$  pN). This behavior is identical to that reported for intrinsic terminators alone and fit by a quantitative model (19, 21). The upstream aptamer sequence in the absence of adenine only slightly lowered the TE (at  $F = 0$ ) relative to that of the isolated terminator. Under saturating adenine levels (Fig. 2B, red), the TE remained near 50% throughout the low-force regime, where the aptamer folds readily, but defaulted to the identical load dependence as data acquired without adenine for forces beyond  $\sim 7$  pN, the equilibrium value for folding of the aptamer region alone. These results imply that, absent an adenine-reinforced aptamer caused by either (i) a lack of adenine or (ii) a load-dependent destabilization of the aptamer, the riboswitch behaves as an unhindered terminator.

We observed transcription directly, including cotranscriptional folding events, by monitoring transcript extension change over time. By shortening RNA tethers into the range of hundreds of bases, which increases stiffness and reduces noise, and lowering the tension below levels sufficient to open short hairpins, we were able to visualize structure formation as it took place. We also revised our protocols to measure transcription without significant delay from the restart of transcription (19). Two antagonistic effects control end-to-end transcript length: The extension tends to increase overall as RNA gets synthesized but may decrease whenever the nascent chain folds. An illustration of such lengthening is found in Fig. 2C, as the first part of the stem and the loop of a simple hairpin are synthesized. This phase is followed by shortening as the complementary portion of the stem is generated, until the folding completes, whereupon lengthening resumes until the roadblock is reached.

In transcription records of the adenine riboswitch obtained at comparatively high loads ( $F =$



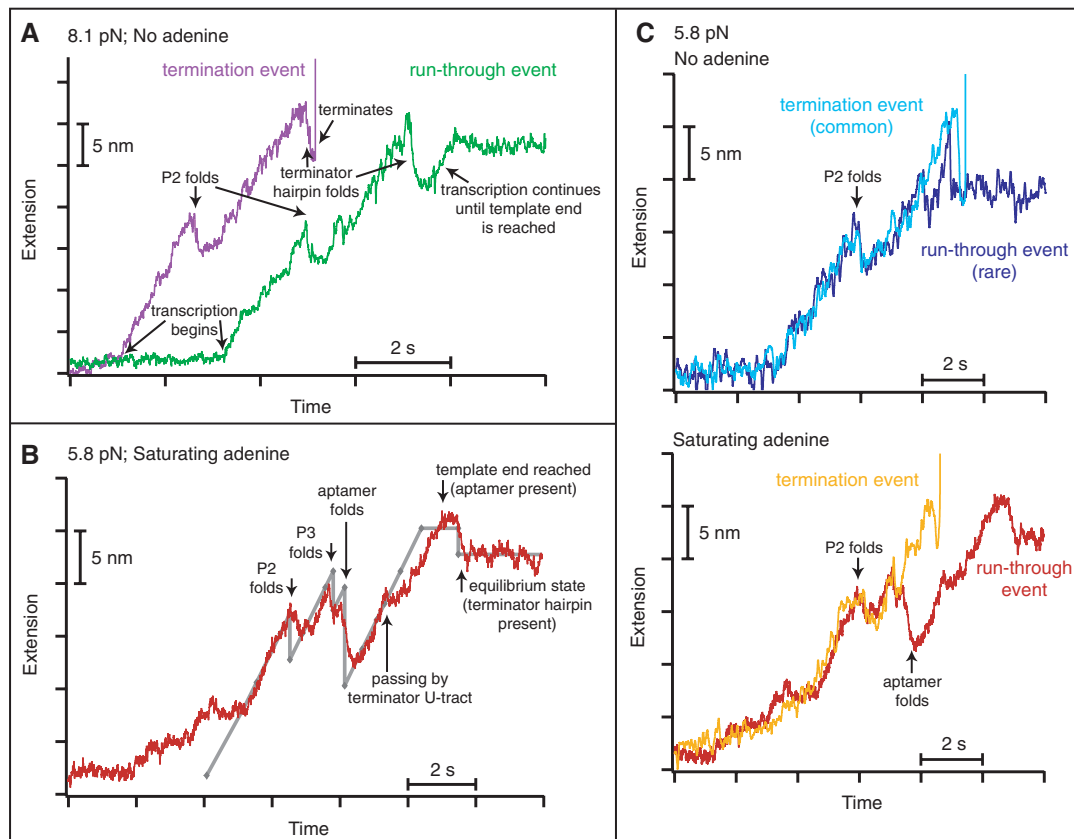
**Fig. 1.** (A) Secondary structure of competing aptamer and terminator conformations of the *pbuE* riboswitch. (B) Schematic of the “dumbbell” assay showing the experimental geometry, with the nascent RNA transcript transcribed in situ (not to scale). (C) Elongation produces the riboswitch, causing RNAP to dissociate at the terminator element or to run through to the roadblock at the template end.



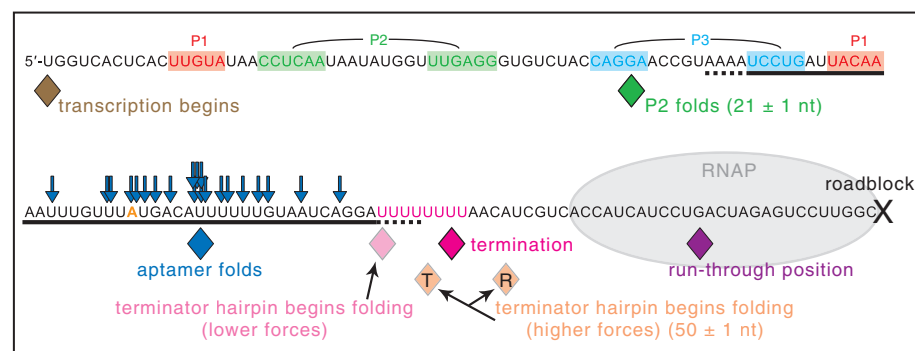
**Fig. 2.** Termination efficiency (means  $\pm$  SEM). (A) TE from a gel-based assay with termination (T) and run-through (R) bands, and (B) as a function of tension applied to the RNA. Data under load were from single-molecule records (filled symbols); zero-force points were from gel assays (open symbols). Blocking RNA upstream of the terminator with a DNA oligomer suppressed aptamer formation and created an isolated terminator, raising TE slightly (gray open square). Data without adenine were fit by a model of intrinsic termination (blue curve). The equilibrium unfolding forces of the adenine-bound aptamer and terminator hairpin are indicated (orange, gray shading and dashed lines, respectively; means  $\pm$  SD). (C) Cotranscriptional folding of a hairpin (left; 72 base pairs, 12 pN load). Stages during transcription are illustrated (right; not to scale): (1) Extension remains constant before transcription restart, then (2) increases (outward arrows) during elongation until (3) the duplex nucleates, causing extension to decrease (inward arrows) as (4) the hairpin forms. Once the hairpin is completed, (5) elongation resumes until RNAP reaches the roadblock (6).



**Fig. 3.** Riboswitch transcription showing cotranscriptional folding. Transcript extension generally increased during transcription, with local decreases induced by folding events. Terminating traces led to RNA release and a sharp upward displacement, corresponding to tether breakage; run-through traces remained at a fixed extension once RNAP encountered the terminal roadblock. Records were collected in 0  $\mu$ M or 300  $\mu$ M adenine (saturating). (A) At high forces, where the aptamer rarely folds (8.1 pN), folding is adenine-independent (fig. S3); there is no outcome-correlated difference in folding patterns (purple versus green traces). Records are offset for display. (B) At lower forces (5.8 pN), run-through traces displayed the characteristic signature of bound aptamer formation. A run-through record is superimposed against a simple folding model (gray; see supplementary materials), with the events indicated. (C) Records displayed distinct motifs correlated with the transcription outcome. Records overlaid: absent adenine (top), run-through events were rare and records did not display an aptamer folding signature following P2 formation, appearing indistinguishable from terminating records. With adenine (bottom), aptamer folding led to transcriptional run-through.



With adenine (bottom), aptamer folding led to transcriptional run-through.



**Fig. 4.** Map of cotranscriptional folding events. The average computed position of the last nucleotide transcribed at the specified event is indicated (solid diamonds; SEM  $\pm$  1 nt, approximately equal to the symbol width), beginning with the resumption of transcription at the 5'-U, ending with termination or run-through and roadblock arrest. The numbers of nucleotides associated with folding elements are indicated. Adenine-bound aptamer folding (blue diamond, average position; blue arrows, sampled individual observations) occurred between P1 and complete terminator transcription. For forces below 9 pN (5.8 pN, 8.1 pN), the terminator hairpin (underlined) was observed to begin folding (light pink diamond) before the termination position (dark pink diamond; transcript release). At higher forces (10.4 pN), the average folding position differed between traces that terminated (T, orange diamond) or ran through (R, orange diamond), occurring too late in the latter to produce successful termination. See table S2 for additional details.

8.1 pN) (Fig. 3A), extension increased monotonically during the synthesis of the early, unstructured sequence, followed by a decrease at the first fold, corresponding to the folding of the P2 stem-loop. Transcription continued until the terminator

was reached, at which point its hairpin folded and transcription was terminated (releasing the RNA) or RNAP ran through to the end of the template. At this high force, the aptamer rarely forms, so the TEs and shapes of records were similar in the

presence or absence of adenine (Fig. 3A and fig. S3).

At lower loads, adenine-dependent folding occurred in a way that decided transcriptional fate. The signature of aptamer folding appeared as a relatively large decrease in extension, after P2 folded and before the terminator hairpin was generated: This resulted in a stable fold, during which other folding events were not observed. A simplified model of cotranscriptional folding serves as a useful guide to track the folding events (Fig. 3B). P2 folded first, followed closely by P3 and then the aptamer. With the aptamer formed, RNAP is able to transcribe past the terminator hairpin without its folding. However, even in such cases, the adenine-bound aptamer was eventually observed to unfold and to be replaced by an equilibrium structure with the terminator hairpin folded. However, because RNAP had already moved beyond the terminator position, the RNA was not released.

Comparisons at lower force (5.8 pN) showed that in the presence of adenine, the vast majority of records destined to run through displayed a distinct folding signature, attributable to aptamer folding. Aptamer folding signatures were not observed in records that ultimately terminated or in rare run-through records obtained without adenine (Fig. 3C and fig. S4). With adenine present, the chance of run-through in a record carrying

the aptamer-folding signature was at least 95%. Conversely, the probability of transcriptional termination when the aptamer-fold signature was not scored but adenine was present was ~85%. The probability of run-through under such conditions (~15%) agrees closely with the probability of run-through in the absence of adenine.

On the basis of the short time window for folding and possible adenine binding in the *pbuE* aptamer before the termination decision (~2 s), and on the relatively long lifetime of the adenine-bound aptamer (~10 s) (16, 19), as well as the dependence of TE on transcription rate (18), it has been conjectured that kinetic (as opposed to thermodynamic) effects would play a dominant role in controlling transcriptional fate. We find unambiguous evidence that the *pbuE* riboswitch is kinetically controlled, because aptamers were observed to fold and bind adenine once, or not at all, precluding any possible thermodynamic equilibration between conformations during the binding window, as transcription proceeded rapidly [~20 nucleotides (nt)/s]. Once formed, the adenine-bound aptamer rarely unfolded before the termination decision (fig. S5A), and it was observed to fold multiple times only in one unusually slow record (fig. S5B). The probability of forming an unproductive, aptamer-like fold (i.e., where the aptamer folds and unfolds at least once before influencing the outcome) is therefore low: 5% or fewer transcripts generated an unproductive, adenine-associated fold.

The flavin mononucleotide (FMN) riboswitch sequence contains two pause sites thought to be important for allowing FMN additional time to bind (7) and, possibly, increasing the time window for folding. In the *pbuE* riboswitch, two short series of uridine (U) residues, situated before the poly(U) tract of the terminator, have similarly been proposed to act as pause sites (16), with pausing reported in one of these regions, but only observed under limiting NTP conditions (18). In our experiments using physiological levels of NTPs, long pauses were not observed at either of these sites; pauses, if any, were too brief to identify (<1 s). This discrepancy might be attributed to the differences in NTP concentrations, to transcription by the cognate *B. subtilis* RNAP (although this is unlikely to increase pausing), or to the involvement of other factors (7, 18, 22), as discussed (19).

Our data show that the folded aptamer can persist briefly after transcription of the terminator hairpin, temporarily trapping the riboswitch far from equilibrium. Taking U8 of the terminator U tract as the position where the terminator is largely completed, we find that in the presence of adenine, run-through records unfolded beyond this point with a dwell time averaging 2.6 s (fig. S7). In the functional riboswitch, the aptamer persisted for a shorter time than it would have without any competing structure (~10 s for the adenine-bound aptamer) (fig. S7B), which implies that some mechanism—for example, branch migration—may facilitate conversion between states

(5, 6). The unfolding aptamer gave way to the terminator conformation instantaneously on the time scale of our apparatus (~50 ms), and the RNA remained thereafter in this equilibrium state (fig. S6).

Using data collected over a range of forces in the presence and absence of adenine, we can construct a map of cotranscriptional folding (Fig. 4 and table S2). P2 is the first significant structure to fold, sequestering  $21 \pm 1$  nt (as expected, based on sequence) once RNAP reached a position  $12 \pm 1$  nt after the last nucleotide of P2 was transcribed, which agrees closely with the ~12-nt RNA footprint estimated for RNAP (23, 24). The aptamer region is transcribed by nt 74, and it folded, on average, when RNAP reached nt  $90 \pm 1$ , soon after the RNAP footprint cleared the aptamer. At low forces, if the aptamer failed to fold, the terminator hairpin formed later in transcription, once a segment of its complementary RNA hairpin sequence became available. Termination subsequently occurred at nt  $112 \pm 1$ , around U7 of the U tract, which is typical for intrinsic terminators (25). Notably, at higher forces for records that terminated, the terminator hairpin folded at nt  $110 \pm 1$ , whereas for records that ran through, the terminator folded later, at nt  $117 \pm 1$  (after transcription of the downstream U tract), which supported the notion that the energy of hairpin formation assists in releasing the RNA. For loads close to the unfolding force of the terminator, records showed no evidence of cotranscriptional hairpin folding, and the vast majority ran through. The few records that terminated appeared to do so at the “slippery” U tract (21). In all records that ran through, RNAP arrested at nt  $134 \pm 1$ , a position ~15 nt upstream of the roadblock, consistent with the downstream footprint of RNAP (26, 27).

These experiments provide a real-time window into transcript formation and functional switching in a riboswitch. Cotranscriptional folding is fundamental to the action of the *pbuE* adenine riboswitch, which exerts its modulatory effects far from equilibrium. Once the aptamer sequence is formed, the adenine-bound aptamer is able to briefly lock down the riboswitch conformation until RNAP has successfully passed the termination signal. Conversely, if the ligand-bound aptamer fails to become stabilized by the time RNAP reaches the decision point, the terminator hairpin forms instead, which leads to prompt transcript release at the terminator U tract. Cotranscriptional folding events follow a sequential progression, with associated structures forming within seconds (or less) of the times that their sequences clear the RNAP footprint. Nascent transcript structures that get trapped out of equilibrium are later found to switch conformations nearly instantly. The sensitivity of this system to kinetic details therefore poses a serious challenge in the quest for synthetic mimics of riboswitches in a bioengineering context. The experimental approaches presented here provide the opportunity to explore the dynamics of cotranscriptional folding directly in a variety of RNA structures. We anticipate that

force spectroscopy will become a useful tool in the study of cotranscriptional events and, possibly, cotranslational events as well.

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## Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6105/397/DC1  
Materials and Methods  
Figs. S1 to S7  
Tables S1 and S2  
References (28–34)

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## Stability in the Face of Change

Biopharmas that have fared well despite global economic turmoil have done so by making smart acquisitions, paying attention to global opportunities, investing in R&D, looking beyond the bottom line—and valuing and respecting the scientists who work for them, according to this year's *Science* Careers Top Employers Survey.

By Anne Harding

## Annual Top Employers Survey



Is it possible to have a successful career in science, and have a life too?

Yes, if you're working for the right company and you're ready to ask for what you need, says **Lori Morton**, associate director of cardiovascular research at Regeneron Pharmaceuticals, Inc., which is #1 on the 2012 *Science* Careers Top Employers Survey—after making the list for the first time ever, at #2, last year. “I really believe that having an engaged parenthood and career success are not incompatible ideas here,” says Morton, who has worked at the Tarrytown, New York-based biotech for 10 years and has two young children.

Despite continued gloom on the global economic front, Regeneron, Novo Nordisk (up to #4 from #9 last year), Celgene (#12, first time in the top 20), and many more of the companies that made this year's list are continuing to hire scientists and invest in research and development. Not only that: In an environment where layoffs, benefit cuts, and hiring freezes still seem to be the rule, these well-respected companies are focused not just on getting the most out of their employees, but on helping them live balanced, healthy lives. These companies have recognized that these two things are far from mutually exclusive.

“We of course focus on innovation and the development of new products, and therefore we focus very much on people,” says **Mario Watzke**, head of human resources marketing and employer branding at Roche (excluding Genentech), which moved up to #8 on the list this year from #15, making the top 10 list for the first time. (Genentech, a member of the Roche group, is #3 this year). Roche's concern for its employees shows itself in benefits ranging from an Olympic-size swimming pool for employee use at the company's Basel headquarters to flexible schedules, on-site kindergartens, a babysitting service, and a nanny finder. Other unusual benefits offered by this year's top 20 employers include health insurance for pets (Novo Nordisk); in-office massage and visits from the ice-cream truck (Regeneron); deliveries of farm-fresh vegetables to the office (Novartis Institute for Biomedical Research, the research and development division of Novartis, #11); and annual health checks for all employees (Biocon Limited, #19).

### WHAT MAKES A TOP EMPLOYER

Every year, *Science* commissions a survey to identify the biotech and pharma companies considered to be the top employers in the industry, and also to determine which qualities scientists use to make

this judgment. This year's results are based on 4,276 responses to a web-based survey (see chart, page 404, for a description of survey methodologies).

Two companies, Monsanto Company (from #16 to #5) and Roche (from #15 to #8), made the top 10 list for the first time this year, while Biocon is a newcomer to the top 20. Vertex Pharmaceuticals Incorporated (#2), Genentech (#3), Millennium: The Takeda Oncology Company (#6), Boehringer Ingelheim (#7), Biogen Idec (#9), and DuPont (#10) round out the top 10. (See chart for a full list of the top 20.)

### INNOVATION IN EVERYTHING

“Innovative leader” doesn't just describe the research the top 20 companies do or the products they make; it also characterizes a company's approach to recruiting and retaining the best talent, their strategies for growth, and their corporate culture.

It can even mean becoming a player in social media, like Boehringer Ingelheim, which has won recognition for its Facebook-based Health-Seeker social game, which helps people with diabetes learn how to make healthy lifestyle changes with their Facebook friends' support.

Novartis Institutes for Biomedical Research (NIBR), which currently employs more than 6,500 scientists in over 10 different locations across the globe, got its start a decade ago when the Basel-based pharma giant decided to headquarter its research and development operations in Cambridge, Massachusetts—a bold and innovative move in and of itself. And NIBR's mission is also unique, explains **Mark Sawyer**, global head of human resources for NIBR. “It's not the pursuit of the blockbuster; it's simply pursuing unmet medical needs, and not being driven by the market.”

Project teams are built around key scientific questions, Sawyer adds, not market opportunities, and NIBR works hard to foster collaboration and keep the organization as flat as possible. And it works: Only 2 percent of hires leave the company within their first 12 months on the job, giving NIBR the lowest turnover of any division within Novartis. As of 2011, the company had more [continued>](#)

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European Regional Focus—November 9

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## Annual Top Employers Survey

### Top Twenty Employers

2012 Rank 2011 Rank Employer (Global Headquarters)

|    |    |                                                         |   |   |   |   |   |
|----|----|---------------------------------------------------------|---|---|---|---|---|
| 1  | 2  | Regeneron Pharmaceuticals, Inc. (Tarrytown, NY)         | • |   |   | • | • |
| 2  | 1  | Vertex Pharmaceuticals Incorporated (Cambridge, MA)     | • |   | • | • |   |
| 3  | 3  | Genentech (South San Francisco, CA)                     | • |   | • | • |   |
| 4  | 9  | Novo Nordisk (Bagsvaerd, Denmark)                       |   | • | • | • |   |
| 5  | 16 | Monsanto Company (Creve Coeur, MO)                      | • |   |   | • | • |
| 6  | 5  | Millennium: The Takeda Oncology Company (Cambridge, MA) |   | • |   | • | • |
| 7  | 7  | Boehringer Ingelheim (Ingelheim, Germany)               |   |   | • | • | • |
| 8  | 15 | Roche – excluding Genentech (Basel, Switzerland)        | • |   | • |   | • |
| 9  | 10 | Biogen Idec (Weston, MA)                                | • |   |   | • | • |
| 10 | 4  | DuPont (Wilmington, DE)                                 |   | • |   | • | • |
| 11 | 14 | Novartis (Basel, Switzerland)                           | • |   | • |   | • |
| 12 | –  | Celgene (Summit, NJ)                                    |   | • |   |   | • |
| 13 | 6  | Amgen (Thousand Oaks, CA)                               |   | • | • |   | • |
| 14 | 12 | Merck KGaA/Merck Serono/EMD Serono (Darmstadt, Germany) | • | • | • |   |   |
| 15 | 11 | Abbott (Abbott Park, IL)                                |   |   | • | • | • |
| 16 | 13 | Genzyme, a Sanofi company (Cambridge, MA)               |   | • | • |   | • |
| 17 | 8  | Syngenta (Basel, Switzerland)                           |   | • | • |   | • |
| 18 | 18 | Gilead Sciences (Foster City, CA)                       | • | • |   |   | • |
| 19 | –  | Biocon Limited (Bengaluru, India)                       |   | • |   | • | • |
| 20 | 20 | Bayer (Leverkusen, Germany)                             |   | • | • |   | • |

Innovative leader in the industry  
Treats employees with respect  
Is socially responsible  
Has loyal employees  
Does important, quality research  
Makes changes needed



### Survey Methodology

Science Careers conducted its annual web-based survey of individuals familiar with pharmaceutical and biotech employers to determine the best employers in the field. This survey was conducted from March 7 to March 21, 2012. Roughly 23,000 individuals from the AAAS database, as well as former survey participants, were invited via e-mail messages to take this survey. Individuals working in human resources at biotech and pharma companies (Science Careers sales database) were also contacted by e-mail and asked to promote the survey within their organization. Lastly, banner ads and e-mail blasts were run with two outside providers.

In all, 4,276 surveys were submitted, which served as the basis for the analysis. The top 20 companies were selected using a statistical process that calculates a unique ranking score for each company rated. Only companies that were rated by 30 or more respondents were eligible to become part of the top 20 best employers.

The 20 companies with the best reputations as employers and the top three driving characteristics for each company, according to respondents in the 2012 survey undertaken for the Science/AAAS Custom Publishing Office. The companies without a 2011 rank did not receive enough mentions to qualify or did not receive a high enough ranking during the 2011 survey.

than 130 projects in clinical development, and is well-prepared for the expiration of the patent on its blockbuster drug Diovan in 2013, according to Sawyer. “Nearly 30 percent of revenue is now gleaned from recently launched products, and we’re seeing significant growth in emerging markets.”

### SUPPORTING HARD WORK, FAMILY LIFE

Since its founding, Regeneron leadership has focused on making the company a science-driven blend of academia and industry. “We give our scientists much greater freedom to go where the science takes them to do innovative things; we’re not constantly hounding them about expenses and minor administrative details,” says **Ross Grossman**, Regeneron’s vice president of human resources. Among the most innovative benefits the company offers to employees, he adds, is plenty of support for their lives outside the company. “Because we demand so much of our people, we offer highly subsidized in-home or in-facility child care and adult day care, so that if something happens and your child can’t go to school or you have a problem with your aged parent, we can help.”

Leonard S. Schleifer, a neurologist and assistant professor at Columbia University, founded Regeneron in 1988 with the goal of

using gene technology to regenerate neurons. The following year, **George Yancopoulos**, then a molecular immunologist also at Columbia and now the company’s executive vice president and chief scientific officer, opened Regeneron’s labs in Tarrytown. Schleifer continues to lead the company as president and chief executive officer.

In 2008, the Food and Drug Administration (FDA) approved the company’s first drug, the interleukin-1 inhibitor Arcalyst (rilonacept), for treating cryopyrin-associated periodic syndromes, which are extremely rare diseases. In 2011, the FDA approved Regeneron’s Eylea (aflibercept), an antibody fragment that helps maintain vision in patients with wet age-related macular degeneration. The drug is expected to reach hundreds of millions of dollars in sales annually. Both drugs were created with Regeneron’s Trap technology for making “decoy receptors.”

The company now has an array of fully human antibodies in its pipeline created with its proprietary VelocImmune mouse, which has been engineered to express human antibody genes while still mounting a robust immune response by making antibodies with fully human variable regions and mouse constant regions. “This is by orders of magnitude the largest genetic engineering project **continued**”





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agricultural  
sustainability  
through our innovations\*

A photograph of three female scientists in white lab coats and safety glasses working in a greenhouse. They are looking at a clipboard. In the foreground, there are rows of green plants in a hydroponic system.

**syngenta**

**\*Our work matters**

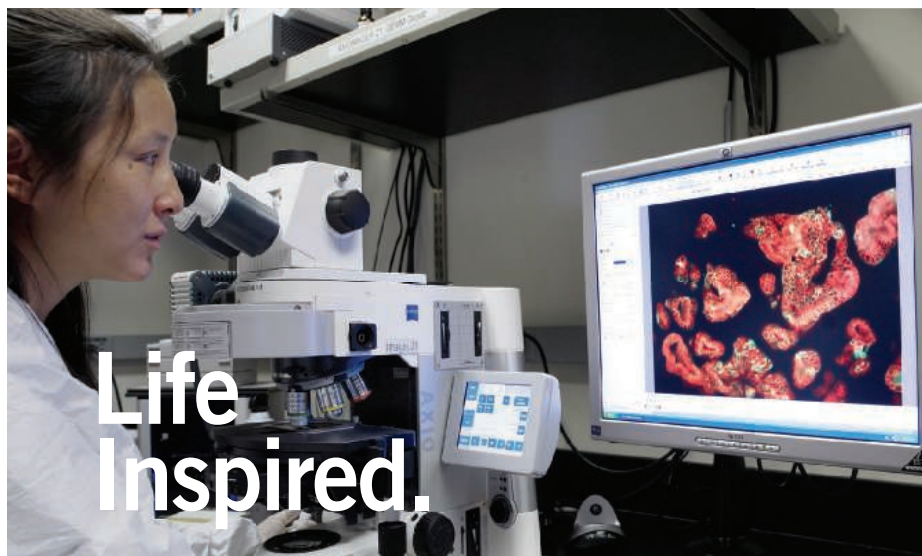
R&D is at the heart of Syngenta which is in a strong growth phase. We are looking to recruit scientists from a broad variety of scientific disciplines including agronomy, biology, chemistry, biochemistry, breeding and genetics. Syngenta is also proud to be ranked among the Top Employers according to Science's 2012 Top Biotech and Pharma Employers survey.

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Brenda, Patient

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## Annual Top Employers Survey

## SURVEY DEMOGRAPHICS



ever undertaken by scientists, and it worked,” says Yancopoulos.

“During the first half of our existence we were criticized for being a ‘research boutique,’” explains Yancopoulos. “They said, ‘Oh, you’re doing great science, but the company doesn’t know how to produce products.’” At the same time, he added, many in the industry were so heavily focused on products that they were cutting in-house research and development and licensing in new drugs.

Now, while the drying up of pipelines has become a common lament for the industry, Yancopoulos adds that Regeneron has in development more than 10 therapeutics in the areas of inflammation, metabolism, oncology, ophthalmology, and pain. And thanks to the VelocImmune mouse—which has brought in \$2 billion in licensing and collaboration fees over the past five years alone, according to Yancopoulos—these therapeutics are being developed at record speed. This means, he says, that the scientists get to see the results of their work in real time.

“Many of us grow up with the dream that we’re going to be able to do important science, to help and advance humankind in some way,” Yancopoulos says. “We haven’t just said it and dreamt it, but we’ve been doing it, over and over again.”

### THINKING, AND ACTING, GLOBALLY

Bangalore-based integrated biopharmaceutical company Biocon made the top 20 list for the first time this year, in the #19 spot. Syngene, Biocon’s contract research arm, and Clinigene, its clinical development branch, have long collaborated successfully with companies overseas, but Biocon has become an R&D power in its own right, with its own global reach. The company, founded in 1978 by Kiran Mazumdar-Shaw, began setting up its first overseas manufacturing facility in Malaysia in 2011, and recently launched the world’s first humanized anti-epidermal growth factor receptor (EGFR) monoclonal antibody, nimotuzumab, which is also the first novel biologic to be developed in India.

Biocon’s story underscores the increasingly global nature of the biopharma industry—and the importance of India, China, and other emerging markets not just as testing grounds and markets for drugs, but as resources for scientific and commercial collaboration. In April, the company opened a state-of-the-art, 200,000-square-foot biologics R&D complex that employs more than 300 scientists and was inaugurated by Chemistry Nobel Laureate Kurt Wuthrich.

A host of other deals between leading biopharma companies and their counterparts in the developing world have taken place over the past few months. Amgen (#13 this year, down from #6 in 2011) purchased Turkey’s Mustafa Nevzat Pharmaceuticals, a maker of injectable generic drugs, for \$700 million. Merck KGaA (which includes Merck Serono/EMD Serono and is #14 this year) announced it would invest \$1.5 billion in R&D in China over the next five years, and Novartis (back up to #11 after being #14 in 2011 and #11 in 2010) has cut jobs in the United States and Europe while adding employees in China and India.

**Don Foster**, head of research in immunology at Novo Nordisk’s R&D center in Seattle, says working with colleagues across cultures—and continents—is a very effective way to get scientists thinking outside of the box. “In research and development, especially in research, getting diversity of thought is one of the most critical things that you can do to get innovation,” he explains. Scientists at the company are encouraged to shuttle among the company’s R&D sites in Denmark, the United States, and China, by doing job rotations overseas, taking expat assignments, and going on extended business trips, according to **Rebecca Capuano**, the company’s senior director of human resources.

“Diversity is so important to us, and this gift of being in so many sites around the world enables the diversity,” says NIBR’s Sawyer. The company’s “mini-sabbaticals,” which offer NIBR associates the opportunity to work at other sites for up to three months, have been “extremely valuable and popular,” he adds.

Africa has also emerged as an important partner for the three agricultural chemical producers in the top 20. All have made major commitments to expand their business on the continent. DuPont (#10) announced it would invest \$3 million over the next three years to help Ethiopian smallholder farmers achieve food security. In July of this year, the company opened a new office in Lagos, Nigeria, to serve as the hub for its operations in West Africa. Monsanto has said it plans to spend \$50 million over the next 10 years in Africa. Syngenta (#17), the world’s largest agrochemical company, said it will invest \$500 million in Africa, from which it expects to reap \$1 billion in revenues over the next decade.

### MAKING A DIFFERENCE

The responses to this year’s survey make it clear that helping patients, doing important science, and maybe even changing the world are important to scientists working in biopharma. When asked about the advantages of working in the industry, “positively impacting lives” came first, followed by “fulfilling careers,” “stability,” and “salary/benefits.”

And the companies on this year’s top 20 list make it clear that scientists working in the industry can indeed “do well by doing good.”

Celgene, a Summit, New Jersey-based company and **continued**>

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## Annual Top Employers Survey

## Driving Characteristics of Top Employers

\* Driving characteristics are listed in descending order of impact on overall employer rankings.

## 2012

1. Innovative leader in the industry
2. Treats employees with respect
3. Socially responsible
4. Loyal employees
5. Does important, quality research
6. Makes changes needed

## 2011

1. Innovative leader in the industry
2. Treats employees with respect
3. Socially responsible
4. Loyal employees
5. Makes changes needed
6. Does important, quality research

Numbers  
5 & 6  
reversed  
order in  
2012.

a top 20 newcomer, was spun off by Celanese Corporation in 1986. "In the early part of our history we developed our focus on orphan diseases," explains **Greg Geissman**, director of public relations at the global biopharmaceutical company.

The company in-licensed thalidomide in 1992 and received FDA approval to market the drug (as Thalomid) for treating severe cutaneous manifestations of leprosy in 1998 and for treating multiple myeloma in combination with dexamethasone in 2006. Over its history, Celgene has made a number of diverse acquisitions to expand its portfolio of cancer drugs, most recently purchasing Abraxane maker Abraxis Pharmaceuticals in 2010.

Celgene invests an unusually large 30 percent of its revenue back into research and development, Geissman points out. (The industry average in 2010, according to a Pharmaceutical Research and Manufacturers of America survey, was 17 percent).

"The continued significant investment shows up in the fact that currently we have more than 25 phase 3 and pivotal studies ongoing in a range of diseases," Geissman says.

Scientists at Celgene see their work as a vocation, and they also feel supported in taking constructive risks in their research, says **Carol Thompson**, senior director of human resources at the company. Both Thompson and Geissman say the company's focus on connecting scientists with patients is also unique.

"It's the only place I've worked at where employees have the opportunity to directly connect with the patients," Thompson says. "That makes what we do come to life. We've had patients actually at our town hall meetings. We've had a number of opportunities to see and hear our patients and their gratitude, and to see their hope and their encouragement for surviving that much longer because they're taking our drugs."

## PROUD TO BE "PATIENT-CENTRIC"

Novo Nordisk, the world's largest maker of insulin, is also a company that's proud of its "patient-centric" approach. "We look at everything we do from the lens of the patient," Capuano says.

In 2009, the company opened an R&D facility in Seattle dedicated to producing biologics for treating autoimmune diseases. Foster says he agreed to head up this research at the facility because he believes in what Novo Nordisk does, and how the company goes about doing it. "I've had type 1 diabetes myself for 35 years, and I've seen the impact that innovation can have on the quality of life of people

with a chronic disease," he says. "We can manufacture the missing component and provide a relatively normal life and normal life span for people with fatal diseases."

"I'm a big believer in biologics," Foster adds. "They have huge specificity advantages over small molecules. Biologics are fundamentally nature's way of regulating disease, so we're manufacturing almost the perfect drug."

Clinical data is now coming in from one of the company's first antibodies, for treating rheumatoid arthritis. "It's encouraging us to believe that there

are therapeutics that can not only manage the pain and inflammation of diseases like that, but also potentially impact bone destruction," Foster says.

Scientists working at Novo Nordisk's Seattle facility benefit from the excitement of working in an environment that has a biotech startup feel, while at the same time enjoying the stability of being employed by a large, financially healthy organization, which just marked its 40th consecutive quarter of double-digit revenue growth. "It's thriving and it's stable, and that means the company has the ability to be not only short-term but long-term focused," says Foster.

"We're probably one of the few companies right now that are making long-term commitments in focused research and development, and are fortunate to have the ability to do that," Capuano says. At around \$1.5 billion, she points out, the company's R&D spending rivals the National Institutes of Health's funding for diabetes research.

And Novo Nordisk's financial health means it can offer some unique benefits, including a concierge, health insurance for pets, and "lunch and learn" sessions with college coaches to help parents preparing to send their kids off to university, she adds. "Although the pharmaceutical industry might, at least in the past, have gotten beat up for their extravagant ways," Capuano continues, "I think at Novo we give back to our employees and we give back to our communities."

## STABILITY, CHANGE, OR BOTH?

This year's survey respondents listed "stability," including job security and industry growth, as one of the key advantages of working in biopharma. But respondents also named "change"—mergers and acquisitions, reorganizations, outsourcing, market forces, employees moving between companies, and decreasing innovation—as the main disadvantage of employment in the industry, followed by "negative image," "workplace conditions," and "government influence."

Nevertheless, just one in five survey respondents said they were likely to look for a new job in the next 12 months. A third of those who expected to begin a job search said their goals were career advancement and professional growth; 17 percent were seeking new challenges and experiences; 13 percent wanted to leave their current job due to leadership, management, or supervision issues; 11 percent were planning to look for work because they were not happy with the work environment or culture or the stress of working for their current employer; while 11 percent wanted a **continued**>





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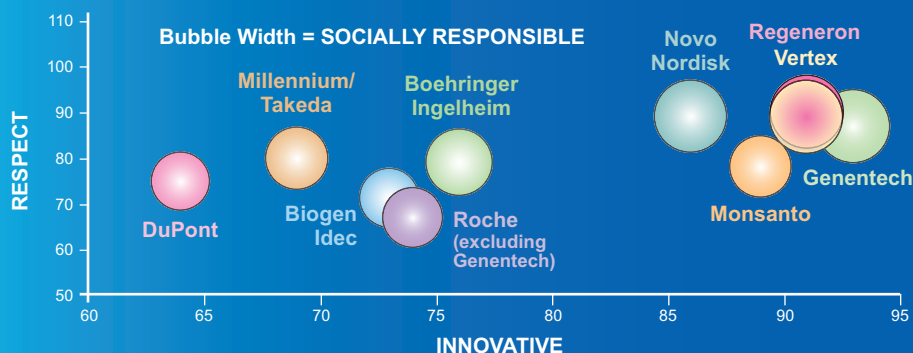
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## Annual Top Employers Survey

## COMPARISON OF TOP TEN'S TOP CHARACTERISTICS



Comparison of the top 10 companies on the basis of the top three drivers (scored out of 100): *Socially responsible* (bubble width), *Innovative leader* (x-axis), and *Treats employees with respect* (y-axis).

better salary and benefits.

Working in biopharma can definitely be a wild ride, and many scientists will find themselves involved in multiple mergers, acquisitions, and spinoffs throughout their careers (see “Navigating Biotech/Pharma Mergers and Acquisitions” article, June 8, [scim.ag/QfNmuM](http://scim.ag/QfNmuM)).

But the health of the industry means that talented, experienced scientists can expect to land another—maybe better—job after being laid off. R&D spending was essentially flat between 2007 and 2009, but has begun to climb again, according to Pharmaceutical Research and Manufacturers of America (PhRMA) data. While the number of U.S. jobs overall fell by 6 percent between 2007 and 2009, according to data from Battelle, biotech jobs were up by 0.2 percent during the same period. Big pharma employment shrank 4.8 percent, while there was a 3.6 percent increase in jobs at research, testing, and medical labs.

### LETTING EMPLOYEES FOLLOW THEIR PASSION

NIBR and several other top 20 employers take pride in allowing their scientists to create their own career paths within the company. One of the most innovative things about Monsanto, says **Kelly Franklin Brendel**, chemistry strategy and operations lead at the St. Louis-based Fortune 500 company, is the freedom the company gives its employees to follow their interests and passions—even if that means going into a totally different division. And if an employee wants to stay where they are and become a “super expert” in that area, their wishes will be honored as well, she says.

Brendel started at Monsanto as an engineer in manufacturing 20 years ago. “It does not feel like I’ve been around that long,” she adds. “It says a lot about the opportunities the company provides when you can feel renewed all the time.”

“Our culture is one that ensures people have multiple development opportunities,” says **Melissa Harper**, vice president of talent acquisition and diversity lead at Monsanto. “We certainly, in our culture, recognize that our competitive advantage relies on our ability to attract great talent, but at the same time develop and retain that talent.”

people who are fitting into the working culture, and that’s the most important thing...they have to fit into the team, they have to like it.” So the company works hard to show potential recruits just what that working culture is all about—and employees are happy to share this information too. Of their own accord, several scientists at the Basel site put together a video showing themselves talking about what it’s like to work at the company, Watzke adds.

The social factor is important for Regeneron, as well. “Being the best or being the most accomplished or the most brilliant is not enough here,” Morton says. “To be a Regeneron person you also have to have personality. Personality and charisma are highly valued here. Because in addition to wanting everyone to have a healthy balance of their personal life and their work, they want to work to be a pleasant place to be, so I think there’s really a strong effort to recruit people who are nice to be around.”

And while Regeneron obviously values hard work, it doesn’t demand face time. “We really encourage people to be as efficient with their time as possible, because it doesn’t help anybody to work endless hours. It’s not healthy. I don’t think anyone who has that work ethic is really working efficiently.”

While Morton says she certainly works hard—typically logging a “reasonable nine-hour day”—she feels comfortable taking the time to call her child’s school, make a doctor’s appointment, or, as she did this spring, leave early to go to her daughter’s preschool graduation.

“The key to that is feeling comfortable, is saying that this is what I need and this is why I’m leaving early, to not be vague about it and say ‘I have an appointment,’ but to feel comfortable saying ‘I’m going to my daughter’s preschool graduation,’ and have them say ‘Great, bring pictures back,’” Morton says. “That is the attitude and the culture, and that’s what I think makes Regeneron such a healthy place to be.”

*Anne Harding is a freelance science writer based near New York City.*

To read the extended content, please see the online version at [www.sciencecareers.org/TopEmployers2012](http://www.sciencecareers.org/TopEmployers2012)

DOI: 10.1126/science.opms.r1200125

A cluster of red blood cells, appearing as bright red, biconcave discs, is positioned on the left side of the page, partially overlapping the main title text.

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Red blood cells. David Gregory and Debbie Marshall/Wellcome Images



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**Profit from excellent conditions for research**

**The Alexander von Humboldt Professorships are Germany's way of creating a beacon effect and energising its research landscape. Every year, the Alexander von Humboldt Foundation is offering ten of the world's leading researchers up to five million EUR each to create new or consolidate existing internationally visible research focus areas at German universities.**

Academics of all disciplines are eligible for an Alexander von Humboldt Professorship, provided that they are established abroad and recognised internationally as top-class researchers. They will be nominated by German universities – where appropriate in cooperation with non-university research institutions. Each Alexander von Humboldt Professorship will be sponsored for a period of five years on the premise that the university presents a convincing strategy to sustain the position once the funding period has come to an end.

This will allow new, long-term research groups to be established, conducting cutting-edge international research. The German Ministry of Education and Research is supporting this programme.

The Humboldt Foundation actively promotes equal opportunities and therefore particularly welcomes nominations on behalf of leading female academics.

More information: [www.humboldt-foundation.de/ahp-en](http://www.humboldt-foundation.de/ahp-en).

**Closing dates for applications: 15 April and 15 October.**

**Exzellenz verbindet –  
be part of a worldwide network.**



Alexander von Humboldt Foundation  
Jean-Paul-Str. 12  
53173 Bonn  
Germany  
[info@avh.de](mailto:info@avh.de)

**[www.humboldt-foundation.de](http://www.humboldt-foundation.de)**



## Jefferson Science Fellowship



The National Academies is pleased to announce a call for nominations and applications for the 2013 Jefferson Science Fellows program. Initiated by the Secretary of State in 2003, this fellowship program engages the American academic science, technology, engineering and medical communities in the design and implementation of U.S. foreign policy.

Jefferson Science Fellows (JSF) spend one year at the U.S. Department of State or the U.S. Agency for International Development (USAID) for an on-site assignment in Washington, D.C. that may also involve extended stays at U.S. foreign embassies and/or missions.

The fellowship is open to tenured, or similarly ranked, academic scientists, engineers and physicians from U.S. institutions of higher learning. Nominees/applicants must hold U.S. citizenship and will be required to obtain a security clearance.

The deadline for 2013-2014 program year applications/nominations is **January 14, 2013**. To learn more about the Jefferson Science Fellowship and to apply, visit the JSF web at:

[www.nas.edu/jsf](http://www.nas.edu/jsf)

**THE NATIONAL ACADEMIES**  
Advisers to the Nation on Science, Engineering, and Medicine

## UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

### Faculty Positions in Virology

The Department of Microbiology is expanding its existing diverse virology research program as part of the UAB strategic plan by inviting applications for up to four tenure track/tenured junior or senior faculty positions in virology. Successful candidates will have demonstrated records of originality and productivity in research, and concerned interest in graduate and medical education. UAB Microbiology is a national leader in NIH research funding and offers a uniquely interactive research environment. Collaborations among basic science disciplines and between basic and clinical faculty are stimulated by multidisciplinary centers at UAB: Center for AIDS Research, Comprehensive Cancer Center, Center for Structural Biology, Center for Clinical and Translational Sciences, Center for Emerging Infections and Emergency Preparedness, Liver Center, and a campus-wide Program in Immunology. State-of-the-art BSL3/ABSL3 containment facilities are available. UAB is one of the top clinical and research institutions in the nation, located in a beautiful, livable, and affordable city with many cultural and outdoor attractions.

Applicants may provide expertise in new, complementing or existing areas (<http://www.microbio.uab.edu/UABvirology>). Investigators in the areas of viral pathogenesis, HIV, viral latency, HCV, viral effects on microbiome or metabolome, and select agent research are encouraged to apply.

Review of applications will continue through January 15, 2013. Applicants should invite three individuals who are familiar with their work and potential for success to send letters directly via email. Applicants can submit their CV, a 2-4 page summary of their research accomplishments and future plans, and the names and contact information of three references as a single pdf file to: **Frances Lund, Ph.D., c/o Kristina Sinclair, email: [ksinc@uab.edu](mailto:ksinc@uab.edu)**. A pre-employment background investigation is performed on candidates for employment.

*UAB is an Equal Opportunity/Affirmative Action Employer committed to fostering a diverse, equitable and family-friendly environment in which all faculty and staff can excel and achieve work/life balance irrespective of ethnicity, gender, faith, gender identity and expression as well as sexual orientation. UAB also encourages applications from individuals with disabilities and veterans.*

## GRADUATE PROGRAM



The Leipzig School of  
Human Origins

### The Leipzig School of Human Origins

- An International Max Planck Research School -

by

The University of Leipzig

and

The Max Planck Institute for Evolutionary Anthropology

The Leipzig School of Human Origins offers a unique interdisciplinary graduate program to study the evolutionary history of humans and great apes. Graduate students are accepted into one of the following areas, but are encouraged to take part in courses and seminars from all three disciplines:

**Comparative and Molecular Primatology**

**Evolutionary and Functional Genomics, Ancient DNA,  
Molecular Anthropology and Genome Bioinformatics**

**Human Paleontology, Prehistoric Archaeology  
and Archaeological Science**

The language of the school is English. Visit [www.leipzig.de](http://www.leipzig.de) for information on living in Leipzig, Germany, in the center of Europe.

For project and application details go to [www.leipzig-school.eva.mpg.de](http://www.leipzig-school.eva.mpg.de) or contact us at:

e-mail: [leipzig-school@eva.mpg.de](mailto:leipzig-school@eva.mpg.de)  
phone: ++49 (0) 341 3550-0  
fax ++49 (0) 341 3550-119

**Application deadline: January 31, 2013**



## NORTHWESTERN UNIVERSITY

Northwestern University invites exceptional candidates to apply for the position of **ASSOCIATE DIRECTOR** of The Center for Early Cancer Detection Technologies, which is being formed by the University.

The mission of the Center is to develop novel optics technologies for the characterization and imaging of biological tissue. The focus is on the nanoscale, microscale and molecular levels. The Center also utilizes biophotonics to gain new insights into biological systems and their response to disease. Furthermore, the Center aims to translate these technological and biological innovations into clinical practice.

The Associate Director will work in close collaboration with the Center Director, Professor Vadim Backman. S/he will play a leadership role in the Center overseeing administration and ongoing research activities. In particular, the candidate will be responsible for identifying new research targets and help define the Center's strategic development. The candidate is expected to help secure new research funding for the Center. The candidate will manage scientists involved in Center projects, provide creative scientific input and coordinate the overall execution of projects. The candidate will build relationships and coordinate external collaborations with academia and industry.

The successful candidate will have a Ph.D. and 4+ years of experience in biophotonics or a related field. The candidate will have demonstrated an outstanding record of research accomplishment and leadership documented by peer-reviewed publications and external reputation. The candidate is expected to have excellent interpersonal and writing skills.

Candidates should submit their Curriculum Vitae and the names of four referees by e-mail to the following address: **[b-keane@northwestern.edu](mailto:b-keane@northwestern.edu)**. Questions of a scientific nature should be addressed to Professor Vadim Backman at **[v-backman@northwestern.edu](mailto:v-backman@northwestern.edu)**. Applications will be accepted until the position is filled.



## Call for International Research Center Proposals

Skolkovo Tech invites collaborative proposals from research and educational institutions around the world in the following areas:

- ▶ Biomedicine
- ▶ Energy
- ▶ Information Technology
- ▶ Nuclear
- ▶ Space

Skolkovo Tech is a private graduate research university located outside Moscow, Russia. Established in 2011 in collaboration with MIT and the Skolkovo Foundation, the university is founding 15 multidisciplinary Centers for Research, Education and Innovation (CREI) to address critical scientific and technology challenges.

With three centers in negotiations, the Second Round Call for Proposals offers an opportunity to partner with Russian investigators in a world-class research program.

Visit [www.skolkovotech.ru/research](http://www.skolkovotech.ru/research) for details.

**30 November 2012 deadline**

**\$6-12M USD annually, up to five years**

**SkTech/MIT**  
INITIATIVE

Skolkovo Tech is currently hiring tenure-track and tenured faculty. Details available at: [www.skolkovotech.ru/faculty](http://www.skolkovotech.ru/faculty)

World-class research in Russia

Education

Innovation



# WAYNE STATE UNIVERSITY

## Tenured/Tenure Track Faculty Positions in Biological Sciences

The Department of Biological Sciences at Wayne State University (<http://www.clas.wayne.edu/biology/>) anticipates hiring two tenure-track faculty at the full, associate or assistant professor levels. One position is in **microbiology**. Areas of interest include, but are not limited to, bacteriology, virology, immunology, host-pathogen interactions, environmental microbiology and infectious disease processes. The second position is in **evolutionary biology or population genetics**. Preference will be given to candidates working in areas complementing the department's existing strengths in transcription and gene regulation, organismal and evolutionary development, intra- and intercellular signaling, genomics, and community and landscape ecology.

Wayne State University is a large, comprehensive, nationally ranked research institution that offers state-of-the-art research facilities and highly competitive start-up packages. The metropolitan Detroit area offers a rich cultural and educational environment, an excellent standard of living, and easy proximity to Michigan's lakes, forests and recreational sites. Applicants must have a Ph.D. degree, postdoctoral experience and an outstanding record of research achievement. Successful applicants are expected to establish and maintain vigorous, externally funded research programs and to participate in graduate and undergraduate education. Please apply on-line at [jobs.wayne.edu](http://jobs.wayne.edu). In addition to the online application that includes cover letter and curriculum vitae, applicants must submit a 2-page statement of their research plans and have three letters of reference sent directly to the Faculty Search Committee: [ad5348@wayne.edu](mailto:ad5348@wayne.edu). Please apply by **November 15, 2012** for full consideration. Applications will be considered only when all materials have been received.

*Wayne State University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are especially encouraged to apply.*

# Assistant Professor

## Department of Biological Chemistry and Molecular Pharmacology Harvard Medical School

The Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School invites applicants for a tenure-track faculty position at the rank of Assistant Professor. We are seeking individuals with a demonstrated potential for imaginative research and who propose to work on exciting problems in any area of chemical, structural, and/or mechanistic biology. The successful candidate will be expected to direct innovative and independent research and participate in the teaching of graduate and/or medical students. Our highly interactive environment provides the opportunity to engage and collaborate with other dedicated researchers both within the Department and throughout the diverse Harvard research community. Significant scholarly and scientific resources will be made available for this appointment. For further information about our Department, please see our Web Page: <http://bcmp.med.harvard.edu>.

Applicants should submit electronic copies of their curriculum vitae, a description of research accomplishments and future research interests (three pages maximum), and ask at least three references to provide letters of recommendation. These materials should be submitted using the following link: <https://academicpositions.harvard.edu/postings/4340>.

Applications will be considered starting December 1, 2012.

Harvard Medical School is an Equal Opportunity/Affirmative Action employer. We are actively committed to increasing the diversity of our faculty. Women and members of underrepresented minority groups are therefore strongly encouraged to apply.



**HARVARD  
MEDICAL SCHOOL**

**Duke**

NICHOLAS SCHOOL OF THE  
ENVIRONMENT



## MARY DERRICKSON McCURDY VISITING SCHOLAR AT THE DUKE UNIVERSITY MARINE LABORATORY, BEAUFORT, NC

Recharge while on sabbatical at the Duke Marine Laboratory. The Nicholas School of the Environment at Duke University invites you to apply to become the **Mary Derrickson McCurdy Visiting Scholar** resident at the Marine Laboratory in Beaufort, on the North Carolina coast. The McCurdy Scholar will engage in the intellectual life of the Marine Laboratory, including research, teaching, and mentoring in an intimate world-class multidisciplinary research and teaching environment. Our ideal candidate for the position is a gregarious natural or social science scholar in the field of Ocean Science, broadly construed. We strive to understand environmental processes and human behavior broadly framed (e.g., social-ecological systems, human dimensions of natural resource management, human-environment interactions). We are particularly interested in individuals with insights or new perspectives on conservation and enhancement of the environment and its natural resources and especially with clear or potential applications to society. The McCurdy Scholar carries an appointment as Visiting Research Professor appropriate to the rank of the successful applicant. The term of the appointment is for one or two semesters (preferably the nine-month academic year), with the possibility of expansion to one full year. The appointment includes funds that may be used to augment salary, offset living expenses and enable research.

Interested individuals should send curriculum vitae, summary of research interests, reprints of three recent papers and names of three references to the search chair. Electronic submission is best, but the search chair will accept hard copies. Send to: **Dan Rittschof, Chair, Search Committee, McCurdy Visiting Scholar, 135 Duke Marine Lab Road, Beaufort, NC 28516-9721; [Ritt@duke.edu](mailto:Ritt@duke.edu)**. The search committee will begin reviewing applications on **December 15, 2012**. The search will remain open until the position is filled.

*Duke University is an Affirmative Action/ Equal Opportunity Employer.*



UNIVERSITY OF  
NOTRE DAME  
College of Science

## Tenure-Track Faculty Positions in Stem Cell Biology and Rare Diseases

**Stem Cells.** We invite applications for three endowed junior positions in stem and iPS cell biology with interests consistent with one or more broad areas in our initiative in adult stem cell and iPS cell biology, including, but not limited to: developmental and regenerative biology, function and regulation of the stem cell niche, mechanisms underlying cellular dedifferentiation and reprogramming, regulation of gene expression in stem cells and reprogrammed iPS cells, tissue engineering, and stem cells in tumor formation and progression.

**Apply by November 15.**

**Rare Diseases.** We invite applications for an endowed junior position in rare diseases. We seek outstanding faculty candidates who use integrative and innovative approaches in the study of any rare disease, but we particularly encourage applications in areas of immune dysfunction and neurological diseases.

**Apply by December 15.**

[science.nd.edu/faculty/jobs](http://science.nd.edu/faculty/jobs)

The University of Notre Dame, an international Catholic research university, is an equal opportunity employer.

## Fellowships for Postdoctoral Scholars

AT WOODS HOLE OCEANOGRAPHIC INSTITUTION

New or recent doctoral recipients with research interests associated with the following are encouraged to submit scholarship applications prior to Jan. 5, 2013.

**Departments** - Awards related to the following areas are anticipated: Applied Ocean Physics & Engineering; Biology; Geology & Geophysics; Marine Chemistry & Geochemistry; Physical Oceanography

**Institutes** - Each Institute fosters interdisciplinary research addressing critical issues, and will award a scholarship to support related research: Ocean and Climate Change Institute; Coastal Ocean Institute; Deep Ocean Exploration Institute; Ocean Life Institute

**The NOAA-WHOI Cooperative Institute for the North Atlantic Region** will award a Fellowship in one of five theme areas: Ecosystem Forecasting, Ecosystem Monitoring, Ecosystem Management, Protection and Restoration of Resources, Sustained Ocean Observations and Climate Research.

**The National Ocean Sciences Accelerator Mass Spectrometer Facility** will award a fellowship in the development and implementation of new techniques in radiocarbon studies in marine science.

Awards are competitive, with primary emphasis placed on research promise. Scholarships are 18-months with an annual stipend of \$57,000, a research budget and eligibility for health and dental insurance. Recipients are encouraged to pursue their own research interest in association with resident staff. Communication with potential WHOI advisors prior to submitting an application is encouraged.

Further information may be obtained at: <http://www.whoi.edu/postdoctoral>, or by contacting the Postdoctoral Fellowship Committee at: (508) 289-2950, or [postdoc@whoi.edu](mailto:postdoc@whoi.edu).

An Equal Opportunity/  
Affirmative Action  
Employer



## Two Tenure-Track Faculty Positions in Physiology and Neurobiology

The Department of Physiology and Neurobiology in the College of Liberal Arts and Sciences at the University of Connecticut (<http://www.pnb.uconn.edu>) invites applications for TWO tenure track positions at the assistant professor level. We encourage applications from individuals who use innovative approaches to study fundamental physiological or neural processes at the molecular, cellular, or systems level. For details on the position, qualifications, and application instructions please visit <http://www.jobs.uconn.edu>. (Search #2013024)

*The University of Connecticut is an  
EEO/AA Employer.*

# UNIVERSITY OF Nebraska Lincoln

## Cognitive Neuroscientist Center for Brain, Biology and Behavior

The Center for Brain, Biology and Behavior at the University of Nebraska-Lincoln is recruiting a cognitive neuroscientist with fMRI expertise at the Associate or Full Professor rank. The successful candidate is expected to demonstrate excellence in research including an established record of grant funding. We have preference for candidates who can create and connect to initiatives in traumatic head injury and related areas. The interdisciplinary Center engages a broad spectrum of investigators, including a unique research collaboration with University Athletics, and is housed within a new 26,000 square foot building. The facility's centerpiece is a new Skyra 3 Tesla Siemens scanner, as well as 12 high-density EEG/ERP, NIRS and TMS systems. The Skyra is integrated with a 256-electrode high-density EEG system and an eye tracker that enable simultaneous recordings. The Center is adjacent to the University's Holland Computing Center with supercomputer resources and support. The tenure home for the successful candidate will be based on a match between candidate background, preferences and departmental interest. Candidate must have an earned Ph.D. Review of applications will begin **December 2, 2012** and continue until the position is filled. To be considered for the position, please go to <http://employment.unl.edu>, requisition #120845 and complete the Faculty/Academic Administrative Form. Interested applicants should then submit a letter of application, curriculum vitae, research and teaching statement, pdfs of completed research, and letters of recommendation sent directly from three referees to: **Dr. Dennis L. Molfese, Director, The Center for Brain, Biology and Behavior, 238 Burnett Hall, University of Nebraska, Lincoln, NE 68588-0308.**

## MR Physicist/Scientist Research Assistant Professor

The University of Nebraska - Lincoln is seeking to hire a MR Physicist/Scientist at the Research Assistant professor level to oversee a new state-of-the-art fMRI facility that includes a new Skyra 3T Siemens fMRI scanner within the Center for Brain, Biology and Behavior at the University of Nebraska, Lincoln. This interdisciplinary Center engages a broad spectrum of investigators and occupies a new 26,000 sq. ft. building. In addition to the Skyra scanner, Center facilities include 12 high-density EEG/ERP and NIRS systems, genetics and endocrine labs, as well as 256-electrode high density EEG/ERP and eye tracker systems integrated with the fMRI that enable simultaneous recordings within test sessions. The Center is adjacent to the University's Holland Computing Center with supercomputer resources and support. The position will include responsibility for ensuring smooth day-to-day operation of the scanner as well as development of new pulse sequences and hardware to support neuroimaging research conducted on the scanner. The successful candidate will have a Ph.D. in physics, chemistry, biomedical engineering, electrical engineering, or a related field, with at least five years of experience in pulse sequence development, MRI hardware, image reconstruction neuroimaging, or a combination of these fields. Previous experience on Siemens scanners is preferred. The candidate will be expected to develop his or her own successful research and grant-funded programs in neuroimaging, pulse sequence development, image reconstruction, or related academic domains. There will also be opportunities to mentor graduate students and teach courses on MR Physics and other topics of interest to the candidate. In support of these goals, collaboration with Siemens and other research groups/institutions will be encouraged. Compensation is dependent on experience. The successful candidate will be affiliated with an academic department based on a match between candidate background, preferences and departmental interest. Review of applications will begin **December 2, 2012** and continue until the position is filled. The start date for this position is July 1, 2013. To be considered for the position, please go to <http://employment.unl.edu>, requisition #120842 and complete the Faculty/Academic Administrative Form. Interested applicants should then submit a letter of application, curriculum vitae, a research statement, pdfs of completed research papers, and letters of recommendation sent directly from three referees to **Dr. Dennis L. Molfese, Director, The Center for Brain, Biology and Behavior, 238 Burnett Hall, University of Nebraska, Lincoln, NE. 68588-0308.**

Lincoln, Nebraska is a vibrant college town of approximately 260,000 that combines the cultural richness of a large university with the affordability of a Midwestern city.

*The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through Affirmative Action, Equal Opportunity, work-life balance, and dual careers. Inquires should be sent to Nicole Earnest, [nearest2@unl.edu](mailto:nearest2@unl.edu).*



## ANNOUNCEMENTS



**SAO PAULO SCHOOL OF ADVANCED SCIENCE  
FOR PREVENTION OF MENTAL DISORDERS**  
March 25<sup>th</sup> to 29<sup>th</sup> 2013 • Sao Paulo/Brazil

**Y-MIND**



Important note:

For every approved student/participant the organization will cover all travelling costs for one week stay in Sao Paulo.

**“Call for action: a vanguard school of multidisciplinary studies on prevention of mental, emotional and behavioral disorders”**

### Selection of students/participants:

100 positions will be offered for graduate students and post-doctoral fellows working in the field of prevention of mental, emotional and behavioral disorders. Half (50) of these positions are reserved for applicants coming from outside of Brazil. The other half will be distributed among applicants from Sao Paulo state (25 positions) and students from other Brazilian states (25).

The applications have to be made from the 15<sup>th</sup> of October to the 15<sup>th</sup> of November in the website:

<http://www.ymind.com.br>

Additional information can be obtained in the e-mail: [ymind@ymind.com.br](mailto:ymind@ymind.com.br)



## POSITIONS OPEN



**GEISEL**  
— SCHOOL OF —  
**MEDICINE**  
AT DARTMOUTH

### Department of Pathology

The Geisel School of Medicine at Dartmouth seeks to fill a tenure-track faculty position within the Department of Pathology, with a secondary appointment to the Department of Microbiology and Immunology. The successful candidate must have an MD or PhD degree, with postdoctoral training, and will be expected to establish and sustain an independent, extramurally-funded research program in immunology, and to participate in teaching. Rank will be commensurate with experience. We seek to foster the development of a group of independently funded but collaborative researchers who will work strategically toward securing programmatic funding in the area of autoimmunity/inflammation. MD-PhD's are particularly encouraged to apply. Participation in clinical service within the Department of Pathology is not required, but is encouraged up to 20% effort. Generous start-up funds, competitive salary, modern laboratory space, and ample core facilities are available.

Applicants should submit a cover letter, a curriculum vitae and a description of research plans, and should arrange to have three letters of reference sent to:

**James D. Gorham, MD, PhD**

**Professor of Pathology and of Microbiology & Immunology**

**Department of Pathology**

**The Geisel School of Medicine at Dartmouth**

**One Medical Center Drive**

**Lebanon, New Hampshire 03756**

**[Pathology.Faculty.Search@Dartmouth.edu](mailto:Pathology.Faculty.Search@Dartmouth.edu)**

Review of applications will begin **November 1, 2012**

*The Geisel School of Medicine is an Affirmative Action,  
Equal Opportunity Employer. Women and minorities are  
encouraged to apply.*

## POSITIONS OPEN



**UNIVERSITY of MARYLAND**  
**SCHOOL of MEDICINE**

### Neurobiology Faculty Position University of Maryland School of Medicine Baltimore, Maryland

The Department of Anatomy and Neurobiology (<http://neurobiology.umaryland.edu>) is recruiting for tenured/tenure-track faculty positions in Neuroscience. We are particularly interested in candidates whose research will complement existing strengths in the Department, including: chemical senses, peptidergic circuits, sensorimotor systems, neurodegeneration and neural circuits subserving motivated behaviors and cortical functions. Candidates should have a strong record of scholarly activity and an independent funded research program that can catalyze multi-PI initiatives within the department.

We offer an outstanding intellectual and collaborative environment with highly competitive salary and recruitment packages. All department faculty are members of the Graduate Program in Life Sciences and the interdisciplinary Program in Neuroscience (<http://neuroscience.umaryland.edu>).

Candidates should submit the following as one single PDF file to [facsearch@umaryland.edu](mailto:facsearch@umaryland.edu): detailed curriculum vitae, a brief statement of research interests and goals, and names/contact information for three references. For best consideration candidates should submit their application by **February 1, 2013** and should be addressed to the attention of: **Dr. Joseph Cheer, Chair of Faculty Search Committee.**

*University of Maryland is an Equal Opportunity, Affirmative Action  
Employer. Minorities, women, veterans, and individuals with  
disabilities are encouraged to apply.*



### Basic/Translational Scientist in Head & Neck Oncology

The Division of Head & Neck Oncologic Surgery and Head and Neck Tumor Center in the Department of Otolaryngology and the Hollings Cancer Center at the Medical University of South Carolina are pleased to announce openings for junior and senior investigators with research interests and experience in models of head and neck cancer. We are seeking either physician and/or laboratory scientists with independent research programs in areas related to cancer biology that would complement and expand existing research efforts in both the department and in the Hollings Cancer Center. State-of-the-art laboratories, outstanding resources and research support are available (endowed chair available for qualified candidates).

The Hollings Cancer Center is a National Cancer Institute Designated Center and ensures a research environment that promotes and fosters basic and translational research. MUSC also holds an NIH-funded Clinical and Translational Science Award. Located on the Atlantic coast in South Carolina, Charleston boasts one of the nation's most historic downtown areas, beaches and international cultural events such as the Spoleto Festival USA.

Interested candidates (MD, PhD, or MD/PhD) should send their CV, a summary of future research plans and the names of three references via the following website [www.jobs.musc.edu](http://www.jobs.musc.edu) and apply to requisition #048704.

**Terry A. Day, M.D.**

**Director, Head and Neck Oncologic Surgery**

**Director, Head and Neck Tumor Center**

**Department of Otolaryngology/Head and Neck Surgery**

**Medical University of South Carolina**

**135 Rutledge Avenue, Box 250550**

**Charleston, SC 29425**

**843-792-0719 FAX 843-792-0546**

**[dayt@muscedu](mailto:dayt@muscedu)**

*MUSC is an Equal Opportunity Employer,  
promoting workplace diversity.*



澳門大學  
UNIVERSIDADE DE MACAU  
UNIVERSITY OF MACAU



## International Search for Academic Positions of Assistant Professor or above

The University of Macau is a leading higher educational institution in Macao and is making strides towards becoming internationally recognized for its excellence in teaching, research and service to the community. The University is growing rapidly with a number of new strategic initiatives including the relocation to a new campus and the establishment of the largest Residential College system in Asia. The new campus will be 20 times larger than the present one with a projected increase of 40% in student intake and faculty size. English is the University's working language.

We plan to develop a strong team of top-notch scholars to help us realize our vision. Applications are therefore invited from those with excellent academic achievements in the following disciplines:

- Business
- Sciences and Mathematics
- Liberal Arts and Humanities
- Engineering and Technologies
- Law
- Education
- Social Sciences
- Health Sciences

Remuneration and appointment rank offered will be competitive and commensurate with the successful applicants' academic qualification, current position and professional experience. The current local maximum income tax rate is 12% but is effectively around 5% - 7% after various discretionary exemptions.

For details about the above open positions and related information, please visit the following websites:

Job vacancy website: <http://www.umac.mo/vacancy>

University website: <http://www.umac.mo>

Macao government website: <http://www.gov.mo>

Further particulars about the job openings are available at <http://www.umac.mo/vacancy>. Kindly apply online through the E-application system. Applications will be accepted until the positions are filled. Other contact points are

### Human Resources Office

University of Macau, Av. Padre Tomás Pereira, Taipa, Macau

Website: <https://isw.umac.mo/recruitment>;

Email: [vacancy@umac.mo](mailto:vacancy@umac.mo)

Tel: +853 8397 8593 or +853 8397 8592;

Fax: +853 8397 8694

The effective position and salary index are subject to the Personnel Statute of the University of Macau in force.

The University of Macau reserves the right not to appoint a candidate. Applicants with less qualification and experience can be offered lower positions under special circumstances.

\*\*\*Personal data provided by applicants will be kept confidential and used for recruitment purpose only\*\*\*

University of Macau -  
An ideal place to pursue your career

<http://www.umac.mo>

## Innovative Engineering for Health

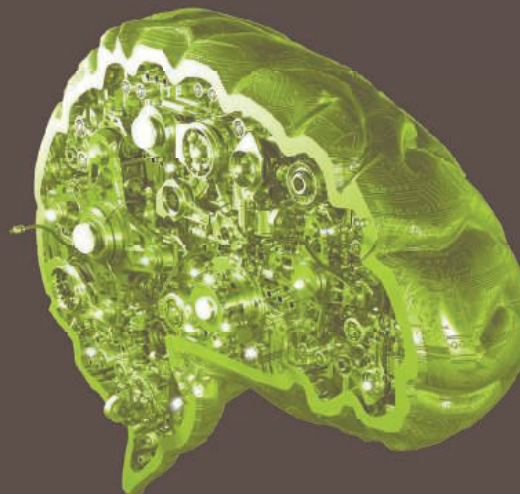
# Supporting ambitious research and development in biomedical engineering

A £30 million partnership between the Wellcome Trust and the EPSRC to fund engineering solutions to healthcare problems.

Applications invited from companies and academic researchers worldwide.

Apply by **10 December 2012**.

[www.wellcome.ac.uk/engineering](http://www.wellcome.ac.uk/engineering)



**EPSRC**

Engineering and Physical Sciences  
Research Council

**wellcome**trust

Mechanical brain. Oliver Burston/Wellcome Images



## Faculty Positions in Neuroscience



The California Institute of Technology invites applications for tenure-track professorial positions in the Division of Biology. Positions in Systems Neuroscience are open to investigators studying the neural mechanisms and circuit properties that underlie behavior. Positions in Cellular and Molecular Neuroscience are aimed at applicants whose research programs concern molecular and/or cellular aspects of physiology, behavior, or neural development. Successful applicants are expected to develop innovative research programs and should also have a commitment to undergraduate teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Initial appointments at the assistant professor level are for four years, and are contingent upon completion of the Ph.D. degree.

### Cellular, Developmental, or Regulatory Biology

The California Institute of Technology invites applications for a tenure-track professorial position in the Division of Biology. The successful applicant is expected to develop an innovative research program aimed at deciphering the molecular mechanisms that underlie biological phenomena at the level of molecules, cells, or organisms, and to be committed to high quality teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Initial appointments at the assistant professor level are for four years, and are contingent upon completion of the Ph.D. degree.

Please submit on-line application at <http://biology.caltech.edu/Positions> and include a brief cover letter, curriculum vitae, relevant publications, and a description of proposed research. Instructions will be given for submission of letters of reference when you apply on-line. Application deadline is **December 15, 2012**.

*The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.*



## TWO POSITIONS: BIOINFORMATICS/COMPUTATIONAL BIOLOGY AND PLANT EVOLUTIONARY BIOLOGY DEPARTMENT OF BIOLOGY COLORADO STATE UNIVERSITY



The Biology Department at Colorado State University (Fort Collins, Colorado) invites applicants for two tenure-track positions (**ASSISTANT PROFESSOR**): one in bioinformatics / computational biology and one in plant evolutionary biology. Areas of interest for the bioinformatics/computational biology position include (but are not limited to) epigenomics, evolutionary genomics, metagenomics, molecular or physiological imaging, systems biology, or synthetic biology. This is one of three positions in a cluster hire in Bioinformatics and Computational Biology launched in the College of Natural Sciences.

For the plant evolutionary biology position we seek a creative and broadly trained plant biologist who addresses fundamental and integrative questions in evolutionary biology. Examples of research interests include adaptation, bioinformatics, conservation biology, evolution of morphology and life histories, evolutionary ecology, genomics, hybridization, invasive species, mating systems, molecular evolution, population genetics, and speciation.

Successful candidates will be expected to develop extramurally funded and innovative research programs that complement existing strengths of the department, and to contribute to undergraduate and graduate teaching.

Applicants must have a Ph.D. by the time of application, a research program in bioinformatics/computational biology or plant evolutionary biology, respectively, and peer-reviewed publications. Postdoctoral experience and evidence of successful grant writing are preferred. Applications received online by **December 3, 2012** will be given full consideration. Use these links to access the full position descriptions and online applications: bioinformatics/computational biology: <http://cns.natsci.colostate.edu/employment/BioInformatics/>; plant evolutionary biology: <http://cns.natsci.colostate.edu/employment/BioPE/>. Include a cover letter, CV, statements of research and teaching interests, representative publications, and the names and contact information for three references. References will be sent instructions by e-mail for submitting letters online by the target date. Application materials of finalist candidates, including letters of reference, will be made available for review by the entire faculty of the Department of Biology.

*CSU is an EO/EA/AA Employer. Colorado State University conducts background checks on all final candidates.*

## POSITIONS OPEN

### FACULTY POSITION in Eukaryotic Genetics

The Department of Biological Sciences ([website: http://biology.uark.edu](http://biology.uark.edu)) at the University of Arkansas solicits applications for a tenure-track **ASSISTANT PROFESSOR** working in eukaryotic genetics (**Position Y13930**). The successful candidate will have a Ph.D. and postdoctoral experience, and will be expected to establish an extramurally supported research program, supervise graduate and undergraduate research, and teach undergraduate general genetics, and advanced offerings. Review of completed applications will begin November 09, 2012, and will continue until the position is filled. Applications must include curriculum vitae, statement of current and future research plans, teaching philosophy/interests, and three letters of recommendation sent independently. Electronically send applications in PDF form to: **Dr. Michael Lehmann** (e-mail: [mlehmann@uark.edu](mailto:mlehmann@uark.edu)), Department of Biological Sciences, SCEN 601, 1 University of Arkansas, Fayetteville, AR 72701. *The University of Arkansas is an Equal Opportunity/Affirmative Action Institution. All applicants are subject to public disclosure under the Arkansas Freedom of Information Act and persons hired must have proof of legal authority to work in the United States.*

**POSTDOCTORAL POSITION** available to participate in NIH-funded research project to study molecular mechanisms underlying Treg dysfunction and impairment of immune tolerance in early life caused by respiratory virus infection. The successful candidate will have the opportunity to employ state-of-the-art techniques in immunology, molecular biology including RNA sequencing technology, immunofluorescence microscopy, pulmonary function tests and other related techniques using wild-type and conditional gene knockout mice using animal model and human samples. Strong written and verbal skills in English are essential. Fresh Ph.D.s from Molecular Biology, Immunology, or Pathology program are highly encouraged to apply. Special consideration will be given to individuals with strong expertise in molecular biology and bioinformatics. Please send a brief cover letter, curriculum vitae, and contact information of two references to: **Dr. Prabir Ray**, Professor of Medicine & Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213. E-mail: [rayp@pitt.edu](mailto:rayp@pitt.edu); see website <http://www.dept-med.pitt.edu/pacmc> and *Nature Medical* 14:565, 2008; *PNAS* 108:5360, 2011; *Nature Medical* 18:1525, 2012.

### FACULTY POSITION

#### Department of Chemistry University of North Carolina at Chapel Hill

The Chemistry Department at the University of North Carolina (UNC) at Chapel Hill is seeking a tenured faculty member with research interests in solar energy science and solar fuels or in closely related fields. The appointment will be at the **FULL PROFESSOR** level. The successful candidate is expected to be an internationally recognized scholar with demonstrated science management skills and ability to provide high-level leadership to university initiatives in energy including the UNC Energy Frontier Research Center in Solar Fuels.

Interested applicants should upload current curriculum vitae to the following website: <http://unc.peopleadmin.com/postings/9152>. A confidential review of applicants will start 1 November. Questions should be directed to: **Chair, EERC Search Committee, Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3290; e-mail: chemsearch@unc.edu.**

*UNC is an Equal Opportunity/Affirmative Action Employer, and strongly encourages applications from women and minorities.*

### BIOINFORMATICS ASSISTANT PROFESSOR Tenure Track

Department of Plant Pathology, Kansas State University. Develop independent research program in bioinformatics with emphasis on the genomics of plants, microbes, and/or their interactions. Teach one course annually in bioinformatics. Ph.D. required, postdoctoral experience preferred. Full announcement available at website: <http://www.plantpath.ksu.edu/p.aspx?tabid=485>. Application review begins 23 November 2012.



# LUNDBECK FOUNDATION FELLOWSHIPS

The Lundbeck Foundation hereby invites applications for five fellowships within biomedicine and two within natural sciences which will be granted to especially promising young researchers and their research groups. Within biomedicine, the Foundation has special focus on neurology, psychiatry and allergology/immune modulation.

The fellowships are awarded for five years and each fellowship amounts to DKK 10 million (approx. Euro 1,3 million).

The subject area should be frontline basic- or applied research within the scope of the Foundation's grant strategy, which can be seen at [www.lundbeckfonden.com](http://www.lundbeckfonden.com)

The fellowships may well attract Danish or foreign researchers from abroad who wish to move to Denmark and continue their research here. The call is also open for applicants at Danish universities and university hospitals.

The fellowships are intended for seven researchers who are qualified to establish or develop their own research groups within the health or natural sciences and who have received their Ph.D. degree within the last 5-7 years.

The application should include an account of the project's research plan, collaborators, budget and how the research group is envisioned to be placed within a Danish research institution. In addition, it should include a letter of intent from a resident researcher at the host institution, who makes him- or herself available as a mentor to facilitate the applicant's establishment of the research group as an integral part of the host institution. Further guidance is provided in the application form.

The application, written in English, should be sent via the Foundation's Electronic Application System for fellowships at [www.lundbeckfonden.com](http://www.lundbeckfonden.com) no later than December 16, 2012.

For further information please contact Ulla Jakobsen, Science Manager at the Lundbeck Foundation, phone: +45 39 12 80 11 or at [application@lundbeckfonden.com](mailto:application@lundbeckfonden.com)



The Lundbeck Foundation has controlling shareholdings in its subsidiaries H. Lundbeck, ALK and Falck. In addition, the Foundation manages financial investments of approx. € 1.3 billion. The Foundation supports research within the medical and natural sciences. In 2011, the Foundation had a profit after tax of approx. € 217 million and made research grants of approx. € 68 million.

Lundbeckfonden  
Vestagervej 17, DK-2900 Hellerup  
Tel. +45 39 12 80 00  
[www.lundbeckfonden.com](http://www.lundbeckfonden.com)

THE LUNDBECK FOUNDATION



## Department of Health and Human Services National Institutes of Health National Institute of Neurological Disorders and Stroke

### Tenure Track Investigator Surgical Neurology Division of Intramural Research

The Division of Intramural Research of the National Institute of Neurological Disorders and Stroke, NIH is searching for outstanding neurosurgeons for tenure track positions in the Surgical Neurology Branch to establish or continue independent and imaginative research efforts integrating laboratory and clinical research. The Surgical Neurology Branch offers intramural basic, translational and clinical neuroscience/neurosurgery research opportunities in central nervous system disorders including (but not exclusive to) tumors, cerebrovascular disease, degenerative diseases, spinal disorders, stereotactic and functional approaches, drug delivery and epilepsy. An MD or MD/PhD with neurosurgical training and at least three years of research experience are required. Board eligibility or certification by American Board of Neurological Surgery is required. An individual selected for a tenure-track position is expected to build a dynamic and productive research group. The successful candidate will join a diverse, collegial group of investigators at NINDS. The position includes a highly competitive research support package that will be commensurate with the qualifications and experience of the individual.

Applicants should send curriculum vitae, including bibliography, statement of research interests and a list of three references to: **Dr. Avindra Nath, Clinical Director, NINDS c/o Caren Collins at Building 10, Room 7C103, MSC 1430, Bethesda, MD 20892** or [Caren.Collins@nih.gov](mailto:Caren.Collins@nih.gov). Review of applications is expected to begin on **October 29, 2012** but applications will be accepted until the position is filled.



*HHS and NIH are Equal Opportunity Employers.*



## POSITIONS OPEN



### TENURE TRACK FACULTY POSITIONS in Gene Expression and Signaling Department of Biochemistry and Molecular Biology

The Department of Biochemistry and Molecular Biology ([website: http://www.bmb.msu.edu](http://www.bmb.msu.edu)) at Michigan State University (MSU) seeks to recruit two outstanding tenure system **ASSISTANT/ASSOCIATE PROFESSORS** in gene expression and signaling. The individuals will employ innovative and multidisciplinary approaches to address central questions in gene expression and signaling in development and disease. Systems may include stem cell, vertebrate, or invertebrate models. It is anticipated that the successful candidate will contribute to the highly collaborative research environment on campus as exemplified by the Gene Expression in Development and Disease, Molecular Metabolism and Disease, Mitochondrial Science and Medicine, and Bio/computational Evolution in Action Consortium groups. The position is intended to build upon existing research focus areas in gene regulation, metabolism and metabolic disorders, neuroscience, and cancer.

Review of application materials will begin on November 15, 2012, and continue until suitable candidates are identified. Applicants should upload a single PDF file with cover letter, curriculum vitae, concise description of research interests and future directions, and teaching philosophy to [website: https://jobs.msu.edu](https://jobs.msu.edu) (position #6878). Three or more letters of reference should be sent to [e-mail: gesfacultysearch@cns.msu.edu](mailto:gesfacultysearch@cns.msu.edu). Questions about the position can be addressed to David Arnosti at [e-mail: gesfacultysearch@cns.msu.edu](mailto:gesfacultysearch@cns.msu.edu).

*Michigan State University is an Affirmative Action/ Equal Opportunity Employer. MSU is committed to achieving excellence through cultural diversity. The university actively encourages applications and/or nominations of women, persons of color, veterans, and persons with disabilities.*

### NEUROBIOLOGIST

Loyola University Chicago (LUC), College of Arts and Sciences, Department of Biology, invites applications for a full-time tenure-track position in Neurobiology at the rank of **ASSISTANT PROFESSOR**, beginning August 2013. We are a large department that serves more than 1,500 undergraduate majors and 25 M.S. students with a strong affiliation with the Interdisciplinary Neuroscience Minor. Please visit our [website: http://www.luc.edu/biology](http://www.luc.edu/biology). We have modern laboratory space and research support and imaging facilities. Candidates must have a Ph.D. and postdoctoral experience, and will be expected to establish a vigorous, externally funded research program involving undergraduates and M.S. students. Candidates will also contribute to department, college, and university service as requested. Primary research areas of interest include development and/or function of the nervous system using molecular, cellular, and/or physiological approaches. Teaching responsibilities include courses in neurobiology and/or neurobiology laboratory, and/or an advanced course in the candidate's area of specialization. Candidates for the position must clearly demonstrate the potential for excellence in research and teaching and have a record of (or clear potential for) distinguished scholarship, grant-funded research, and student mentorship. Candidates should submit cover letter, curriculum vitae, research plan, teaching philosophy statement, and names and contact information for three references online at [website: http://www.careers.luc.edu](http://www.careers.luc.edu). Review of applications will begin on November 1, 2012, and continue until the position is filled. Written inquiries about the position can be sent to: **Neurobiologist Search Committee, Loyola University Chicago, Department of Biology, 1032 West Sheridan Road, Chicago, IL 60660.**

*Loyola University Chicago is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversifying its faculty. Applications from women and minority candidates are especially encouraged. For information about LUC visit [website: http://www.luc.edu](http://www.luc.edu).*

## POSITIONS OPEN

### FACULTY POSITIONS

#### Massachusetts Institute of Technology

The Department of Materials Science and Engineering (DMSE) seeks candidates for open tenure-track faculty positions to begin July 2013 or thereafter. Appointments would be at the **ASSISTANT** or untenured **ASSOCIATE PROFESSOR** level. In special cases, a senior faculty appointment may be possible. Faculty duties include teaching at the graduate and undergraduate levels, research, and supervision of student research.

Candidates should hold a Ph.D. in Materials Science and Engineering or a related field by the beginning of the appointment period. Candidates with deep knowledge of the core of Materials Science and Engineering are desired. DMSE seeks to broaden its research portfolio in several key areas, including: materials selection and materials economics, microstructure characterization and design, materials chemistry and surface science, photonic and optical materials. Research portfolios that cut across these areas and other aspects of materials science and engineering are desired. However, the present search is broadly defined and applicants with expertise in any and all areas of materials science and engineering are encouraged to apply. MIT has a number of institute-wide initiatives underway or in development, on topics that include Manufacturing, Energy, Environment, and Health. Individuals who can connect to these initiatives are of interest.

Interested candidates should submit application materials electronically at [website: https://school-of-engineering-faculty-search.mit.edu/dmse/](https://school-of-engineering-faculty-search.mit.edu/dmse/). Each application should include: curriculum vitae, a statement of research interests, and a statement of teaching interests. We request that each candidate arrange for three letters of reference to be uploaded at [website: https://school-of-engineering-faculty-search.mit.edu/letters/](https://school-of-engineering-faculty-search.mit.edu/letters/). Questions should be addressed to [e-mail: dmse-search-master@dmsefacsrch.mit.edu](mailto:dmse-search-master@dmsefacsrch.mit.edu). Responses received by December 31, 2012, will be given priority.

*We especially encourage minorities and women to apply because of MIT's strong commitment to diversity in engineering education, research, and practice.*

### AQUATIC ECOLOGIST

Loyola University Chicago (LUC), College of Arts and Sciences, Department of Biology, invites applications for a full-time tenure-track position in Aquatic Ecology at the rank of **ASSISTANT PROFESSOR**, beginning August 2013. We are a large department that serves more than 1,500 undergraduate majors and 25 M.S. students ([website: http://www.luc.edu/biology](http://www.luc.edu/biology)). We have modern laboratory facilities, a 2,100 square-foot artificial stream facility and a recently established field station ([website: http://www.luc.edu/retreatcampus](http://www.luc.edu/retreatcampus)). Candidates must have a Ph.D. and postdoctoral experience, and will be expected to establish a vigorous, externally funded research program involving undergraduates and M.S. students. Preference will be given to candidates working in freshwater ecology with research expertise complementing existing research strengths in the department. Teaching responsibilities shall include general biology, general ecology, and an advanced course in the candidate's area of specialization. Candidates for the position must clearly demonstrate the potential for excellence in research and teaching and have a record of (or clear potential for) distinguished scholarship, grant-funded research, and student mentorship. Candidates should submit cover letter, curriculum vitae, research plan, teaching philosophy statement, and names and contact information for three references online at [www.careers.luc.edu](http://www.careers.luc.edu). Review of applications will begin on November 1, 2012, and continue until the position is filled. Written inquiries about the position can be sent to: **Aquatic Ecologist Search Committee, Loyola University Chicago, Department of Biology, 1032 West Sheridan Road, Chicago, IL 60660.**

*Loyola University Chicago is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversifying its faculty. Applications from women and minority candidates are especially encouraged. For information about LUC visit [website: http://www.luc.edu](http://www.luc.edu).*

## POSITIONS OPEN

### TENURE-TRACK POSITIONS

#### RNA Therapeutics Institute

The new RNA Therapeutics Institute (RTI), co-directed by **Craig C. Mello**, **Victor Ambros**, **Melissa J. Moore**, and **Phillip D. Zamore**, invites applications for tenure-track positions in the areas of RNA Biology, Disease, and Therapy. Successful candidates will conduct innovative basic or clinical research to understand normal and disease mechanisms involving RNA or develop nucleic acid-based diagnostics or therapies.

Areas of specific interest include (but are not limited to): genetics and biochemistry in model organisms, pre-clinical models, and human disease of regulatory RNAs, non-coding RNAs and RNA-protein complexes; chemical biology of RNA-mediated cellular processes; engineering of nucleic acids for controlled delivery and improved efficacy in vivo. Physician scientists, materials scientists, computational researchers and bioengineers, as well as molecular, genetics, and biochemical researchers, are encouraged to apply.

The RTI will be housed in a new state-of-the-art research building opening in January 2013. The Institute is part of a campus-wide initiative in RNA therapeutics, stem cell biology and gene therapy, which is designed to promote collaboration among basic scientists, preclinical investigators, and clinicians. As a recipient of a Clinical and Translational Science Award (CTSA) from the NIH, UMass Medical School (UMMS) seeks to build an environment in which basic research informs our understanding of human disease, accelerating the development of novel diagnostics and therapies. To further foster collaboration, RTI investigators will hold joint appointments with other departments on campus.

Applicants should submit a cover letter, curriculum vitae, a two-page statement of research interests and contact information for three references to [website: https://academicjobsonline.org/ajob/jobs/2025](https://academicjobsonline.org/ajob/jobs/2025). Applications will be reviewed expeditiously, with interviews commencing in November. The position is open until filled. Inquiries, but not application materials, may be directed to **Tiffany Covello** at [e-mail: tiffany.covello@umassmed.edu](mailto:tiffany.covello@umassmed.edu). Applications from both junior level and more senior researchers will be considered.

*As an Equal Opportunity/Affirmative Action Employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.*

### FACULTY POSITION in Molecular/Cellular Neuroscience The Ohio State University Wexner College of Medicine

The Departments of Neuroscience and Molecular and Cellular Biochemistry at The Ohio State University invite applications for a joint tenured faculty position in molecular/cellular neuroscience at the **ASSOCIATE** (or potentially **FULL PROFESSOR**) level. We are seeking an investigator with a well established and productive research program focused on diseases of the nervous system. We are particularly interested in research that focuses on tissue microenvironment or expression genetics and that synergizes with our current strengths in cell and developmental neuroscience using zebrafish and/or mice as model systems. We offer generous space and startup funds, mouse and fish facilities, and the opportunity to join a vibrant and rapidly growing biomedical research community. The successful candidate will have a joint appointment in the Department of Neuroscience ([website: http://biomed.osu.edu/neuroscience/](http://biomed.osu.edu/neuroscience/)) and Department of Molecular and Cellular Biochemistry ([e-mail: http://biomed.osu.edu/mcbiochem/](http://biomed.osu.edu/mcbiochem/)).

Please submit a curriculum vitae and statement of research interests in PDF format to **Cheryl Yoder** ([e-mail: cheryl.yoder@osumc.edu](mailto:cheryl.yoder@osumc.edu)) and arrange for three letters of reference addressed to **Randy J. Nelson**, Chair, Department of Neuroscience to be sent to the same e-mail address. Review of applications will start 1 December 2012. The Ohio State University has a strong commitment to diversity and actively encourages applications from candidates from individuals of underrepresented groups. The Ohio State University is an Equal Opportunity/Affirmative Action Employer.

**Cleveland Clinic**  
Lerner Research Institute  
**Faculty Positions**  
**Department of Cellular & Molecular Medicine**

We are seeking established investigators who use multidisciplinary approaches to understand causes of and treatments for human diseases. Individuals will receive appointments as Associate or Full Staff (Full Professor equivalent) in the Department of Cellular & Molecular Medicine. Applicants with PhD, MD, or dual degrees will be considered. Exceptionally qualified junior faculty may be considered for appointment at the Assistant Staff level. Applicants must have a track record of high quality funded research and productivity.

Recruits will join a highly collaborative environment with outstanding opportunities for collaborations with basic and translational researchers and clinicians from multiple specialties. Outstanding facilities, generous start-up funds, and ongoing operational support are available. The Molecular Medicine Graduate Training Program, School of Medicine, and other training programs provide access to excellent PhD students.

Please submit a CV and a 2-page summary of research interests by **December 15, 2012** to Ms. Raquel Assal at [assalr@ccf.org](mailto:assalr@ccf.org); <http://www.lerner.ccf.org/jobs/faculty/view.php?id=528>.

*The Cleveland Clinic Foundation is an Equal Opportunity/Affirmative Action Employer in a smoke/drug free environment.*

**ASU** SCHOOL OF  
**Life Sciences**  
ARIZONA STATE UNIVERSITY

**Faculty Position in Biomarker Research at Arizona State University –Tempe, AZ**

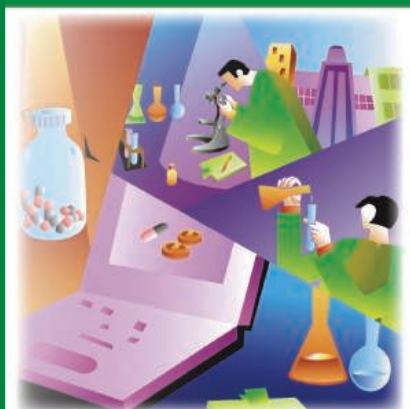
The School of Life Sciences (SOLS) at Arizona State University (ASU) invites applications for a tenure-track position at the rank of Assistant Professor in the area of biomarker research. We seek imaginative, creative individuals with strong interest in discovery of biomarkers and their mechanistic validation. The successful candidate will be expected to develop an innovative, extramurally-funded, independent research program, fulfill teaching requirements at both the undergraduate and graduate levels and have a commitment to outreach and service at levels within and outside the University community. The successful candidate will be expected to mentor undergraduate and graduate students, postdoctoral trainees, and interact with the multidisciplinary consortium of faculty of SOLS as they interface with other groups on campus such as the Biodesign Institute, Department of Chemistry/Biochemistry, the Schools of Engineering, the Metabolic/Vascular Biology Center at Mayo-Scottsdale, and the NIH-funded Center of Excellence for Membrane Proteins of Infectious Diseases. While we encourage applications of top candidates **with a demonstrated record of significant publications** within the realm of biomarker research, strong preference will be given to applicants who have **particular** interests in molecular mechanisms of disease (e.g. infectious, neurodegenerative, metabolic or neoplastic diseases) that complement expertise of existing faculty and who will expand our overall research and instructional capabilities. A competitive start-up package and a teaching load compatible with high research productivity will be provided.

Candidates must have a doctoral degree in biology, biochemistry, engineering, or a related field at the time of appointment and two or more years of relevant postdoctoral experience with demonstrated research and teaching/mentoring excellence.

To apply, send a cover letter, your curriculum vitae, three representative publications, separate statements of future research plans and teaching philosophy and interests, and contact information for three references to be sent to **Tsafrir Mor, Chair, Biomarker Faculty Search Committee, School of Life Sciences, PO Box 874501, Tempe, AZ 85287-4501**. Electronic applications sent as pdf files to [solsfacultysearch5@asu.edu](mailto:solsfacultysearch5@asu.edu) are preferred. The initial closing date for receipt of applications is **November 18, 2012**; applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on this position and the School of Life Sciences, please visit <http://sols.asu.edu>.

*Arizona State University is an Affirmative Action, Equal Opportunity Employer committed to excellence through diversity. We especially encourage women and minorities to apply.*

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**GROUP LEADER, COMPUTATIONAL BIOLOGY**

To apply, visit <http://jobs.ornl.gov/>

**Purpose:** The Biosciences Division at the Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee is seeking applicants for the Computational Biology Group Leader position. The group leader will provide direction of the BioEnergy Science Center's (BESC) computational biology initiatives, as well as direct ORNL's involvement in the Plant-Microbe Interfaces Project (PMI). The group leader will report to the Biosciences Division Director.

**Major Duties/Responsibilities:**

- Promote and facilitate the execution and dissemination of high-quality research in a manner that encourages creativity and capability while demonstrating a strong commitment to scientific integrity.
- Collaborate with research staff across the Laboratory on advancing the scientific mission of the Laboratory.
- Select and retain highly qualified staff, develop capabilities of staff, and ensure that staff members are competent, trained, and qualified for assigned work.
- Provide effective and meaningful performance feedback to individual staff members while ensuring that staff are effectively utilized, rewarded, and motivated.
- Support the professional development of staff consistent with Laboratory and individual development plans.
- Develop, support, and lead successful bioinformatics and computational biology programs that both leverage and further the mission of the organization. Lead the bioinformatics efforts for key DOE projects (e.g., BESC and PMI) Interact with relevant DOE program offices, other Federal agencies, universities, and industry. Represent and promote Bioinformatics and Computational Biology Group research activities to sponsors and visitors as appropriate. Develop and maintain research programs and substantial programmatic funding.

**Qualifications Required:** A Ph.D. degree and 10 or more years of related professional experience in one or more of the following fields: computational biology, bioinformatics, biological databases, plant or microbial genomics, or other relevant scientific areas plus relevant management and leadership experience. Dynamic leadership skills are required with the ability to plan, organize, and manage activities of a diverse organization within a major R&D division. Excellent oral and written communication skills are required to support regular interactions with sponsors, peer reviewers, and others; and to prepare reports, proposals, publications and journal articles. Strong interpersonal and communication skills are required to support team building, partnering, and leadership.

*UT-Battelle is recognized by our employees and the community as an inclusive environment where diversity is valued and individuals and teams are inspired to contribute fully to the organization's success. ORNL is an Equal Opportunity Employer.*



## POSITIONS OPEN

### ECOLOGIST POSITION Fordham University

Applications are invited for a tenure-track position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in the Department of Biological Sciences. We seek an ecologist conducting hypothesis-driven research in urban ecology. Experience using molecular tools is desirable. The successful applicant will establish a research program at Fordham's biological field station, the Louis Calder Center, and participate in our Center for Urban Ecology (CUE). There are also opportunities to collaborate with scientists at the New York Botanical Garden, Wildlife Conservation Society, and American Museum of Natural History. A commitment to undergraduate and graduate teaching and research is required. Assistant Professor candidates must demonstrate potential to develop an externally funded research program. Associate candidates must have a record of external, peer-reviewed funding and indicate future directions using regional resources. Applicants should electronically send one PDF application file containing a cover letter, curriculum vitae, contact information for three references, teaching and research statements, and three reprints to e-mail: [jdlewis@fordham.edu](mailto:jdlewis@fordham.edu). Address the cover letter to: **Dr. J.D. Lewis, Chair, Department of Biological Sciences, Fordham University, Bronx, NY 10458**. Review of applications will begin October 29, 2012. *Fordham University is an independent, Catholic university in the Jesuit tradition that welcomes applications from men and women of all backgrounds. Fordham is an Equal Opportunity Employer.*

### FRESHWATER BIOLOGIST

The Department of Biology at William Paterson University invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level. Ph.D. required. Postdoctoral research and teaching experience preferred. We are seeking a broadly trained Freshwater Biologist with a strong field orientation and additional expertise in development, physiology, or molecular biology. Preference will be given to candidates working with animal systems. The successful candidate will teach in the B.S./M.S. Biology program, in the B.S./M.S. Biotechnology program if appropriate and will contribute to teaching of core biology courses and upper-level and graduate courses in the candidate's specialty. Candidate will also develop a strong program of research involving undergraduate students in both field and laboratory settings. Demonstrated commitment to excellence in teaching and research required.

Applicants should submit curriculum vitae, statements of research interests and teaching philosophy, names, addresses and telephone numbers of three (3) references to: **Dr. Lance S. Risley (e-mail: [risleyl@wpunj.edu](mailto:risleyl@wpunj.edu)), Chairperson, Department of Biology, Science Hall East, William Paterson University, 300 Pompton Road, Wayne, NJ 07470**. Review begins immediately and continues until the position is filled.

*William Paterson University is an Equal Opportunity Employer committed to diversity. Women, minorities, and members of underrepresented groups are encouraged to apply.*

### U.S. DEPARTMENT OF AGRICULTURE Agricultural Research Service

Job Announcement opens October 9, 2012 thru November 2, 2012. Salary: \$68,809.00 - \$147,857.00/year.

The USDA-ARS, is seeking a **RESEARCH SCIENTIST** (Basic or Clinical) to work at the Arkansas Children's Nutrition Center in Little Rock, Arkansas.

Interdisciplinary Research Position: Research Nutritionist; Research Molecular Biologist; Research Physiologist; Research Microbiologist

To review the full Vacancy Advertisement and application requirements please go to website: <https://www.usajobs.gov/JobSearch/Search/GetResults?OrganizationID=AG03>.

Search (Top Right) for **Control #327489400**.

## POSITIONS OPEN

### FACULTY POSITION in Theoretical Biological Physics Purdue University

The Department of Physics at Purdue University (website: <http://www.physics.purdue.edu>) seeks applications for a faculty position at the rank of **ASSISTANT PROFESSOR** in the area of theoretical biological physics. Applicants must have a Ph.D. in physics, biophysics, or a related field, an outstanding record of research accomplishments, and potential for excellence in teaching at both the undergraduate and graduate levels. Salary and benefits are highly competitive. Candidates are expected to develop vigorous research programs, supervise graduate students, and teach undergraduate and graduate courses. Interested candidates should submit their curriculum vitae, publication list, and brief descriptions of their planned research program and teaching philosophy. Electronic submission is preferred at website: <https://www.physics.purdue.edu//searches/app/>. At least three letters of recommendation should be sent to: **Biophysics Search, Department of Physics, Purdue University, 525 Northwestern Avenue, West Lafayette, Indiana 47907-2036, USA**. Applications completed by December 1, 2012 will be given full consideration, although the search will continue until the position is filled. A background check is required for employment in this position.

*Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce.*

### ASSISTANT PROFESSOR—GENETICIST Tenure-Track Position Dickinson College

The Biology Department at Dickinson College seeks a Geneticist to fill a new tenure-track assistant professor position beginning July 1, 2013. Teaching responsibilities will include introductory biology, Genetics (with lab), upper-level courses in the candidate's area of expertise, and occasional participation in our First-year seminar program. The ability to create inclusive learning environments for an increasingly diverse student body will be an important characteristic of the successful candidate. The research area is open, but should use modern molecular, cellular, genetic, genomic, and/or biochemical approaches. The successful applicant will contribute to the Biochemistry & Molecular Biology program, have laboratory and office space in the newly completed Rector science complex, have access to start-up funds, and be expected to develop a vigorous research program involving undergraduates. A Ph.D. is required; postdoctoral experience is preferred. Review of applications will begin on November 5, 2012. To apply, send a letter of application, curriculum vitae, statements of teaching philosophy and research interests, and three letters of reference to website: <https://jobs.dickinson.edu>. Located in south central Pennsylvania, Dickinson (enrollment of 2,300) is a highly selective national liberal arts college with an emphasis on innovative science teaching and student/faculty research. *The College is committed to building a representative and diverse faculty, administrative staff, and student body. We encourage applications from all qualified persons.*

### FACULTY POSITION in Biological Chemistry

Bentley University's Natural and Applied Sciences Department invites applications for a tenure-track faculty position in biological chemistry. The successful candidate must have a Ph.D. or equivalent degree in a relevant field of science. Areas of interest may range broadly, including biological chemistry (pharmaceutical or environmental applications), cheminformatics, or toxicological health risks. Individuals with industry or government science experience are encouraged to apply. This is a tenure-track position with salary commensurate with qualifications. All application materials should be submitted through Bentley's online employment site at website: <https://jobs.bentley.edu/applicants/Central?quickFind=51899>. *Equal Opportunity/Affirmative Action Employer.*

## POSITIONS OPEN

### DEPARTMENT CHAIR Pharmacological and Physiological Science

Saint Louis University, a Catholic Jesuit institution dedicated to education, research, service, and health care, has started a national search for the next William Beaumont Professor and Chair of the Department of Pharmacological and Physiological Science (website: <http://medschool.slu.edu/pharmphys>). The department encompasses multidisciplinary research in the cardiovascular, endocrine, and neuroscience areas and has an outstanding record in graduate education supported in part by an NIH-T32 training grant currently in its 22nd year. The department seeks an individual with the vision and leadership to build and maintain robust basic and translational research programs, and continue the strong commitment to graduate and medical education. The successful applicant will have a Ph.D. and/or M.D. degree, a strong record of academic achievement, a solid level of extramural research funding, and experience in both graduate student and medical student education. Interested candidates should submit a cover letter, curriculum vitae, and a description of their leadership vision to website: <http://jobs.slu.edu> (Req. ID# 20120062). Letters of nomination may be sent electronically to **Enrico Di Cera, M.D.**, Chair of the Search Committee (e-mail: [enrico@slu.edu](mailto:enrico@slu.edu)).

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### CHAIR, DEPARTMENT OF PHYSICS The University of Memphis

Applications are invited for the position of Chair of the Department of Physics at The University of Memphis starting fall 2013. Applicants must have a Ph.D. in Physics or closely related field, an established research program, a record of teaching and service, a commitment to academic excellence, and strong interpersonal and administrative skills. Applicants at the **FULL** or **ASSOCIATE PROFESSOR** level will be considered. Individuals whose research interests complement the department's research strengths in experimental, theoretical, or computational materials physics are encouraged to apply.

Interested individuals should upload to website: <https://workforum.memphis.edu> (1) a letter of application that describes the applicant's vision for promoting research and education, (2) a detailed curriculum vita that includes the applicant's education, experience, scholarly activities, and related information, and (3) a list of three or more professional references.

To learn about the department, go to website: <http://www.memphis.edu/physics>. For additional information about the position, contact the search committee chair, **Dr. Omar Skalli**, at e-mail: [oskalli@memphis.edu](mailto:oskalli@memphis.edu).

Review of applications will begin on December 1 and may continue until the position is filled.

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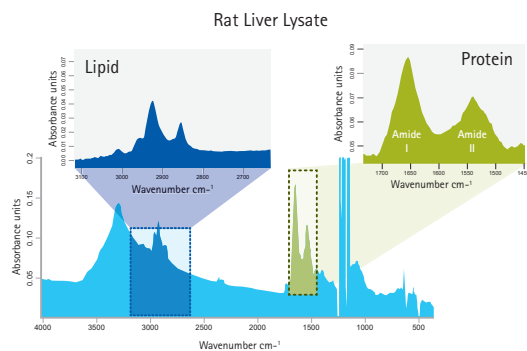
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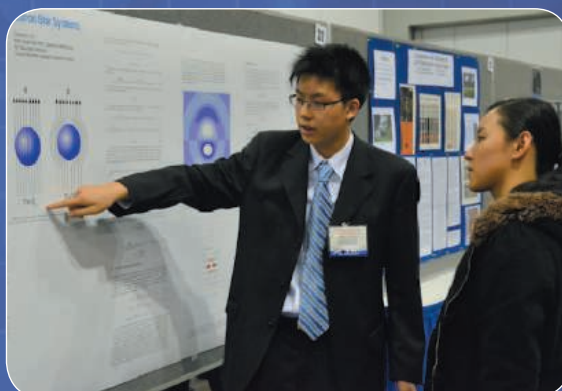
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## 2012 Top Employers Survey Stability in the Face of Change

### In This Issue

Biopharmas that have fared well despite global economic turmoil have done so by making smart acquisitions, paying attention to global opportunities, investing in R&D, looking beyond the bottom line—and valuing and respecting the scientists who work for them, according to this year's *Science* Careers Top Employers Survey.

See full story on page 403.

### Upcoming Features

Regional Focus on Europe—November 9

Faculty—February 1, 2013

Regional Focus on Asia—February 22, 2013

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- 13 Amgen
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- 15 Abbott
- 16 Genzyme, a Sanofi company
- 17 Syngenta
- 18 Gilead Sciences
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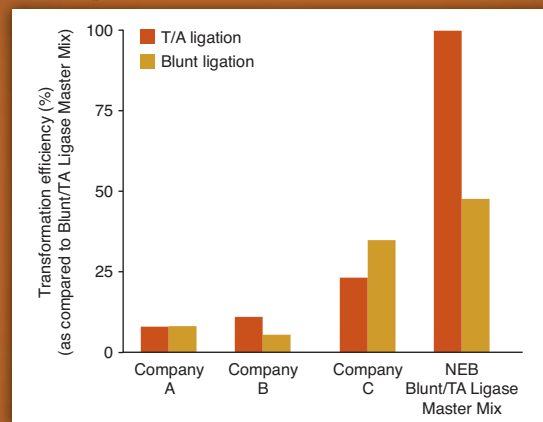
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