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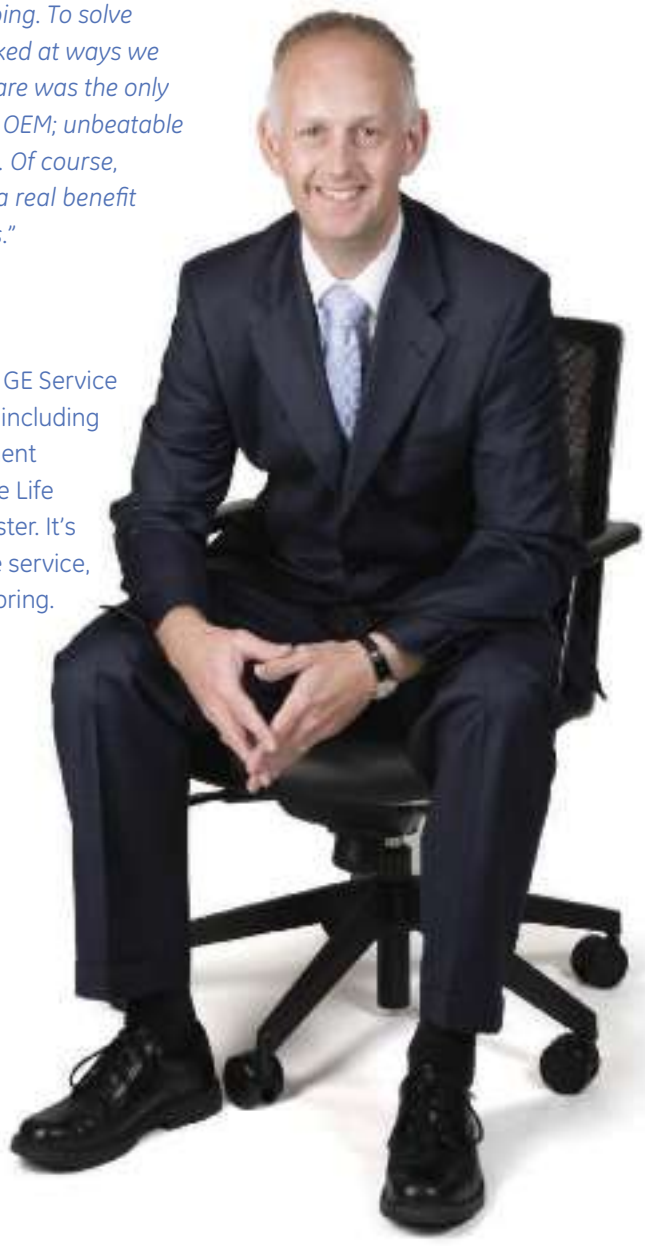

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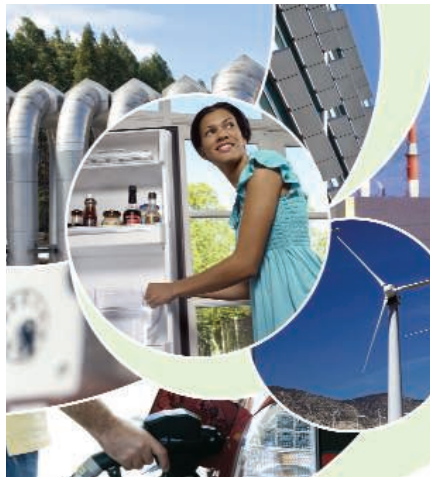

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COVER

Pulsars in our Galaxy newly discovered by the Fermi Gamma-ray Space Telescope, launched in June 2008. The objects emit broad gamma-ray beams that have enabled Fermi to locate a never-before-seen population. Fermi has also seen pulsars with millisecond periods and has detected at least one globular cluster (center left). See the Research Article and two Reports beginning on page 840.

Image: NASA/U.S. Department of Energy/Fermi LAT Collaboration/Sonoma State University/Aurore Simonnet

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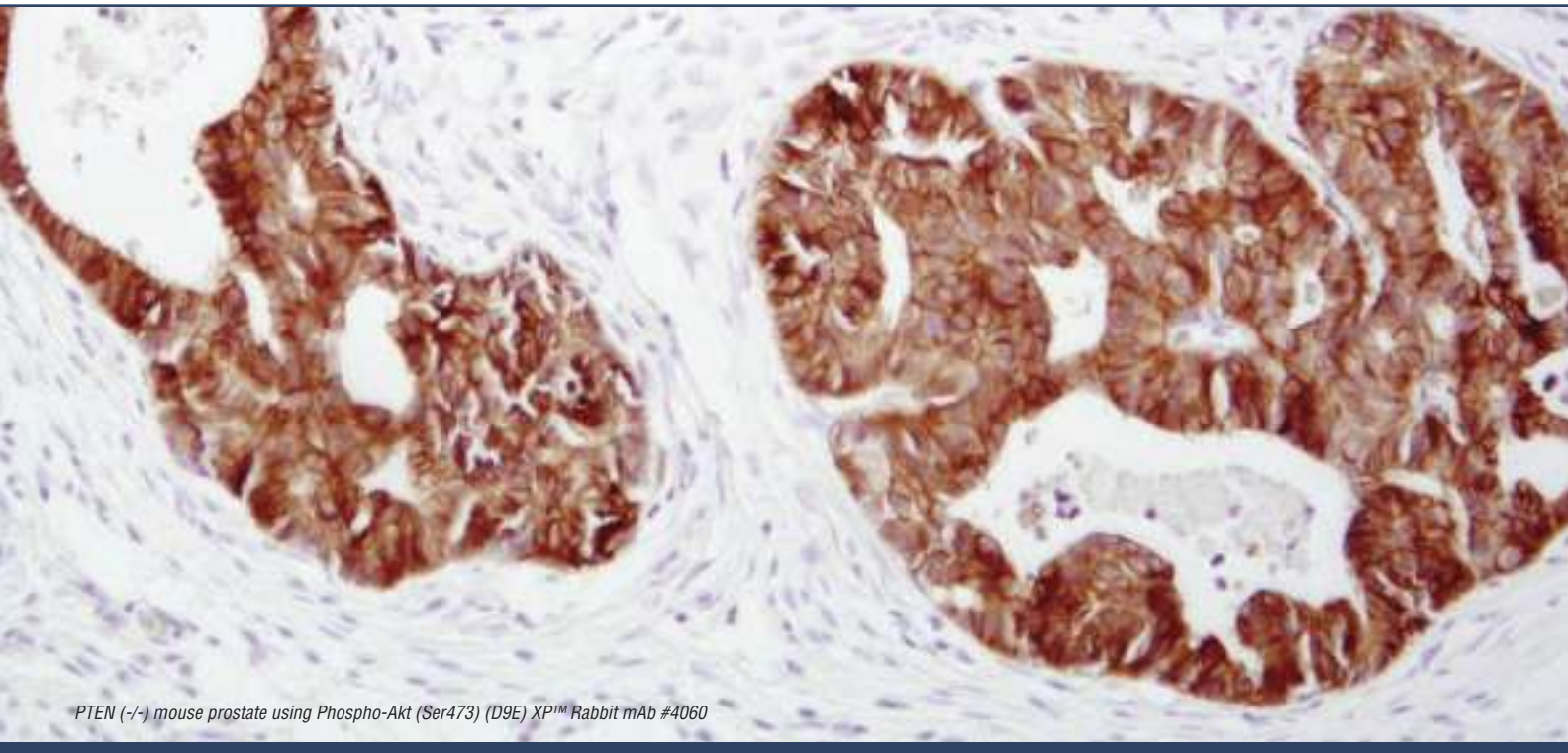

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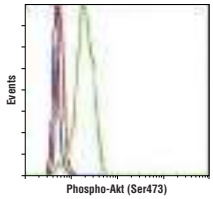

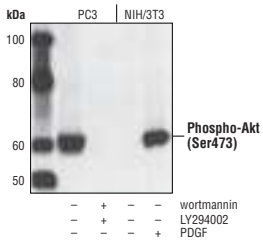

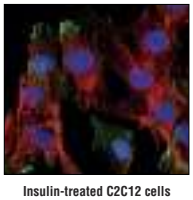

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The Molecular Basis of Nacre Formation
N. Kröger

A protein complex guides the intricate process of nacre formation in mollusk shells.
10.1126/science.1177055

Formation of ArF from LPdAr(F): Catalytic Conversion of Aryl Triflates to Aryl Fluorides
D. A. Watson et al.

A catalyst enables versatile carbon-fluorine bond formation using simple fluoride salts.
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Evidence for Obliquity Forcing of Glacial Termination II
R. N. Drysdale et al.

Marine records suggest that the early onset of the penultimate deglaciation was due to changes in Earth's obliquity.
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RESEARCH ARTICLE: Neuronal and Intestinal Protein Kinase D Isoforms Mediate Na^+ (Salt Taste)–Induced Learning
Y. Fu et al.

Protein kinase D signaling in the intestine and nervous system enables worms to learn to avoid salt.

RESEARCH ARTICLE: Cooperativity Between T Cell Receptor Complexes Revealed by Conformational Mutants of CD3ε
N. Martínez-Martín et al.

A mutant CD3ε subunit that cannot change conformation in response to ligand binding exerts dominant-negative inhibition of T cell responses.

PERSPECTIVE: Actin and Microtubule-Based Cytoskeletal Cues Direct Polarized Targeting of Proteins in Neurons
D. B. Arnold

Delivery of proteins to axons or dendrites depends on interactions between molecular motors and the cytoskeleton.

PERSPECTIVE: New Complexity in Differentiating Stem Cells Toward Hepatic and Pancreatic Fates
S. S. Huppert and M. A. Magnuson

Programming of the liver and pancreas is controlled by a dynamic, temporally coordinated signaling network.

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H. Akil

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L. Chiu

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Perspective: Top 10 Tips to Maximize Your Mentoring
J. Lakoski

Cultivating and nurturing mentoring relationships is an essential career skill.

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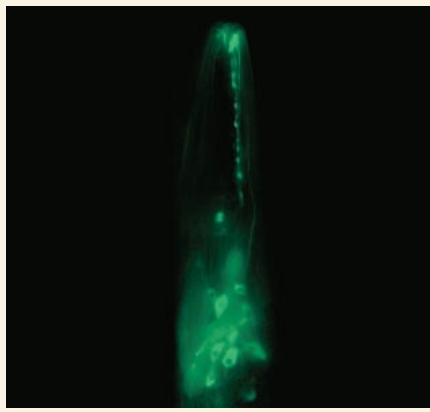

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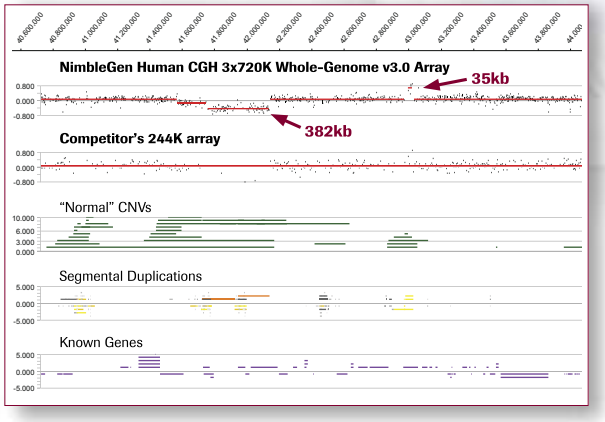

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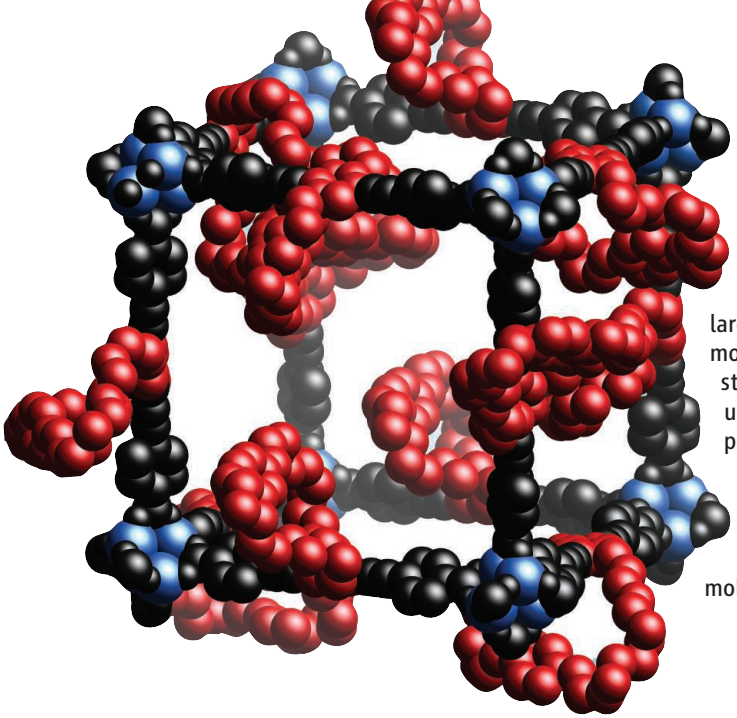


<< MOFs with Guest Rooms

Metal organic framework (MOF) solids typically boast large pores in their crystal lattices that can accommodate molecular guests and are useful as compact media for gas storage. However, guest binding has generally relied upon nonspecific interactions with the framework components. **Li *et al.*** (p. 855) have now prepared MOFs that incorporate macrocyclic ethers into the structural ligands comprising the framework walls within which certain cationic guests can bind quantitatively and site-specifically, akin to the molecular docking of drug molecules within protein receptors.

Friendly Fire

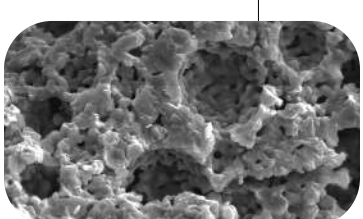
Hints of the use of more advanced materials by humans, including symbolic marking and jewelry, appear about 75,000 years ago or so in Africa. **Brown *et al.*** (p. 859; see the Perspective by **Webb and Domanski**) now show that these early modern humans were also experimenting with the use of fire for improved processing of materials. Replication experiments and analysis of artifacts suggest that humans in South Africa at this time, and perhaps earlier, systematically heated stone materials, including silcrete to improve its flaking properties in making tools.

Gamma-Ray Pulsar Bonanza

Most of the pulsars we know about were detected through their radio emission; a few are known to pulse gamma rays but were first detected at other wavelengths (see the Perspective by **Halpern**). Using the Fermi Gamma-Ray Space Telescope, **Abdo *et al.*** (p. 840, published online 2 July; see the cover) report the detection of 16 previously unknown pulsars based on their gamma-ray emission alone. Thirteen of these coincide with previously unidentified gamma-ray sources, solving the 30-year-old mystery of their identities. Pulsars are fast-rotating neutron stars. With time they slow down and cease to radiate; however, if they are in a binary system, they can have their spin rates increased by mass transfer from their companion stars, starting a new life as millisecond pulsars. In another study, **Abdo *et al.*** (p. 845) report the detection of gamma-ray emission from the globular cluster 47 Tucanae, which is coming from an ensemble of millisecond pulsars in the cluster's core. The data imply that there are up to 60 millisecond pulsars in 47 Tucanae, twice as many as predicted by radio observations. In a further companion study, **Abdo *et al.*** (p. 848, published online 2 July) searched Fermi Large Area Telescope data for pulsations from all known millisecond pulsars outside of stellar clusters, finding gamma-ray pulsations for eight of them. Their properties resemble those of other gamma-ray pulsars, suggesting that they share the same basic emission mechanism. Indeed, both sets of pulsars favor emission models in which the gamma rays are produced in the outer magnetosphere of the neutron star.

Porous Anodes for Solid Oxide Fuel Cells

Fuel cells that use ion-conducting oxides as the electrolyte can be highly efficient and use hydrocarbon fuels directly. However, their very high operating temperatures (usually above 700°C) can lead to unwanted reactions with their electrode materials and premature degradation of their performance. In order to improve fuel-cell electrochemical performance, **Suzuki *et al.*** (p. 852) describe a route for increasing the porosity of the anode material, which contains nickel oxide and zirconia doped with scandium and cerium and is fabricated as a cylinder. Subsequent coating and firing steps added a layer of a zirconia-based electrolyte and the $(La,Sr)(Co,Fe)O_3$ cathode. The resulting fuel-cell power density exceeded 1 watt per square centimeter at 600°C, and its performance improved as hydrogen fuel velocities were increased through the cell.



Lysine Acetylation Catalog

Covalent posttranslational modification is an essential cellular regulatory mechanism by which the activity of proteins can be controlled. Advances in mass spectrometry made it possible for **Choudhary *et al.*** (p. 834, published online 16 July) to assess the prevalence of lysine acetylation throughout the whole proteome. Acetylation is much more widespread than previously appreciated and occurs on proteins participating in all sorts of biological functions. Acetylation can influence susceptibility of proteins to phosphorylation and occurs frequently on enzymes that control the modification of other proteins by covalent ubiquitination and on proteins that form large macromolecular complexes. The findings also help to characterize the actions of lysine deacetylase inhibitors, which have shown clinical promise in treatments for cancer.

Strategic Reading

Scanning the scientific literature these days is more akin to flicking through TV channels and trying to watch everything at once. Often the outcome of the scan will not have located any specific paper and it is unlikely to have involved any in-depth reading, but it achieves an undefined "assimilation" of knowledge from a melange of titles and abstracts, tables, indexes, databases, and figures. **Renear and Palmer** (p. 828) review how scientists use new forms of the "literature" and how the ascendance of novel computing technologies will combine to revolutionize the way scientific data is accessed, synthesized, and turned to practical use.

Continued on page792

CREDITS (TOP TO BOTTOM): QIAOWEI LI; SUZUKI *ET AL*

Continued from page 791

Why Birds of a Feather Flock Together

The biological determination of sociality, that is, why one might choose to associate with others and how many, has been unclear. **Goodson *et al.*** (p. 862) show that in gregarious finches, oxytocin-like receptors and their cognate ligand, mesotocin, are associated with group size choices. Receptor distributions clearly differentiate territorial species from flocking species. Furthermore, these compounds appear to play a role in affecting choice in affiliation in mammals, and thus may be conserved across evolutionary distant taxa.

Reducing Sleep Length

Humans, like most animals, need their beauty sleep. But the preferred amount and quality of sleep varies between individuals—and some individuals exhibit a heritable, lifetime tendency to sleep less then 6 hours per night. **He *et al.*** (p. 866; see the Perspective by **Hor and Tafti**) identified a mutation in humans associated with people who regularly require shorter than usual sleep duration. The mutation is found in the gene encoding a transcriptional repressor, DEC2, already implicated in regulation of circadian rhythms. Related mutations introduced into mice and flies similarly resulted in shortened sleep phases.

Friction in Microscopic Motor

Friction arises because adhesive bonds between two bodies must be broken in order for them to move relative to each other. Now **Bormuth *et al.*** (p. 870; see the Perspective by **Veigel and Schmidt**) have used single molecule measurements to characterize the frictional drag force of kinesin-8 motor proteins interacting with their microtubule track. Friction, arising from rupture of bonds with the track, constrains the speed and efficiency of the motor protein.

Monkey See Human Do

Imitation has been put forth as one mechanism through which cultural learning occurs. **Paukner *et al.*** (p. 880; see the Perspective by **Call and Carpenter**) now demonstrate that imitation may also contribute to prosocial behaviors. Capuchin monkeys behaved in a more affiliative manner—as assessed by direction of gaze, physical proximity, and token exchange—toward humans who imitated them as compared to humans who performed the same movements, but did not do so simultaneously.

Ironing Out Stress

The peptide hormone, hepcidin, is secreted from the liver in response to extracellular factors, including inflammation, and regulates iron homeostasis by controlling transmembrane iron transport. **Vecchi *et al.*** (p. 877) showed that intracellular stress signals in the endoplasmic reticulum also control hepcidin expression and can thus modulate local or systemic iron traffic. This mechanism occurs through the transcription factor CREBH, which is a known mediator of the inflammatory response. Collectively, the results suggest a direct link between the intracellular stress response, innate immunity, and iron metabolism.

Diverting Asperger Deficit

Placement of Asperger syndrome within the family of autism spectrum disorders (ASD) has always been a bit uneasy; although people with Asperger syndrome do exhibit the core impairments in social interaction and communication that are characteristic of ASD, they nevertheless perform well on tests that are thought to assess the ability to mentalize or to possess Theory of Mind skills. One of the classic tests of mentalizing ability is the false-belief task, in which subjects must be able to represent their own beliefs (true) and another's beliefs, which are false because they have not been given complete information, such as not having seen the transfer of a piece of candy from one drawer to another. People with Asperger syndrome succeed at the verbal form of the false-belief task, yet **Senju *et al.*** (p. 883, published online 16 July) show that this is owing entirely to their having learned how to cope with an existing and still demonstrable deficit in an implicit version of the false-belief task. That is, the core impairment is present, but conscious and explicit learning allows them to compensate.

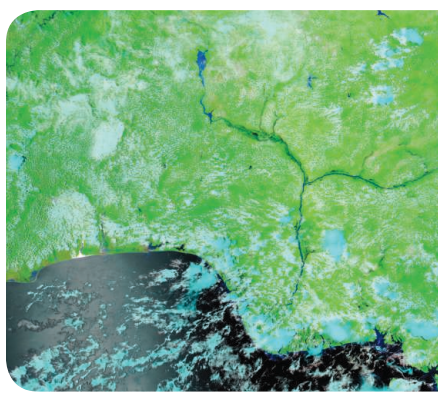
CREDIT: ANNA SCHMIDT

Akin L. Mabogunje is chairman of the Foundation for Development and Environmental Initiatives, Ibadan, Nigeria. E-mail: mabogun@skannet.com

New Land Reform in Nigeria

IN AN INTERVIEW FOR ALLAFRICA.COM THAT WAS CONNECTED TO HIS VISIT LAST MONTH TO GHANA, U.S. President Obama noted that progress was being made in the rule of law, anti-corruption efforts, and the protection of property rights in Africa. Success in these endeavors requires that Africa faces squarely several fundamental impediments to progress. In doing so, it must access the best available science and technologies not only from the natural sciences and engineering, but also from the social sciences. A critical example that combines all of these elements relates to property rights and land tenure reform, which is an issue at the heart of reducing poverty, promoting wide-ranging development, and ensuring the basic rights of African citizens.

The World Bank estimates that about 70% of the population of my country, Nigeria, lives on less than U.S.$1 to $2 a day. In discussions of poverty here and throughout Africa, far too little emphasis is placed on the economic incapacitation of the population due to their having no recognizable property rights. Nigerian citizens thereby miss out on many economically empowering possibilities that such rights confer, including access to the collateral that is required to acquire credit from financial institutions. This ability could bring them into the mainstream of the growing market economy



For several decades, many African countries have engaged in programs aimed at realigning their largely kinship-based, communal land tenure to the demands of an increasingly free-market economy. Initially, in the 1970s and 1980s, such land reform attempts devolved into legislating custodial rights of communal land to governments in a manner reminiscent of land nationalization. The growing inequities of this earlier reform effort, and the recognition that it prevents rural farmers from participating effectively in the evolving market economy, has propelled many of these countries to a new round of land reforms. Nigeria is one such country.

Today, land reform in most African countries is about how to induce or encourage largely peasant land-owning communities to confer individual property rights, rather than communal tenure, to the present tillers of the land. New, ongoing land reforms in countries including Ghana, Rwanda, Namibia, Tanzania, Liberia, Mali, Namibia, Uganda, and South Africa are dedicated to providing individuals, and in particular rural producers, with titles to their land. Many of these programs are supported by bilateral and multilateral agencies such as the British Department for International Development and the World Bank. As also noted by President Obama, modern science and technologies are being drafted for this purpose. These include the use of aerial photographs, satellite images, global positioning systems, geographic information systems, and various types of computer software. Para-surveyors are trained to read and interpret these remotely sensed images. And farmers are trained to appreciate not only the plans of their holdings, but also the many implications of a land title for their economic advancement.

On 2 April 2009, the President of Nigeria inaugurated a Presidential Technical Committee on Land Reform under my chairmanship. The Committee is "to determine individuals' 'possessory rights' using best practices and most appropriate technology to determine the process of identification of locations and registration of title holdings," as a forerunner to a National Land Commission. In order to identify, demarcate, and title the land holdings of individuals, one of the committee's major challenges will be to create mechanisms that are coordinately implemented by state and local governments.

The new Land Reform in Nigeria is not expected to be completed in the short or even the medium term. But as its effects begin to show, the process should accelerate to serve its goal of confirming the property rights of the majority of the population, whose citizens each hold a vital stake in a nation's most important natural resource.

– Akin L. Mabogunje

10.1126/science.1178058

CREDITS: (TOP) MR. ADAMU USMAN/IT DEPARTMENT OF UNITY BANK PLC., ABUJA, NIGERIA; (RIGHT) NASA

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EDITED BY GILBERT CHIN AND JAKE YESTON

EVOLUTION

Rarely Jumping Genes

Horizontal gene transfer (HGT), the movement of genes between distinct lineages, has been proposed as a means by which foreign genetic material can be incorporated and utilized within a species and its descendants. It is believed that most such transfer has occurred either via the engulfment of one organism by another, which generally is restricted to single-celled species, or during the course of host-parasite interactions. Richards *et al.* have identified suspected HGT events by comparing the genomes of six plant species with those of 150 prokaryotes and eukaryotes. Through stringent phylogenetic analyses, they found five fungus-to-plant and four plant-to-fungus transfers, including three cases in which transfer had been presaged by a jump from a prokaryote to a eukaryote. On the basis of these results, they suggest that HGT between eukaryotes does occur and can provide new genes that are useful enough to hang on to. — LMZ

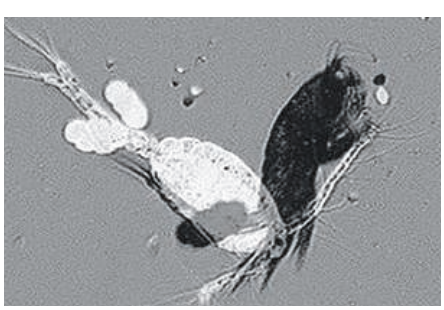
Plant Cell **21**, 10.1105/tpc.109.065805 (2009).

BIOPHYSICS

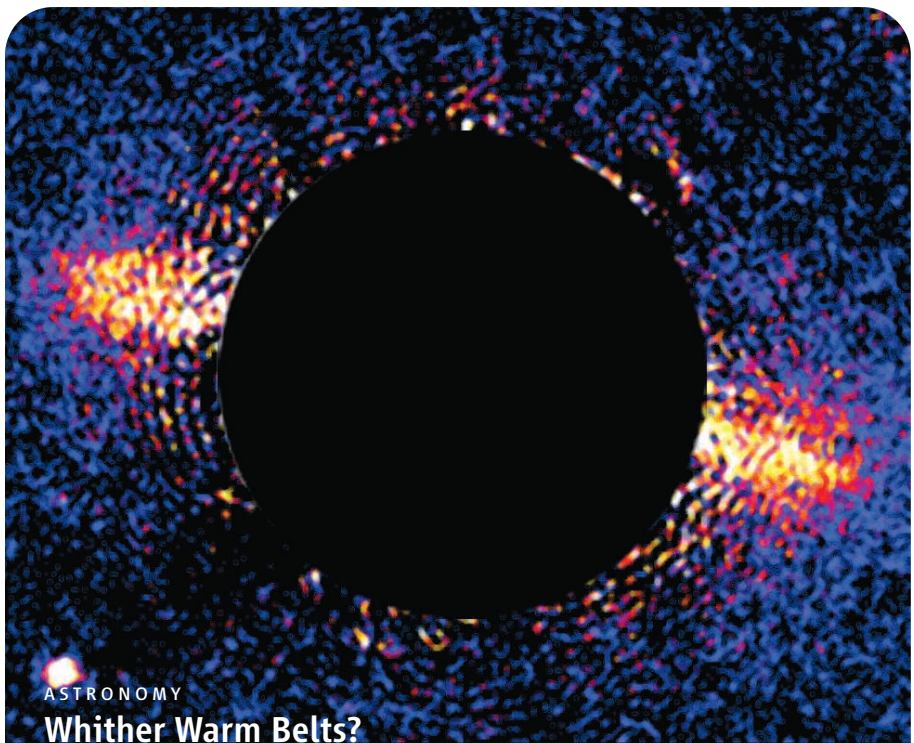
In the Blink of an Eye

The advent of high-speed video microscopy is helping to resolve many of the hitherto unseen mysteries of animal biomechanics. Copepods—crustaceans that typically are smaller than 1 mm and are a vital component of marine foodwebs—feed on even smaller prey that they capture in swift, surprise lunges. What has been a puzzle is how this is achieved without alerting the target or displacing it by fluid flow caused by the leaping copepod. Capturing 2000 images per second, Kiørboe *et al.* show that the attack is so fast (about 100 mm s^{-1}) that the viscous boundary layer ahead of the predator remains thin, thus preserving the element of surprise, as well as maneuverability. In terms of evolutionary feasibility, this strategy appears to be restricted to zooplankton in a limited size range (0.3 to 1 mm), which also possess a powerful musculature. — AMS

Proc. Natl. Acad. Sci. U.S.A. **106,** 12394 (2009).



Ambush of a dinoflagellate by *Oithona davisae*.



ASTRONOMY

Whither Warm Belts?

Is our solar system's architecture—four terrestrial planets, an asteroid belt, four Jovian planets and a Kuiper belt—common across the universe? Space-based infrared observations of circumstellar disks around nearby, Sun-like stars have suggested that cold disks of dust and debris analogous to our Kuiper belt are common, whereas warmer, asteroid belt analogs appear to be much rarer. Chen *et al.* reanalyzed spectra acquired with the Spitzer infrared telescope of three debris disks around nearby stars, and found that the thermal emission from all three is consistent with the presence of two dust populations, one warm and one cold. For one of the stars, HR 8799, the warmer dust lies within the orbits of the three known local planets, whereas the cold dust lies outside—a structure very much resembling that of the solar system. Eleven stars are now known to have multiple dust belts around them, in potentially similar fashion to the solar system's asteroid belt and Kuiper belt; the arrangement could prove even more common. — MJC

Astrophys. J. **701**, 1367 (2009).

PROTEIN CHEMISTRY

The Rag-and-Bone Trade

Sequencing ancient DNA can establish genetic differences among species and can document population changes over long periods, yet a major challenge is to avoid extensive sampling of rare and ancient specimens. One technique used to probe for the presence of well-preserved DNA in such specimens is to measure the extent of racemization of aspartic acid. Shapiro *et al.* have performed replicate amino acid analysis in three laboratories of 91 samples of human and animal bones that had been recovered from a range of burial environments and ages. They found no correlation between racemization and the successful amplification of ancient DNA. Collagen, which constitutes up to 95% of bone protein, contributes most of the aspartic acid in bone, but racemization is slowed by its triple helical structure; after denaturation into soluble gelatin, racemization proceeds readily, but gelatin is more easily lost in the burial environment. — LDC

Proc. R. Soc. B **276**, 2971 (2009).

CREDITS (TOP TO BOTTOM): PAUL KALAS, UC BERKELEY; KIØRBOE *ET AL., PROC. NATL. ACAD. SCI. U.S.A.* **106**, 12394 (2009)

Published by AAAS

PHYSICS

Up for Hours

The natural lifetime of an atomic or molecular excited state is typically measured in millionths, billionths, or trillionths of a second. Traditionally, the challenge in achieving precision has therefore been developing a laser source and detection system with sufficiently fine time resolution. Hodgman *et al.* tackle a challenge at the opposite extreme: precise measurement of the longest atomic excited state lifetime. Promotion of one of the two electrons in helium from the 1s to the 2s orbital, concomitant with a spin flip, produces the 2^3S_1 state, which persists for more than 2 hours before relaxing by emission of a photon in the extreme ultraviolet regime. The lifetime is especially long in this case because both orbital angular momentum and spin considerations render direct relaxation highly improbable in quantum mechanical terms. In measuring the comparatively long duration of such a state, the challenges are twofold: collisional interference by other atoms in the system must be compensated for, and the rare photon-emission events must be detected with great efficiency and matched to a well-determined number of excited atoms. The authors addressed the first of these challenges by isolating the excited helium atoms in a magnetically confined trap. To accurately quantify emission events, they switched from a previously implemented absolute detection scheme to a relative detection mode, comparing the number of photons emerging from atoms in the 2^3S_1 state to those emerging from atoms prepared in a shorter-lived (and thus more easily calibrated) state at somewhat higher energy. The extracted lifetime of 7870 s compares well with theoretical predictions. — JSY

Phys. Rev. Lett. **103**, 53002 (2009).

CANCER

Becoming Homesick

One mechanism by which organisms battle the spread of cancer cells is the process of anoikis—cell death that occurs when a cell's adhesion molecules lose touch with their cognate substrata that line the cell's normal home within the body. A deficit in anoikis, which is mediated in part by the tumor suppressor protein p53, may contribute to cancer metastasis. Cheng *et al.* used RNA interference to identify SIK1 (salt-inducible kinase 1) as necessary for p53-mediated anoikis in a transformed mammary epithelial cell line. Losing SIK1 was associated with an increase in micrometastases formed by transformed cells upon injection into mice. Losing the protein kinase LKB1 is also associated with cancer; LKB1 phosphorylates and activates SIK1, and in a lung tumor cell line that lacked LKB1, a constitutively activated from of SIK1 inhibited invasion and metastasis. An enhanced understanding of cancer metastasis may lead to strategies to prevent this deadly aspect of the disease's progression. – LBR

Sci. Signal. **2**, ra35 (2009).

APPLIED PHYSICS

Focusing Gem

Diamond is a hard, chemically stable, and wear-resistant material, and so constitutes an ideal coating for apparatus used under harsh conditions. At the same time, it is not particularly pliable and therefore would not seem to be the first material of choice for tunable optics. However, Kriele *et al.* show that a thin membrane of nanocrystalline diamond—deposited initially as a thin film on a sacrificial substrate that is subsequently etched away—can be used as a flexible lens. By simply varying the applied pressure, the authors show that the membrane can bend and bow, with the focal length of the diamond lens varying accordingly from infinity to just 3.5 mm. Diamond should thus find use in remote-sensing applications and imaging in harsh environments: no longer just a material to be seen with, but now a material to see with as well. — ISO

Appl. Phys. Lett. **95**, 31905 (2009).

MEDICINE

A Gut Feeling

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract. The exact cause is unknown, but environmental and host genetic factors are known to be important. Jansson *et al.* have carried out a large-scale study of the gut microbiota and detected thousands of metabolites in fecal samples from sets of identical twins that were discordant for Crohn's disease using high-resolution ion cyclotron resonance–Fourier transform mass spectrometry. They identified metabolites that could differentiate between diseased and nondiseased individuals, including some from pathways involved in tyrosine metabolism, which is consistent with genetic polymorphisms that have been associated with Crohn's disease. The data from this metabolomic study could lead to the identification of biomarkers for disease diagnosis. — HP

PLoS ONE **4**, e6386 (2009).

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Double Trouble

The yellow-lipped sea krait (*Laticauda colubrine*), a venomous marine snake that prowls the Western Pacific and Indian oceans, has developed a clever tactic for warding off enemies even nastier than it is: It fools them into thinking it has two heads.

The ruse combines skin markings and clever behavior patterns, biologists report online this month in *Marine Ecology*. While probing crevices and coral for food, the meter-long snakes temporarily drop their guard and become vulnerable to attack. But the krait appears to twist its tail around so that the black-and-yellow tip looks like a second head bearing an extra load of deadly venom. Other sea snakes, including *Hydrophis pachycercos*, seem to bear similar head-mimicking tail markings, the researchers say.

"I think the two-head hypothesis is an interesting and credible idea," says Martin Attrill of the University of Plymouth Marine Institute in the U.K. "Fish that sit around on reefs or the seabed … often have large 'eye' spots at the tail end to distract predators." The krait is odd in another way, says Arne Rasmussen of the Royal Danish Academy of Fine Arts in Copenhagen, who led the study: Unlike most other sea snakes, it spends almost half of its time ashore. "It remains to be confirmed whether the kraits use their sea defense tactic of motioning their tails when on land," Rasmussen says.

Rasmussen, *L. colubrine*, and *H. pachycercos*.

Get a Grip!

Despite their 91-kilogram heft, orangutans know how to live the high life, climbing from tree to tree 45 meters above the ground and snacking on fruit. It's tricky, though. The thinnest branches—twigs just a few centimeters across—tend to flex wildly and can cause fatal falls if they snap. But they're also located where fruit abounds and where the gaps between trees are the smallest, says Susannah Thorpe of the University of Birmingham, U.K.

Over the course of a year, Thorpe and colleagues watched orangutans in a Sumatran rainforest traverse the treetops more than 2800 times, recording their motions and estimating—after much training to ensure accuracy—the diameters of the branches used during the animals' travels. The animals made the most of even flimsy branches, often grabbing handfuls of them to steady themselves as they moved, Thorpe's group reported in the 4 August issue of the *Proceedings of the National Academy of Sciences*. The researchers found that although half the size of male orangutans, females were more conservative, sticking to bigger branches, whereas males were the risk-takers. "Humans would have a really hard time navigating these small branches," says Serge Wich, a primatologist with the Great Ape Trust in Des Moines.

Don't Hold Your Breath

Competitive breath-holders can float face down in a pool for more than 4 minutes, and the world record is 11 minutes 35 seconds. That can't be good for their brains, right?

To find out, researchers at Lund University in Sweden had nine trained breath-holders lie face-up on a couch and hold their breath to mimic the so-called static apnea pool competition. The breath-holders started with their normal warm-up routines: hyperventilation and "lung packing" (using tongue and throat muscles to force extra air into the lungs). Then they stopped breathing for an average of 5.5 minutes.

The volunteers' blood oxygen levels dropped by almost 80%, and concentrations of the protein S100B—a standard marker for brain damage—increased by 37%, the researchers reported online 2 July in the *Journal of Applied Physiology*. S100B is normally found inside brain cells and fluid but seeps into the bloodstream when an injury disrupts the brain's protective blood-brain barrier. The breath-holders' concentrations were lower than those seen in stroke or traumatic brain injury patients, and the levels recovered after 2 hours. "We probably only saw a temporary opening of the blood-brain barrier," says researcher Johan Andersson, but he warns that it is unclear if repeated barrier openings might produce long-term brain damage.

Ralph Potkin, a pulmonologist at the Beverly Hills Center for Hyperbaric Medicine in Los Angeles, California, calls the findings "disconcerting." But John Fitz-Clarke, a diving physiologist at Dalhousie University in Halifax, Canada, says that "it's way too premature to draw any conclusions about brain damage" from these findings.

RIP ISS Tool Bag

The world-famous $100,000 tool bag that astronaut Heidemarie Stefanyshyn-Piper lost in space last November finally met a fiery demise on 3 August. The backpack-sized bag—containing scrapers, two grease guns, some wipes, and a debris container—left Stefanyshyn-Piper's grip while she was cleaning a joint on one of the space station's solar panels. Amateur astronomers tracked the tool bag on Web sites and blogs, and some captured the time-lapse streak of its orbital track on film. In November, a hoaxer claimed to have found the bag on a golf course in Minnesota and briefly tried to sell it on eBay. No such luck for the real thing: NASA says the tool bag disintegrated completely in the atmosphere above the Pacific or Indian Ocean at about 10:30 a.m., EST. The tool bag will not be missed, a spokesperson for NASA said, as astronauts routinely drop space litter and let it burn up in Earth's atmosphere.

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Steven Chu speaks out 801

Margaret Hamburg's FDA agenda 802

HYDROLOGY

Northern India's Groundwater Is Going, Going, Going …

Farming is a thirsty business on the Indian subcontinent. But how thirsty, exactly? Satellite remote sensing of a 2000-kilometer swath running from eastern Pakistan across northern India and into Bangladesh has for the first time put a solid number on how quickly the region is depleting its groundwater. The number "is big," says hydrologist James Famiglietti of the University of California, Irvine—big as in 54 cubic kilometers of groundwater lost per year from the world's most intensively irrigated region hosting 600 million people. "I don't think anybody knew how quickly it was being depleted over that large an area," Famiglietti says.

The big picture of Indian groundwater comes from the Gravity Recovery and Climate Experiment (GRACE) satellite mission, launched in March 2002 as a joint effort by NASA and the German Aerospace Center. Actually two satellites orbiting in tandem 220 kilometers apart, GRACE measures subtle variations in the pull of Earth's gravity by using microwaves to precisely gauge the changing distance between the two spacecraft.

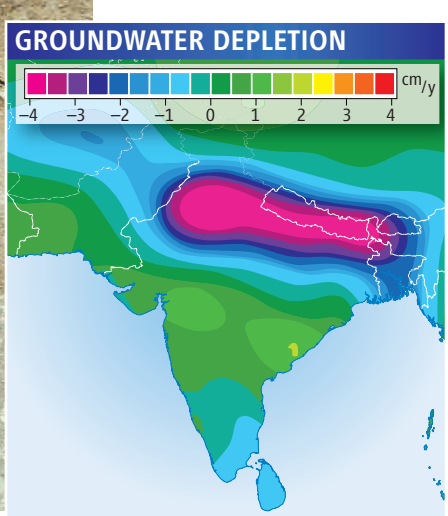
As the lead spacecraft passes over a patch of anomalously strong gravity, it accelerates ahead of the trailing spacecraft. Once past the anomaly, the lead satellite slows back down. Then the trailing spacecraft gets accelerated and again closes on the leader. By making repeated passes over the same spot, GRACE measures changes in Earth's gravity, which are due mainly to water moving on and under the surface. Most famously, GRACE has recorded the shrinking of ice sheets (*Science*, 24 March 2006, p. 1698); it has also detected shifting ocean currents, the desiccation of droughts, and the draining of large lakes.

Outside of wasting ice sheets, the world's largest broad-scale decline in gravity during GRACE's first 6 years came across a 2.7-million-square-kilometer east-west swath centered on New Delhi. That's according to a study in press in *Geophysical Research Letters* by geophysicists Virendra Tiwari of the National Geophysical Research Institute in Hyderabad, India; John Wahr of the University of Colorado, Boulder; and Sean Swenson of the National Center for Atmospheric Research in Boulder. Adjusted for natural variations due to changing precipitation and evaporation, the decline in gravity that GRACE determined equates to a net loss of 54 ± 9 cubic kilometers of groundwater per year, the group reports. That would produce a fall in the water table of about 10 centimeters per year averaged over the entire region.

A paper published this week in *Nature* reaches similar conclusions. Geoscientists combining GRACE data with hydrologic models for part of the same area calculate that groundwater levels fell an average of 4 centimeters per year between 2002 and 2008—more than official estimates and unsustainable in the long run.

A falling water table across the northern Indian subcontinent comes as no great surprise. The GRACE region of sharp groundwater depletion coincides with the world's most intensely irrigated land: 50% to more than 75% of the land is equipped for irrigation with pumped groundwater or reservoir water. And then there are those 600 million people drawing heavily on groundwater. But, the group calculates, the GRACE-determined depletion rate implies that groundwater was being pumped out 70% faster in this decade than the Central Ground Water Board of India estimated it was in the mid-1990s. The apparent surge in withdrawal would have been large enough to turn a once-stable water table into a falling one that demands ever-deeper wells and bigger pumps and may draw in salty or polluted water.

GRACE "has shown us we can do a pretty reasonable job from space" gauging groundwater depletion, says Famiglietti. "We can help regional water managers by giving them a holistic view of a whole system." Still, across the subcontinent, no one knows how far down the water goes. They just know, as Famiglietti notes, that "it's not bottomless." **–RICHARD A. KERR**



Going down. Several centimeters' worth of water (pink) disappears each year from beneath the northern Indian subcontinent.

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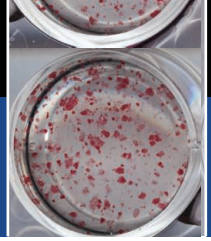

p53 and pluripotency
803

Watch your watts: Energy efficiency
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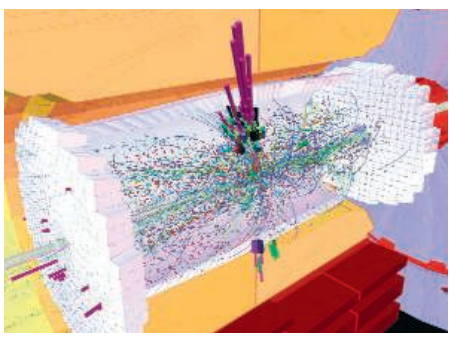
PARTICLE PHYSICS

Running at Half-Energy Keeps LHC in Race for Discoveries

The world's largest atom smasher will finally start blasting particles together this winter, but at only half of its maximum energy, officials at the European particle physics laboratory, CERN, near Geneva, Switzerland, decided last week. Officials say that energy is low enough to ensure that the 27-kilometer-long, $5.5 billion Large Hadron Collider (LHC) will not wreck itself the way it did last fall, just 9 days after circulating its first beams (*Science*, 26 September 2008, p. 1753). But the energy is also high enough, physicists say, to give the LHC a shot next year at surpassing its rival, the aging Tevatron collider at the Fermi National Accelerator Laboratory in Batavia, Illinois, which holds the record for the highest-energy collisions.

CERN officials have dialed down the energy to keep from overloading faulty electrical connections between the thousands of superconducting magnets that guide protons around the LHC (*Science*, 31 July, p. 522). Current doesn't normally flow through the questionable connections, but they need to be able to carry thousands of amps should the superconducting wire in the magnets warm up and lose its ability to carry electricity without resistance. Running at half-energy, "we have a safety margin of 2 or 2.5," says Stephen Myers, director of accelerators and technology at CERN. "It's a very conservative approach."



Get real. A simulation of a collision in the CMS detector. Real data could come by year's end.

Nevertheless, the energy is high enough to give experimenters a chance next year at seeing things that cannot be spotted at the Tevatron, says CERN's Fabiola Gianotti, spokesperson for the 2500-member team working with the LHC's ATLAS particle detector. The Tevatron smashes protons into antiprotons at 2 tera–electron volts (TeV). The LHC is designed to blast protons into protons at 14 TeV but will start at 7 TeV. Running at 7 TeV for a year, the LHC might spot new particles—such as those predicted by a theory called supersymmetry—that are slightly too heavy for the Tevatron to blast into existence. Running at 6 TeV would not suffice, Gianotti says, because the rates for making such particles would be so low that a definite discovery could not be made in a year.

Even at 7 TeV, researchers acknowledge, such a coup is a long shot. But Tejinder Virdee of Imperial College London, spokesperson for the 3600 researchers working with the CMS detector, notes that CERN officials hope to ramp the LHC's energy up 10 TeV later next year. "If the bulk of the data is taken there," he says, "the question [of what can be seen at 7 TeV] becomes moot." **–ADRIAN CHO**

REGULATORY POLICY

U.S. Panel Urges Clearer, Cleaner Role for Science

A bipartisan panel of experts suggested last week how U.S. regulatory agencies can make better use of scientific advice by being more open in selecting and vetting outside experts, clearer in defining the questions they want answered, and more rigorous in reviewing the relevant literature.

Although the 13-member panel included Democrats and Republicans, academics, environmentalists, and industry leaders, its recommendations may still set off political debate. Critics of the Bush Administration say that its proposals for separating scientific and policy issues would go a long way toward correcting the mistakes of the past 8 years, whereas Bush supporters say that it extends efforts initiated by that Administration.

"The report is a sign that it's a new ball game," says Jane Houlihan of the Environmental Working Group, an advocacy organization that battled Bush-era policies on a variety of health and environmental safety issues. "We've seen lots of corruption and closed doors, and the transparency and diversity called for in this report would be a big improvement."

Not quite, says panel member John Graham, who led White House oversight of regulatory efforts under President George W. Bush. Graham, now dean of the Indiana University School of Public and Environmental Affairs in Bloomington, says the report mirrors the Bush Administration's "call for clear disclosure of scientific uncertainties in regulatory analysis … and states clearly that the problems are not unique to any particular Administration."

The report comes from the Science for Policy Project at the Bipartisan Policy Center in Washington, D.C. (bipartisanpolicy.org). "Right now we have a mishmash of policies and no uniformity," says co-chair Sherwood Boehlert, a retired Republican representative from upstate New York. "What we need is a system that's as open as possible and one that's also consistent from one agency to the next."

Panel members also delivered a warning to the Obama Administration: Asking scientists to make policy undermines science and leads to bad policies. "We need to separate the science from the policy," says co-chair Donald Kennedy, a former editor-in-chief of *Science* who led the Food and Drug Administration during the Carter Administration. "Otherwise, groups end up criticizing the science because they don't like the policy."

The report concentrates on the use of advisory panels at regulatory agencies, only one aspect of the broader question of how to maintain scientific integrity across the government. The White House is currently reviewing recommendations from science adviser John Holdren for policy changes on the larger issue based on principles outlined in a 9 March presidential memorandum.

–JEFFREY MERVIS

CREDIT: CERN

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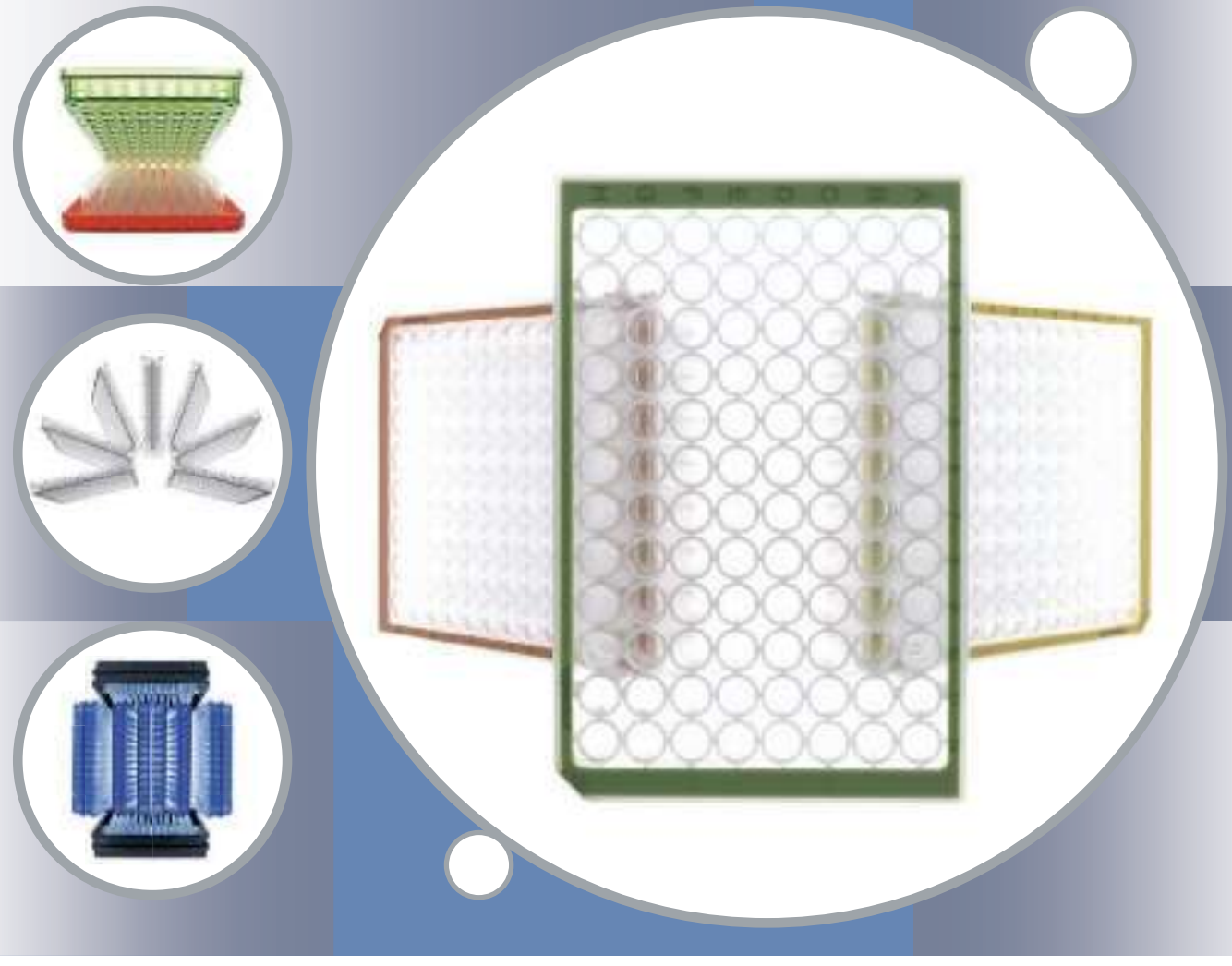

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U.S. SCIENCE POLICY

Chu Lays Out an Agenda for PCAST and Asks for Help

Energy Secretary Steven Chu says that one of his top budget priorities—to spend $288 million on eight break-the-mold energy-research centers modeled after the legendary Bell Labs—was rejected by the U.S. Congress because he failed to deliver the sales pitch in person. It was an uncharacteristic misstep for the 61-year-old Nobelist, who in his 6 months on the job has rarely missed an opportunity to tell policymakers exactly what's on his mind.

Chu described his lapse last week during a freewheeling discussion with the President's Council of Advisors on Science and Technology (PCAST) in Washington, D.C. Meeting for the first time since its 21 members were appointed in late April, PCAST had invited

Staying in touch. Steven Chu is a popular witness at congressional hearings.

Chu and other Obama Administration officials to suggest subjects it might want to tackle. The peripatetic Chu—who 4 hours earlier had keynoted a forum at Harvard University on the House of Representatives–passed climate change legislation with the bill's coauthor, Representative Edward Markey (D–MA)—obliged by giving the star-studded council an earful.

Chu began by fingering two key issues for the new Administration. The first is the difficulty of evaluating and scaling up science-education programs ("How do you analyze what works? And how exportable is it?"), and the second is barriers to industrial innovation ("Wall Street often punishes a company for deciding to invest in research"). Then he waded into more contentious waters. Chu asserted that the Defense Department has retreated from funding basic research over the past 2 decades, pinching support for high-risk, high-payoff ideas. And he tweaked the U.S. biomedical research community for being unduly critical of grant applications to the National Institutes of Health (NIH) from colleagues in the physical sciences. "I think they're afraid some computer scientist might take money from a biologist," he argued, with a nod to PCAST co-chair Harold Varmus, who had pushed to expand such interdisciplinary research while leading NIH during the Clinton Administration.

Chu also wanted something from PCAST. He urged the council, with its three Nobelists as well as industrial titans such as Google CEO Eric Schmidt, to help him assess the strengths and weaknesses of the sprawling Department of Energy (DOE). The agency has been assigned a leading role in meeting the Obama Administration's promises to reduce carbon emissions, lessen the country's dependence on foreign oil, and promote sustainable energy, along with $39 billion in one-time stimulus money to complement its $24-billion-a-year budget. And Chu made it clear that he's already reached some conclusions.

"[DOE's] Office of Science is as good as NSF [in supporting basic research], I think, but I have some questions about the applied areas," says Chu. "I need some ammunition from you guys, … because we're spending billions of dollars [on scaling up research with commercial potential]. I'd love for PCAST to look at what we're doing [in this area]."

Referring to his campaign for research hubs, he said he prefers to call them "Bell Lablets" because he'd like to emulate the old phone monopoly's wildly productive approach to supporting basic research. The idea is to identify an important problem and then work relentlessly at finding a solution, testing a multitude of ideas until one succeeds. In contrast, he says, most scientists funded by federal research agencies are told "you've got 3 years to get refunded, so that's how you work." The result, he says, is too often incremental progress on low-risk ideas.

A 2010 spending bill passed last month by the Senate contains money for three DOE-funded energy research hubs, whereas its House counterpart funds only one. Although Chu hopes that a House-Senate conference will settle on the larger number, he promises to do a better selling job next year.

"Congress asked the people below me, and they didn't know what I was thinking," Chu told PCAST members. "I didn't take the time to explain it personally to each member. That's the way it works around here. And I've learned that."

–JEFFREY MERVIS

CREDITS (TOP TO BOTTOM): AP IMAGES; ESA/NASA

ScienceNOW.org

From *Science*'s Online Daily News Site

Flu Virus May Trigger Parkinson's
Decades after the 1918 influenza pandemic, epidemiologists noted an uptick in the number of people with diminished mobility and other neurological symptoms reminiscent of Parkinson's disease. But despite this and other hints, the idea that viruses can trigger neurodegenerative diseases has remained controversial. Now researchers report new evidence for such a link: Mice infected with the H5N1 avian influenza virus lose the same dopamine-releasing neurons that are destroyed by Parkinson's disease. http://bit.ly/A2zxF

Horses Get Help From Their Friends
If you're a female horse, it pays to have a few girlfriends. Mares who form stronger social bonds produce healthier offspring and more of them, according to a new study. The finding adds to the growing evidence that friendship is an adaptation with deep evolutionary roots. http://bit.ly/IRmuJ

Whale Stranding: Sonar or Lunar?
On the morning of 3 July 2004, more than 150 melon-headed whales tried to beach themselves in Hawaii. A rescue team organized by the National Oceanic and Atmospheric Administration herded the whales back to sea the next day, though a calf died. One study blamed the incident on U.S. Navy sonar; another blamed the moon. Now, researchers believe they've finally gotten to the bottom of this attempted mass stranding. http://bit.ly/pvNTG

Do Clouds Come From Outer Space?

Most of Earth's clouds get their start in deep space. That's the surprising conclusion from a team of researchers who argue that interstellar cosmic rays collide with water molecules in our atmosphere to form overcast skies. http://bit.ly/5rebI

Read the full postings, comments, and more on **sciencenow.sciencemag.org**.

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NEWSMAKER INTERVIEW

Margaret Hamburg Aims to Strengthen FDA Science

Since taking charge of the U.S. Food and Drug Administration (FDA) in May, Commissioner Margaret Hamburg has been a whirlwind of activity. FDA is implementing a new law that gives it the authority to regulate tobacco products, taking a key role in the approval of the planned H1N1 pandemic flu vaccine, revamping its food-safety approach, and trying to make the agency's workings more transparent. Hamburg, a physician and scientist, served as New York City's health commissioner from 1991 to 1997 and led that city's fight against a resurgent TB epidemic. She has also worked as an assistant secretary of the U.S. Department of Health and Human Services and vice president for biological programs of the Nuclear Threat Initiative. In an interview with *Science* last week, she discussed her plans and dismissed rumors that she and Deputy Commissioner Joshua Sharfstein would each manage different FDA functions. This interview has been edited for brevity.

–ROBERT KOENIG

Q: What's being done to bolster the quality of science at FDA?

M.H.: One of my highest priorities … is to strengthen the science base within FDA and also extend our engagement with the scientific community. … We're making huge investments in exciting biomedical research. But if those investments and discoveries aren't married to investments in regulatory science that allow us to understand what products are safe and effective and ready for widespread use, I don't think we can fully realize those investments. … I'd like to see us … working with the Science Board and a range of scientific organizations, professional societies, research universities, industry scientists, and others. I'd like to develop a road map for strengthening regulatory science for the FDA and public health.

Q: Congress has given FDA the mandate to create a new Center for Tobacco Products. What is its focus?

M.H.: There's a very important set of research questions. We need to study the composition of tobacco products and understand both the addictive components of tobacco and the toxic chemical additives. We need to address how these substances are impacting health and ensure that there are not additional innovations by the tobacco industry that will put new products in the market that may be more addictive or more attractive to youth. There's been a new wave of flavored cigarettes and … other things that are of great concern, in terms of their attractiveness to young people as possibly gateways to addiction and smoking. We also want to look at issues of labeling … [and] marketing, with an emphasis on addressing some of the concerns about fraudulent claims and marketing that targets youth.

Busy agenda. Margaret Hamburg wants to emphasize the "science base" at FDA and tackle a range of regulatory issues.

Q: How does FDA approach its role in evaluating H1N1 pandemic vaccines?

M.H.: It's very likely that we will move forward with a vaccine that is licensed using the traditional, standard method of production that's used for seasonal flu vaccines—a "strain change" framework. … Clinical trials to be done by industry and at NIH will provide information on the dose required, whether or not some populations will need two doses, and also the question of adjuvants. We are moving forward with a flexible response on both looking at the data as it emerges and monitoring and tracking the evolution of the virus as it spreads and as we see how cases are evolving.

Q: Is FDA encouraging firms to design gene-based treatments that may only be effective or safe in certain subsets of the population?

M.H.: The issues around personalized medicine are intriguing but also very challenging from a regulatory point of view. I'm excited by the opportunity to work with [NIH Director] Francis Collins on these issues, and I think that, working together, we will be able to put in place a strategic framework to make sure that the scientific opportunities can translate into available products, with appropriate regulatory review.

Q: Some FDA scientists have expressed unhappiness with the agency in the past. Will FDA be open to dissenting views?

M.H.: I hope a hallmark of my tenure as FDA commissioner is to establish that FDA is a science-driven agency and that, as we make critical regulatory decisions, we welcome and foster the input of all of our scientists and outside experts so we can have a robust review of available data and a forum in which all voices can be heard.

Q: You and Joshua Sharfstein recently wrote an article describing FDA as a public health agency at heart. Does that change the way you approach regulation?

M.H.: I think it does. From the earliest days of the FDA's existence, it has always been a public health agency, and its mission is defined as promoting and protecting the health of the public. But because the FDA is also a regulatory agency, that core public health mission has sometimes been lost. Dr. Sharfstein and I want to make that the foundation on which our work is built. … We need to always ask the question: How does this impact health?

Q: How do you and Dr. Sharfstein define your responsibilities at FDA?

M.H.: When our appointments were announced, there was a crazy rumor that he would focus on drugs and I would do food safety and nutrition and tobacco. And there was no basis for that. I'm very much the commissioner, involved in every aspect of FDA activity. But I think we make a good team.

CREDIT: GETTY IMAGES

Published by AAAS

STEM CELL RESEARCH

Recipe for Induced Pluripotent Stem Cells Just Got Clearer

Three years of intense worldwide research efforts into induced pluripotent stem (iPS) cells have failed to decipher exactly how inserting just four or even three genes into an adult mammalian cell causes it to revert to an embryo-like state apparently capable of then diversifying into any of the body's tissues (*Science*, 1 February 2008, p. 560). A better understanding of this reprogramming, which could provide an alternative to controversial embryonic stem cells, might, among other things, enable researchers to boost the efficiency of the process, which works in just a few percent of the targeted cells.

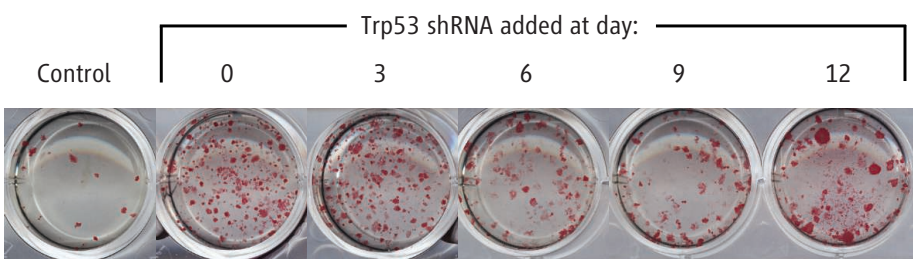
A clutch of five letters published online in *Nature* on 9 August cracks this black box by showing that *p53*, a gene noted for its role in suppressing tumors, hinders the generation of iPS cells. The work suggests ways to control the formation of iPS cells, although that capability will have to be used judiciously because *p53* helps ensure the fitness of generated cells.

Four of the five groups took different approaches in focusing on *p53*. A team led by Shinya Yamanaka of Kyoto University in Japan, who reported the discovery of iPS cells in July 2006, shows that the suppression of *p53* markedly enhances the efficiency of iPS cell generation. Juan Carlos Izpisúa Belmonte of the Salk Institute in San Diego, California, and co-workers improved the efficiency of iPS cell generation by inactivating both *p53* and another tumor suppressor gene while using only two of the original four reprogramming genes. Konrad Hochedlinger of Massachusetts General Hospital in Boston and Harvard University and colleagues report that deleting *p53* from cell populations that normally do not produce iPS cells gave them the ability to do so. And Maria Blasco and researchers at the Spanish National Cancer Research Center in Madrid show that *p53* prevents the reprogramming of cells carrying various types of DNA damage and that blocking *p53* enabled their ability to be reprogrammed. A fifth team led by Manuel Serrano, also of the Spanish National Cancer Research Center, looked at another tumor suppressor locus and found that this also limits reprogramming.

The letters "prove *p53* to be a major barrier in reprogramming … and further describe the underlying mechanisms," says Hongkui Deng, a cell biologist at Peking University, whose group's *Cell Stem Cell* report of last November indicated that blocking *p53* activity dramatically improves the efficiency of generating iPS cells. The additional details on how "the reprogramming process activates *p53* is important, indeed," says Shin-ichi Nishikawa, a stem cell researcher at the RIKEN Center for Developmental Biology in Kobe, Japan.

Although the involvement of *p53* seems to be confirmed, the nature of its role is still up for debate. But "whether p53 and [a gene it regulates] have direct roles in reprogramming, or [whether] they simply regulate proliferation and survival during iPS cell generation, remains to be determined," says Yamanaka.

It does seem that manipulating *p53* to generate iPS cells should be done sparingly. Blasco says it is clear that *p53* is "limiting the reprogramming of suboptimal cells"—normally a good thing, so switching off *p53* could allow cells from aged or diseased patients to be regenerated for research or therapy. But "once the iPS cells are obtained, *p53* has to be turned back on to eliminate damaged and unhealthy iPS cells," she says. She adds that the papers "represent a conceptual advance on what we know about the reprogramming process." But this is still just a peek into the black box of reprogramming. **–DENNIS NORMILE**



CREDIT: JOSE M. POLO AND KONRAD HOCHEDLINGER

Blocked! Adding a molecule (Trp53 shRNA) that inhibits the activity of *p53* at any of several days enables the proliferation of iPS cells (red clusters) as compared to a control (*left*).

ScienceInsider

From the *Science* Policy Blog

Senate appropriators say that staff members of an ambitious **children's health study** at the U.S. National Institutes of Health have committed a "serious breach of trust" in withholding the ballooning costs of the project. The director of the study, which hopes to follow the health of 100,000 children from before birth through age 21, has changed jobs, and there are rumors that Duane Alexander, the 69-year-old head of the institute overseeing the project, may soon retire.

The cash-strapped **University of California plans to lend $200 million** to the state government, which will then give the money back. The university has a better credit rating than the state, which means it can borrow money at a lower interest rate.

Two ships that service the **U.S. Antarctic research program** are likely to fall victim to an expected ban on the heavy-grade fuel **oil** they use. The National Science Foundation has 2 years to come up with a solution.

Congress has given itself 2 months to reconcile differences in **revamping the $2.5-billion-a-year Small Business Innovation Research** and the **Small Business Technology Transfer** programs.

A National Academies panel wants NASA to reopen a research shop that it shut down for budgetary reasons 2 years ago. The report says the space agency could use some of the "creativity" that marked the **NASA Institute for Advanced Concepts.**

Last week, the U.S. Senate confirmed **Francis Collins** to be head of the National Institutes of Health and **David Kappos** to lead the Patent and Trademark Office. The nomination of **Cass Sunstein** to oversee regulatory policy at the White House remains stalled, however, although Majority Leader Harry Reid (D–NV) has promised a vote in September. Meanwhile, bioethics expert **R. Alta Charo** is joining the Food and Drug Administration as a senior adviser to Commissioner Margaret Hamburg.

For more science policy news, visit **blogs.sciencemag.org/scienceinsider.**

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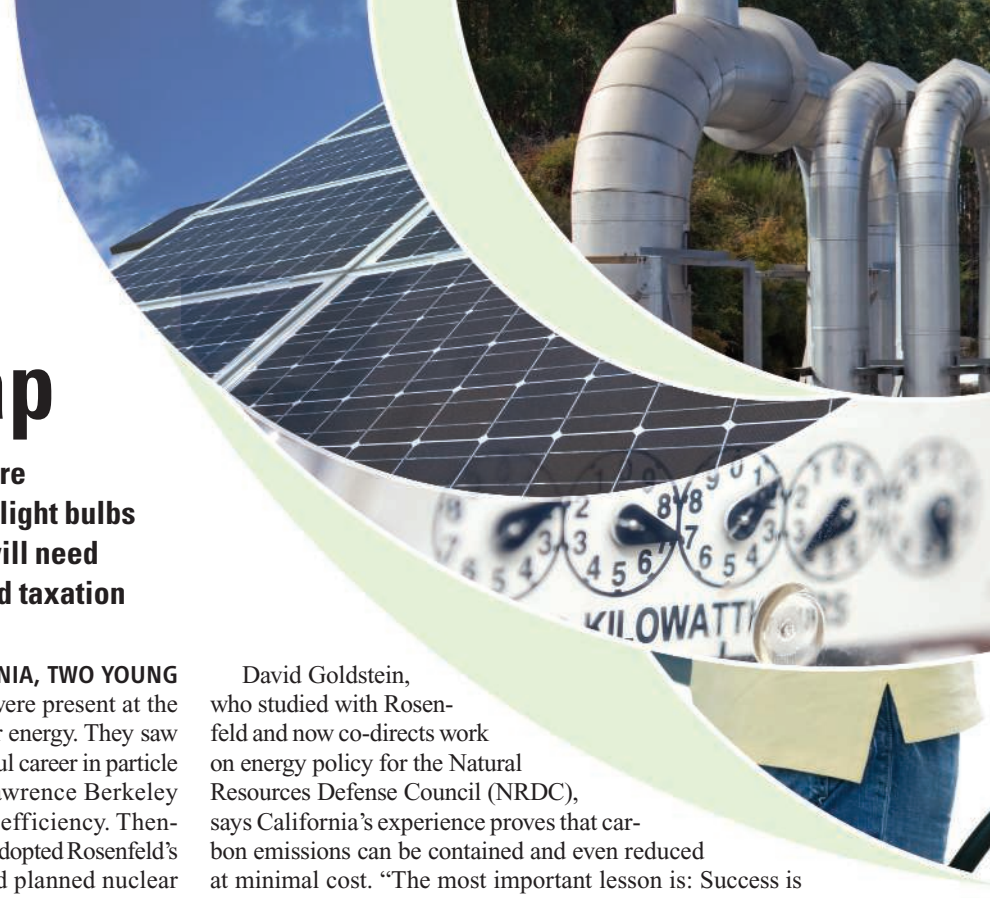


Leaping the Efficiency Gap

Experience has shown that there is more to saving energy than designing better light bulbs and refrigerators. Researchers say it will need a mixture of persuasion, regulation, and taxation

THIRTY-FIVE YEARS AGO IN BERKELEY, CALIFORNIA, TWO YOUNG physicists named Steven Chu and John Holdren were present at the birth of a campaign to curb Americans' appetite for energy. They saw their colleague Arthur Rosenfeld abandon a successful career in particle physics and set up a new research division at Lawrence Berkeley National Laboratory (LBNL) devoted to energy efficiency. Then-Governor Jerry Brown and state regulatory agencies adopted Rosenfeld's ideas with astonishing speed. California canceled planned nuclear power plants, passed pathbreaking efficiency standards for refrigerators and buildings, and ordered electric utilities to spend money persuading their customers to use less power.

Today, Chu, now the U.S. secretary of energy, cites Rosenfeld as a model for scientists and California as a example for the nation. He points out that per capita electricity consumption in California stayed flat for the past 30 years yet rose 40% in the rest of the United States. That flattened curve even has a name: the Rosenfeld Effect. Together with Holdren, now President Barack Obama's science adviser, Chu has made efficiency the heart of the Obama Administration's energy strategy. Tighter appliance standards are on a fast track through the Department of Energy bureaucracy. Billions of dollars from the stimulus package are pouring into programs to weatherize and retrofit homes with energy-saving technology. Chu says such investments quickly pay for themselves in lower energy bills: "Energy efficiency isn't just low hanging fruit; it's fruit lying on the ground."

Online
sciencemag.org
Podcast interview with author Dan Charles.

Efficiency pioneer. Arthur Rosenfeld traded particle physics for cutting-edge research into energy-saving technologies.

David Goldstein, who studied with Rosenfeld and now co-directs work on energy policy for the Natural Resources Defense Council (NRDC), says California's experience proves that carbon emissions can be contained and even reduced at minimal cost. "The most important lesson is: Success is possible, and a fairly limited set of policies gets you most of the way there," Goldstein says. And, he adds, it's not hard to go even further with energy saving: "The practical limits [of increased efficiency] have never been tested."

But not everyone views California's success story as so clear-cut. Alan Sanstad, an LBNL researcher who also worked with Rosenfeld, looks at the same data and concludes that California's efficiency offensive wasn't nearly effective enough. He points out that California's *total* energy use over the past 3 decades grew at almost the same rate as it did in the rest of the country, while the state's population soared. Anant Sudarshan and James Sweeney of Stanford University's Precourt Energy Efficiency Center (PEEC) recently calculated that the state's energy policies can take credit for only a quarter of California's lower per capita electricity use. The rest is due to "structural factors" such as mild weather, increasing urbanization, larger numbers of people in each household, and high prices for energy and land that drove heavy industry out of the state.

For Sanstad, there's a clear lesson: Meeting the more ambitious goal of reducing greenhouse gas emissions will require more aggressive measures that cause some economic pain. "The real potential of energy efficiency is not going to be realized until we get away from the idea that it has to pay for itself," he says.

The biggest challenge is not inventing new technology but persuading more people to adopt technology and practices that already exist. A new generation of researchers and government officials is now examining new strategies for energy efficiency, looking for the key—or a whole ring of keys—that will unlock its full potential. "It's a wonderful opportunity to which we have to rise," says Ashok Gadgil, an energy technology researcher at LBNL. "We were preparing for this for 20 years; now come under the spotlight and sing!"

The human dimension

Rosenfeld and Edward Vine had a friendly, long-running argument during their 2 decades as colleagues at LBNL. Rosenfeld believed in technology. When he testified before the U.S. Congress, as he did

CREDITS (TOP TO BOTTOM): JUPITERIMAGES; LONNY SHAVELSON/WWW.PHOTOWORDS/NEWSCOM

Published by AAAS

Waste not, want not. Everywhere you look, energy can be used more efficiently, but doing so requires care and cash. The potential gains are huge, dwarfing expected increases in production of renewable energy.

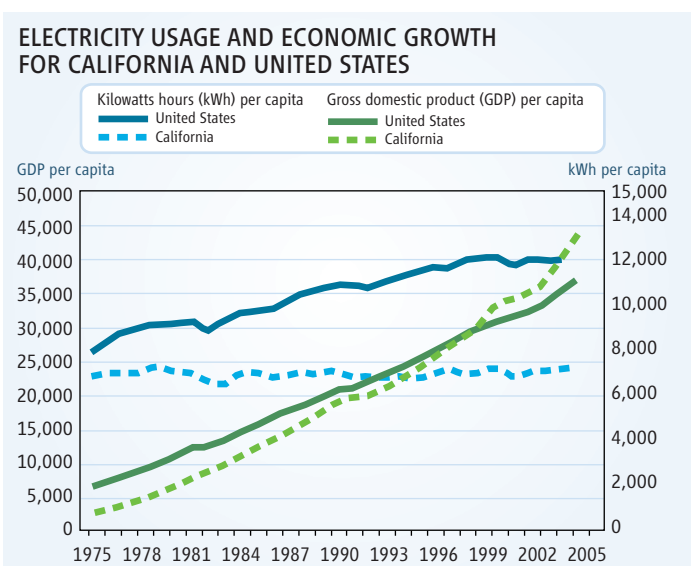
frequently in the early 1980s, he always came with props in hand: compact fluorescent light bulbs, heat-shielding windows, or computer programs for predicting the energy use of new buildings. But Vine, whose Ph.D. is in human ecology, wasn't convinced of technology's power. "We can't assume, if we have a great technology, that people will rush to stores and buy it," Vine says. "We need to find out how people behave, how they make decisions, how they use energy, and we need to work with them."

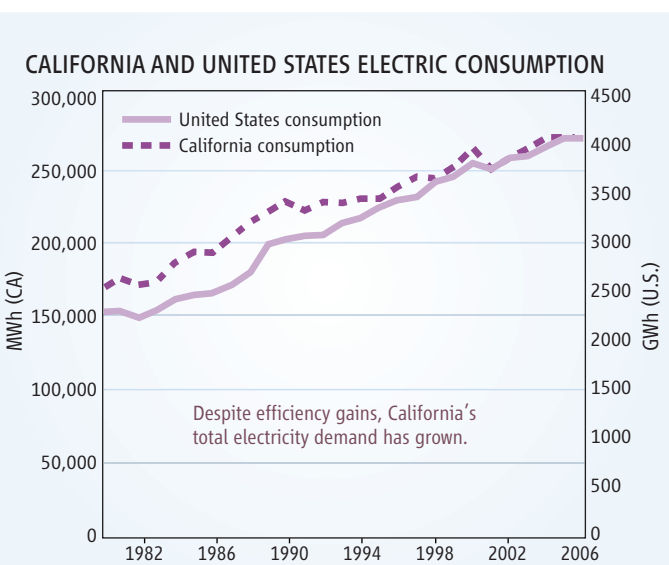
For the most part, energy-efficiency programs around the country have followed Rosenfeld's line. They offer financial incentives for adopting energy-saving, cost-effective technology, and trust that consumers will follow their economic self-interest.

Yet many researchers are now coming around to Vine's point of view. Consumers don't seem to act like fully informed, rational decision-makers when they make energy choices. Many avoid making choices at all. Give them a programmable thermostat, and they won't program it. Offer them an efficient light bulb that pays for itself in 2 years, and they won't buy it. Builders don't take full advantage of the cheapest source of lighting, the sun. Even profit-seeking businesses sometimes make little effort to control their energy use, says Ernst Worrell, who teaches at Utrecht University in the Netherlands and studies companies all over the world. "There are companies that spend 20% of their operating cost on energy, but upper management doesn't know where that money is going," Worrell says. "They see energy costs as an act of God."

Every once in a while, however, circumstances force people to focus on energy. When they do, the results can be astonishing. In April 2008, an avalanche cut a transmission line that supplied Juneau, Alaska, with cheap hydropower. The city switched over to diesel generators, but the electricity they produced cost five times as much. City officials went looking for help and contacted Alan Meier, an LBNL conservation expert.

"In a crisis, you can talk about behavior," says Meier. The city spread the word that "good citizens save electricity." And they did, lowering thermostats, turning off lights, and unplugging electronic equipment. Over 6 weeks, Juneau's electricity consumption fell by 40%, yet Juneau's economy did not falter. The transmission line was repaired within 3 months; electricity use rebounded, but it remains about 6% below its preavalanche level. A similar phenomenon, but on a much larger scale, happened during a 2001





SOURCES (LEFT TO RIGHT): CALIFORNIA ENERGY COMMISSION; 2008 ELECTRIC POWER RESEARCH INSTITUTE

Rosenfeld effect. The average Californian uses less electricity than a typical person uses in the rest of the country. That gap has grown wider over the past 30 years, even though California has become relatively more wealthy.

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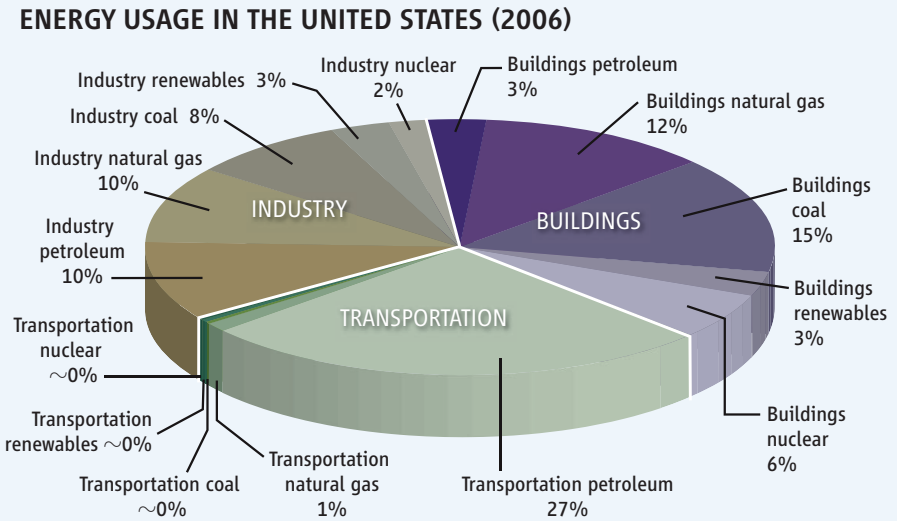


Energy pie. Most energy in the United States is used for one of three purposes: transportation; heating, cooling, and lighting buildings; or industrial production.

"Every person I know who has a Prius, they get a big grin when I mention feedback, and they have to tell me their personal story about how they've reduced their energy use."

—CARRIE ARMEL, PRECOURT ENERGY EFFICIENCY CENTER, STANFORD UNIVERSITY

energy crisis in Brazil. The country "cut its power consumption by 20% in 6 weeks. That shows you how much behavior can get you," Meier says.

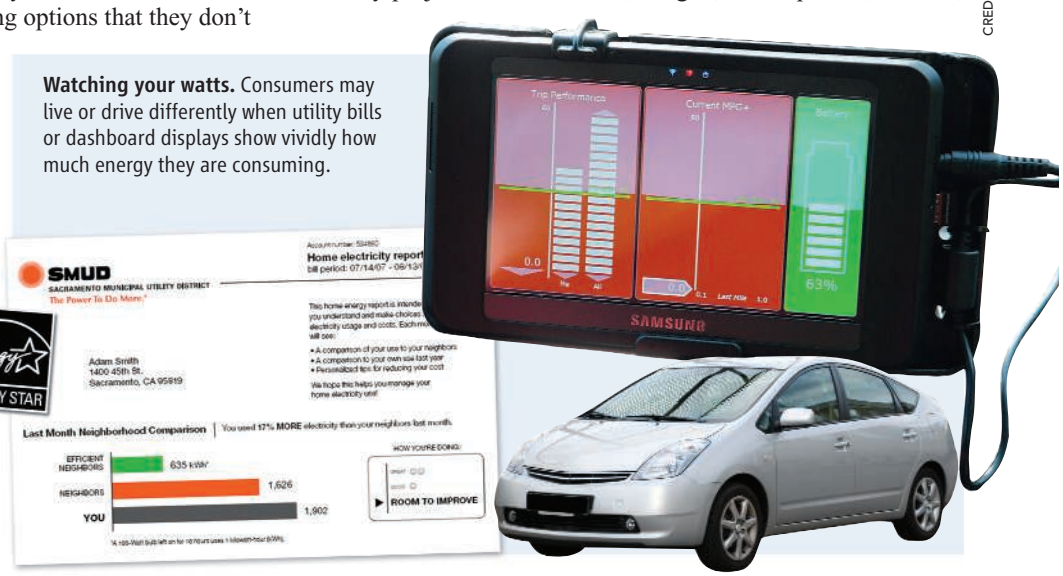
Stories such as this one have fueled a recent explosion of interest in ways to influence people's energy-using behavior. When Carrie Armel, a neuroscientist at Stanford's PEEC, helped organize a conference on the topic in 2007, "we were expecting 150 people and sold out at 500," she says. "Last year we were sold out at 700. This year we're opening it up to 800 people."

Research has produced some intriguing insights. For instance, people believe that others waste energy because of their inner characters, but they regard their own wasteful practices as the product of circumstances. More information doesn't usually produce energy-saving behavior; experts leave the lights on, too. The concrete example of a friend or neighbor who walks her children to school is much more powerful than any impersonal exhortation to drive less. And don't tell someone that he needs to save energy because nobody else does. "It could end up backfiring," Armel says, because most people don't like the feeling of being in the minority.

When people are asked to choose among options that they don't fully understand, such as a list of investment plans, they tend to select the "default option": the one that doesn't require them to change anything or that seems most popular. Right now, that tendency works against efficiency. In appliance stores, says LBNL's Jonathan Koomey, who also works as a consultant for companies, the most efficient "Energy Star" machines are usually aimed at high-end customers. They're manufactured in low volumes and come with additional features that drive up the price. The marketing strategy sends a clear signal that these are not appliances that the store expects most customers to buy. "You can change that," says Koomey. "If Costco or Wal-Mart announce that they are only carrying Energy Star products, suddenly the efficient product becomes the standard product."

A few utilities are now designing programs based on the conclusions of behavioral science. Because people like to keep up with their neighbors, the Sacramento Municipal Utility District (SMUD) began an experiment in competitive fuel saving. Starting in April 2008, 35,000 randomly selected customers got bills showing how their energy use stacked up against the average usage of their neighbors. According to SMUD, the typical customer in the experiment responded by cutting consumption by about 2%. SMUD also got some angry mail. "I resent being told I am below average," one customer wrote. "I pay my bill on time; … leave me alone." SMUD plans to expand the program to 50,000 customers next year.

Some energy-conservation advocates are rediscovering the old-fashioned virtues of porch conversations and town meetings, now renamed "social marketing." "There's more interest now in looking at people as part of a community, a culture, a neighborhood, a church group," says Vine. That approach paid off 20 years ago during energy-efficiency projects in Hood River, Oregon, and Espanola, Ontario,



Watching your watts. Consumers may live or drive differently when utility bills or dashboard displays show vividly how much energy they are consuming.

CREDITS (TOP): APS REPORT (SEPTEMBER 2008); LINDA A. CICERO/STANFORD NEWS SERVICE

Published by AAAS

which reached an impressive proportion of the citizenry. According to Hugh Peach, who helped manage the Hood River project for the energy company Pacific Power & Light, 85% of homes in the community received state-of-the-art energy audits and free efficiency upgrades. In Espanola, more than 90% of homes participated.

Peach compared the process to a political campaign. The utility sat down with local leaders, followed their advice, and relied heavily on local volunteers. The process was time-consuming and labor-intensive but, Peach says, a pleasure. There was "a lot of community spirit. People just saw it as the right thing to do."

The next big force for behavioral change may be technology that brings consumers face-to-face with their energy consumption. A simple version of such energy feedback is the dashboard of a Toyota Prius hybrid car, which displays the rate at which the car is burning gasoline. No one has carried out a controlled study of how drivers react to it, but "every person I know who has a Prius, they get a big grin when I mention feedback, and they have to tell me their personal story about how they've reduced their energy use," says Armel. At the Institute of Transportation Studies at the University of California, Davis, 12 Prius cars have been outfitted with more detailed dashboard displays. Researchers will use them to study how drivers react to different kinds of information, such as energy consumption, emissions, or the cost of fuel being burned.

The same feedback is now becoming available for homes and businesses. About 40 million homes will soon get "smart meters" that record every spike or dip in electricity use, hour by hour. Various companies, including Google, are devising ways to deliver that information directly to consumers, either via the Internet or by using displays that are linked to the smart meters themselves. Studies show that consumers usually respond to such feedback by cutting their energy use by 5% to 10%. But Sanstad thinks that may be only the first step because this information could create new markets for energy efficiency. "I think it will open a lot of doors," he says. "When people have

Soap Operas to Save Energy

In developing countries such as Mexico and Ethiopia, serial dramas on radio and television have proved to be successful tools for social change. Their fictional characters have become role models for real life, encouraging women to use birth control or stay in school.

Filmmaker John Johnson is deploying a similar technique, adapted to the YouTube age, to persuade Americans to act against climate change. Two years ago, he set up the Harmony Institute, an environmental media group based in New York City. Now it is collaborating with the creators of popular video programs on the Web to develop scripts that show people conserving energy and water and considering how their consumption choices might affect the planet. The first programs will go online later this year.

"We were fascinated by this amazing way of reaching people through the medium that they already are using," says the institute's deputy director, Debika Shome. Shome, who previously worked at Columbia University's Center for Research on Environmental Decisions, says the online dramas will harness ideas from behavioral science—for instance, that "people are more likely to make changes if it's not about sacrifice but about community."

Shome won't reveal where on the Web the institute's "product placement for ideas" will appear because she says publicity would make it harder to measure the show's impact. The Harmony Institute plans to survey viewers both before and after the new episodes to see if there's any change in their attitudes and behavior.

–DAN CHARLES

Many More More-Efficient Computers

Few technologies can match the efficiency gains made in computing. Compared with the first personal computers introduced in 1981, today's machines need a millionth as much energy to flip a bit. However, they also flip a million times as many bits per second, and there are more than a billion of them in the world. One watt in every 50 now goes to powering computers, and industry leaders are eager to keep that figure from growing.

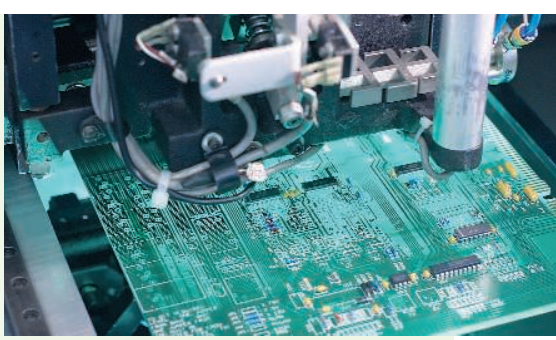
Big savings can still be made by using more-efficient power supplies and automatically putting idling computers into an energy-saving "sleep" mode. The 2-year-old Climate Savers Computing Initiative—begun by search-engine giant Google, chipmaker Intel, and the World Wildlife Fund—asks companies to pledge to do that in hopes of reducing annual carbon emissions from computing by 50%, or 54 million tons, by 2010.

Hardware engineers are applying the turn-out-the-lights strategy within microprocessors themselves. "The number-one issue with processors is that they've become pretty good at what they do, so they spend a lot of time waiting for something to do," says William Swope, vice president and general manager of Intel's Corporate Sustainability Group in Portland, Oregon. To prevent idling, Intel's latest high-end processor runs algorithms that slow down or stop parts of the chip that aren't being heavily used. The new chip consumes 90% less energy per computation than its predecessor from 4 years ago.

At the software level, engineers are making gains by using a process known as virtualization to run several copies of an operating system on a single processor and to shift those "virtual machines" from one physical processor to another. In times of low demand, a big data center can now shift work onto a fraction of its thousands of servers. Erik Teetzel, Google's energy program manager in Mountain View, California, predicts that "cloud computing" will move almost all computing into such highly efficient data centers. "You're going to have a lot of people with 5-watt devices accessing these centralized resources," Teetzel says.

Although computers' energy demand has increased, that expenditure must be weighed against the savings it brings to other machines such as cars and refrigerators, Swope says. "Computers consume about 2% of the power used in the world," he says. "And yet every aspect of computing has made the other 98% [of energy use] more efficient."

–ADRIAN CHO



Nap time. Computer chips can be designed to put subunits to "rest" when not needed.

CREDITS: (BOTTOM LEFTHAND PAGE TO RIGHTHAND PAGE) SMUD; TAI STILLWATER; JUPITERIMAGES

Published by AAAS

Wanted: Help With Building Design

Light, naturally. Movable glass shutters on this office building near Zurich, Switzerland, let in sunlight but keep out unwanted heat.

To create a truly efficient building, don't just buy more insulation, better windows, and efficient lighting. "That gets you a 10 to 30% improvement," says Stephen Selkowitz of Lawrence Berkeley National Laboratory (LBNL) in California. Bigger energy savings, at a lower cost, come from designing a whole building to manage heat and light in an energy-saving way. But current computer-aided design tools are not making it easy for architects to design for efficiency. New software is needed.

An inherently efficient building demands a delicate balance of opposing forces. Big windows provide natural light, for instance, but can place heavy demands on a cooling system in the summer. To make it work, architects need to predict the flow of air and heat through a structure, arranging windows and the heat-storing "thermal mass" of walls and floors in ways that maintain a stable, comfortable temperature inside.

Software can simulate all these phenomena, but the most accurate tools, such as the U.S. Department of Energy's (DOE's) EnergyPlus software, are "unfriendly to nonengineers," says LBNL's Ashok Gadgil. John Haymaker, a specialist on building design at Stanford University in Palo Alto, California, says architects and engineers often use EnergyPlus simply to show that a planned structure will meet building codes or satisfy a client's wishes. What's needed, he says, are more user-friendly tools that let architects experiment with different configurations of a building and find more energy-saving solutions.

Many groups are taking on that challenge. The software giant Autodesk and Integrated Environmental Solutions, based in Glasgow, U.K., are trying to incorporate more sophisticated energy simulations into their design software.

DOE, meanwhile, is funding an effort to mate EnergyPlus with Google's user-friendly SketchUp software. "I am encouraged that this will be the new face of design," says Haymaker.

All simulation tools have one big limitation, however. "You never know how people will use a building," Haymaker says. His own office building at Stanford is an example: It is not living up to its energy-saving promises because its inhabitants brought in unanticipated lighting, computer equipment, and space heaters. So the best design software will predict not just a building's behavior but also the actions of people inside.

–DAN CHARLES

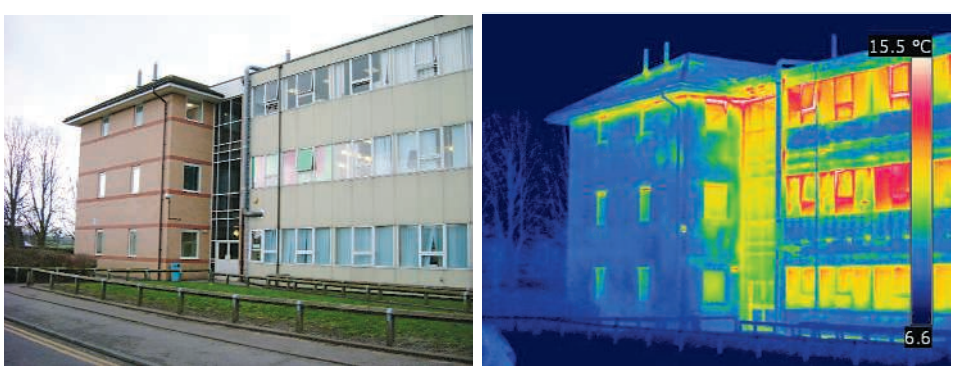
this, what new things will they want?" At a meeting of utility regulators in February, Jason Grumet, executive director of the National Commission on Energy Policy in Washington, D.C., said, "this may change the mood surrounding efficiency. It could make it cool."

Battling perverse incentives

Behavioral change was never a top priority for NRDC's Goldstein. "It's real and important," he says, but it's something "you can only do once." Technological innovation, on the other hand, leads down a path of continuous improvement. What keeps people from adopting efficient technology is not a quirk of human psychology, he says, but institutional roadblocks—what he calls "market failures."

Much has been written about market failures, with little demonstrated success in overcoming them. A prime offender is the "principal-agent problem," which occurs when someone gets to spend another person's money. Hotel guests, for instance, can waste hot water because they don't pay for it. Landlords buy cheap, inefficient appliances because their tenants pay the utility bills. LBNL's Meier found his own favorite example: the humble set-top box that comes with cable TV services. Each box can consume up to 40 watts of electricity continuously—more than an efficient refrigerator. Cable subscribers can't choose which box they get, and cable companies have no incentive to make the boxes more efficient.

"This situation is more important than you might think," says Meier. According to his calculations, some form of the principal-agent problem afflicts a quarter of all residential energy use in the United States. There are ways to solve it, he says. In Japan, companies that deliver a vend-



Snapshot of waste. Infrared cameras quickly show where heat is escaping from a building. The older building on the right, for instance, has leaky windows.

CREDITS (TOP TO BOTTOM): GAETAN BALLY/KEYSTONE/LANDOV; PIXEL THERMOGRAPHICS, WWW.PIXELTHERMOGRAPHICS.CO.UK

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ing machine to a site also pay for the electricity that the vending machine consumes. Not surprisingly, those companies now use more energy-efficient vending machines.

Koomey has studied energy use in big data-processing centers and found similar problems. "The IT department and the facilities department have separate budgets," he says. "The IT department buys the equipment, but they don't pay the electric bill. They don't have an incentive to spend even $1 to buy a more efficient server."

Brazil "cut its power consumption by 20% in 6 weeks. That shows you how much behavior can get you."

—ALAN MEIER, LAWRENCE BERKELEY NATIONAL LABORATORY

Such institutional barriers bedevil the fragmented, tradition-bound construction industry. Buildings account for 40% of the country's energy use. Amory Lovins of the Rocky Mountain Institute, perhaps the country's most eloquent prophet of efficiency, wrote in 2005 that architects, engineers, builders, and maintenance workers are "systematically rewarded for inefficiency and penalized for efficiency." Builders are trained to satisfy the minimal standards of construction codes, but they rarely exceed them.

Energy Secretary Chu told a congressional committee in July that the average new building could use 40% less energy by simply adopting off-the-shelf technology such as automatic controls that turn off lights when they aren't needed and highly insulating windows that also reflect much of the sun's heat in summertime. Retrofits to older buildings, he said, could cut energy use in half and eventually pay for themselves. Innovative architectural designs, arranging windows, shade, and ventilation so as to minimize the need for additional light or cooling, could cut energy use by 80% below today's average (see sidebar, p. 808).

Frustratingly, "green" buildings often don't deliver what their designers promised because of mistakes in design, shoddy construction, or poor maintenance. "No one measures building performance," says Stephen Selkowitz, head of the Building Technologies Division at LBNL. "I'll ask 100 architects, 'How many of you design energy-efficient buildings?' Almost all of them. Then I'll ask, 'How many of you know the measured performance of your last building?' Not a soul! If you don't know how well you did, how will you ever do any better?"

The California Energy Commission plans to require all new buildings in California to consume no net energy by 2030. Rooftop solar panels will generate as much energy as the building requires. In Europe, an even more ambitious model is gaining ground: the superinsulated, airtight "passive house," born in Germany, which consumes 10% of the energy of a typical house.

Such buildings are possible, and hundreds already exist, but most are relatively small. When it comes to large office buildings,

many architects and developers struggle to reach more modest goals, such as cutting energy use in half. "A lot of people start down this path, but they get hung up on cost, they get hung up on complexity, they can't find vendors, they can't find designers who can do it. Owners lose faith," says Selkowitz.

Tougher efficiency standards for buildings could change that, creating a network of architects, equipment suppliers, and construction companies that know how to make highly efficient buildings. Such regulations were the first steps in California's efficiency campaign 30 years ago. The long-term benefits, especially if one includes benefits to the environment, can be substantial. "I'm slowly drifting to the position—let's mandate as much stuff as we can," Selkowitz says.

A few communities in California, including the city of Berkeley, are trying a new approach to overcoming the reluctance of many homeowners to spend money on energy-saving equipment. Instead of using tax breaks or subsidies to get their attention, local governments or counties are going ahead and funding the work themselves. Local

The Quest for White LEDs Hits the Home Stretch

White light–emitting diodes (LEDs) have already cracked several niche lighting markets, such as flashlights and bike lights. But they're still not ready to go head to head with cheaper incandescent bulbs and fluorescents that dominate the nearly $100 billion global lighting market. A new spate of advances, however, suggests that the whitecoats are coming. "There is steady movement and progress in the field," says E. Fred Schubert, an electrical engineer and LED expert at Rensselaer Polytechnic Institute (RPI) in Troy, New York.

Much of that progress is coming from the current generation of white LEDs that use a blue LED in combination with a yellow phosphor to produce white light. In April, North Carolina–based Cree reported that its latest commercial white LED bulb puts out an impressive 132 lumens of light per watt of electricity. Incandescent bulbs, by contrast, put out 15 lumens/watt (lm/W), and compact fluorescents bump that up to about 65 lm/W. And earlier this year, Nichia, a Japanese LED company, reported that in a lab demonstration white LEDs had turned out a stunning 249 lm/W at low current.

Progress is also coming in combining separate blue, green, and red LEDs that not only can combine their primary colors to produce bright white but also can be tuned to shine in any color. The holdup right now is that green LEDs are less efficient than the reds and blues. But over the past 2 years, key advances have come from the University of California, Santa Barbara (UCSB), Purdue University, and RPI.

Bright future? White light–emitting diodes could slash the need for electricity.

If progress continues, the payoff could be enormous. According to UCSB researchers, if an affordable 150-lm/W white LED were developed, the efficiency gains from replacing conventional bulbs would save the United States alone some $115 billion in lighting costs by 2025, alleviate the need for 133 power stations, and prevent the release of 258 million metric tons of carbon dioxide.

–ROBERT F. SERVICE

CREDITS (TOP TO BOTTOM): LBNL; JUPITERIMAGES

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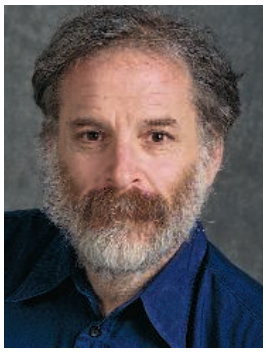
governments recover the cost of the retrofit by adding a small monthly charge to that home's property taxes or utilities bill. But homeowners should still come out ahead as their monthly energy savings are greater than the extra charge.



"There's a battle, and that battle is vicious. It's like abortion, gun control—it's one of those 'apple pie' things."

—LEE SCHIPPER, CENTER FOR GLOBAL METROPOLITAN STUDIES, UC BERKELEY, AND PRECOURT ENERGY EFFICIENCY CENTER, STANFORD UNIVERSITY

Paying the cost

Lee Schipper of Stanford's PEEC is a grizzled veteran of campaigns to save energy around the world. And after many years in the trenches, he's changed his mind. In the early 1970s, when Schipper was studying astrophysics at Berkeley (where he shared a graduate student office with Chu), he started teaching classes and giving lectures on the physics of energy. When the energy crisis hit, he quickly earned a reputation as an efficiency enthusiast of the most irrepressible sort. He eventually joined Rosenfeld's research team at LBNL.

Schipper couldn't restrain himself when, in 1977, President Jimmy Carter urged Americans to conserve energy using arguments that Schipper considered unfounded. Carter said that conserving energy "will demand that we make sacrifices and changes in our lives. To some degree, the sacrifices will be painful." Schipper wrote an angry letter to Representative John Dingell (D–MI), arguing that conserving energy did not, in fact, require painful sacrifices. He explained that new energy-saving lights, windows, and car engines allowed consumers to live just as they always had yet burn less oil and coal. "You know what?" Schipper says today. "I was wrong. Carter was right."

Schipper has worked at the International Energy Agency in Paris, the World Resources Institute Center for Sustainable Transport in Washington, D.C., and now at PEEC. He's seen the push for efficiency repeatedly run into limits. Some of those limits, he says, are perfectly understandable. Energy is not a big-ticket item for most people, and even when new technology is cost-effective, the switch often takes more time and effort than people feel it's worth. And sometimes the technology doesn't live up to its promise. Many con-

Aircraft Designers Shoot for Savings on the Wing

Since jet engines appeared in the mid-1950s, commercial aircraft have become steadily more fuel-efficient—simply in order to fly farther and cheaper. According to the International Air Transport Association, new aircraft are 70% more fuel-efficient than they were 40 years ago. In 1998, passenger aircraft averaged 4.8 liters of fuel per 100 kilometers per passenger; the newest models, the Airbus A380 and Boeing 787, claim 3 liters. Even so, as air travel expands while fuel prices spiral upward, there is more that aircraft designers can do.

The greatest gains in the past, says aerospace engineer Ian Poll of Cranfield University in the United Kingdom, have come from better engines. The earliest engines were turbojets in which all the air sucked in at the front is compressed, mixed with fuel, and burned, providing thrust through a jet out the back. Engineers soon realized that they could get greater efficiency by using some of the power of the jet to drive a fan that pushes some of the intake air through ducts around the core, a design known as a turbofan. Other boosts have come from better compressors and materials to let the core burn at higher pressure and temperature. Poll says engineers might make turbofans yet more efficient by leaving the fan in the open. Such a ductless "open rotor" design—essentially a high-tech propeller—would make possible larger fans, Poll says, if engineers could solve noise problems and figure out how to fit such engines onto the airframe.

Changes in aircraft bodies have led to more modest improvements. Computational fluid mechanics has enabled designers to refine the shape to reduce drag—an enemy of efficiency. Manufacturers have also reduced weight with lightweight materials such as plastic. The Boeing 787 is made of 50% composite materials by weight, mostly carbon fiber–reinforced plastic, and is the first airliner to use them extensively in the fuselage, wings, and tail. But increasing efficiency this way is a hard fight: Each 1% reduction in weight cuts fuel consumption by only about 0.75%.

Poll thinks manufacturers could wring another 50% greater efficiency by using open-rotor engines and more composite materials, but beyond that they may need to radically change the shape of the aircraft. In traditional airliners, the fuselage is a dead weight that contributes no lift. A possible alternative is a blended wing body (BWB) in which the fuselage flows into the wings and is itself a lift-generating airfoil.

NASA and Boeing have been collaborating on an experimental BWB craft known as the X-48B. Since 2007, they have been flight-testing a remotely piloted 6.4-meter-wide model of the plane. Making the jump to such a different technology carries enormous risks for manufacturers and airlines because of development and testing involved. They would take that step only if forced to by high fuel costs. "We know [a BWB] is more fuel efficient," says Poll. "But it's too early to say if it will be the next generation."

–DANIEL CLERY

Delta force. Boeing and NASA are testing fuel-efficient blended-wing body designs to see how they fly.

CREDITS: LEE SCHIPPER; BOEING IMAGE

Published by AAAS

Green campus. Duquesne University, in the heart of Pittsburgh, built a cogeneration plant (*below*, with smokestack) that supplies the campus with electrical power and steam heating.

CREDITS: DUQUESNE UNIVERSITY

sumers haven't been happy with compact fluorescent lighting, either because they don't like the quality of the light or because the light bulbs haven't been as durable as advertised.

More important, efforts to push efficiency ran into intense political opposition, especially in the United States. "There's a battle, and that battle is vicious. It's like abortion, gun control—it's one of those 'apple pie' things," Schipper says.

Schipper's views are shaped by his own particular specialty: transportation, including cars. Since 1980, new cars have doubled the amount of mass they move with a gallon of gasoline, but U.S. car manufacturers used most of that efficiency gain to make cars bigger and more powerful, not more fuel-conserving. The simplest and cheapest way to reduce energy use in transportation, Schipper says, is simply to require cars that are lighter, smaller, and less powerful. But because of fierce resistance to that idea, "we get all these interesting technological fixes, like plug-in hybrids, that are actually quite expensive."

So Schipper has come around to the idea that conserving energy really does demand that people change their attitudes and the way they live. The single most important step in that direction, he says, is to make energy more expensive. "We're still playing 1970s games, thinking that we don't have to confront consumers and industries with the real price of energy and carbon," he says.

Some efficiency advocates are wary of such talk. "I'm a nonenthusiast about price. Low energy prices and efficiency can coexist," says Goldstein. He points to the example of Seattle, where electricity is cheap but people use relatively low amounts of it. Goldstein credits Seattle's tough building codes, cooperative electric utility, and a strong conservation ethic in the population. Koomey thinks conventional economic thinking may underestimate efficiency's growth potential. Perhaps, he says, it's more like the Internet: As more people adopt energy-conserving practices, the infrastructure of efficiency becomes more widespread, making it easier and cheaper for others as well. The phenomenon, he thinks, could gain momentum like a ball rolling downhill.

Rosenfeld, the man who once provided a professional home to many of these efficiency researchers, quietly agrees with Schipper. "Of course we need an energy tax," he says simply. The "father of energy efficiency" is modest in physical stature and demeanor. He still lives in Berkeley but spends just as much time in Sacramento, where he's a member of the California Energy Commission. It's a sad time in his life; his wife, Roz, died suddenly of a stroke in early June.

But it makes him feel "very well," he says, to hear Energy Secretary Chu extol his accomplishments. He's as devoted to saving energy today as he was 35 years ago. His latest cause: promoting white roofs that reflect sunlight, reducing the load on air conditioners and cooling the planet. "I get listened to," he says with a smile. "So I continue to say, 'Energy efficiency is the first thing you want to do, and I know a lot of tricks for doing it.' Steve Chu does answer my phone calls."

–DAN CHARLES

Making Use of Excess Heat

The single biggest opportunity to increase the "energy productivity" of American industry, according to a report issued in July by the consulting group McKinsey & Co., lies untapped in the furnaces of ethanol refineries, paper mills, and other heat-consuming industries. The key is to make use of heat that would otherwise be thrown away.

One way to do that is via cogeneration, or "combined heat and power" (CHP), a technique that is more than a century old but newly fashionable. "District heating," common in Scandinavia and Eastern Europe, uses leftover steam from power plants to heat nearby buildings. Alternatively, a factory that needs steam can build a gas-fired generating plant, sell the electricity or use it on-site, and use the waste heat to produce the steam it needs.

Such combined operations are very efficient. The McKinsey study estimates that linking heat and power generation in U.S. industry could save nearly a trillion megajoules of energy over the next 20 years, and the average project would generate a healthy financial return of 36%.

Currently, Denmark is the world's cogeneration leader; more than half of the country's electricity is produced in CHP plants. In many other countries, including Brazil, Canada, France, the United Kingdom, South Africa, and India, it's less than 8%. According to the International Energy Agency, these countries could double their CHP output in 10 years and triple it by 2030 if they set up the right incentives.

The Netherlands showed that it's possible. Starting in the mid-1980s, the Dutch government guaranteed favorable prices for electricity from CHP plants, then encouraged electric utilities to set up CHP plants as joint ventures with industrial companies. "Suddenly, utilities started to build cogen plants—because they weren't competition anymore!" says Ernst Worrell, an energy researcher at Utrecht University in the Netherlands. Between the early 1980s and the late 1990s, the share of Dutch electricity generation that came from CHP plants rose from 8% to almost 30%.

–DAN CHARLES

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LETTERS

edited by Jennifer Sills

Evolution of the Monkey Crouch

T. PFAU *ET AL*. ("MODERN RIDING STYLE IMPROVES HORSE RACING times," Brevia, 17 July, p. 289) nicely document the effectiveness of the "monkey crouch" riding style on race times and horse-jockey biomechanics. This style produced measurable speed benefits to winning race times at the English Epsom Derby Stakes (1900–1910).

The change in riding style across a decade of different jockeys prompts the question: How did the monkey crouch originate? Many authors credit two American jockeys—Willie Simms and Tod Sloan—with bringing this style to England in 1895 and 1897, respectively. However, English rider Harding Cox claimed to have adopted the monkey crouch still earlier. Cox even described how he developed the style and what benefits it conferred: "When hunting, I rode very short, and leant well forward in my seat. When racing, I found that by so doing I avoided, to a certain extent, *wind pressure*, which … is very obvious to the rider. By accentuating this position, I discovered that my mount had the advantage of *freer hind leverage*" (original italics) (*1*). Measurements taken by Pfau *et al*. support Cox's impressions.

Did Cox intend to design his new riding position? Did he purposefully reposition himself on his horse after painstaking mathematical calculations? Did he record wind pressure scores or take biomechanical readings to assess his new riding style? Probably not. It is more likely that he merely proceeded by trial and error, much as did Olympic champion Dick Fosbury when he invented his famous high jumping "flop" (*2*).

Inventive behavior is often attributed to creativity or to genius when a simpler explanation suffices. The origin of the monkey crouch perfectly fits the Law of Effect: Successful behavioral variations are retained and unsuccessful variations are not. This positively Darwinian process works for human inventions just as it does for earthly organisms—mechanically and without design or purpose.

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Energy Strategies and Efficiency

J. E. CAMPBELL *ET AL*. ("GREATER TRANSPORTation energy and GHG offsets from bioelectricity than ethanol," Reports, 22 May, p. 1055) compared the efficiency of using biomass to power vehicles through either ethanol production or electricity production (for electric vehicles). However, it is premature to conclude that biomass use should focus on the latter simply because it boasts greater overall efficiency.

Some energy uses, such as air travel and long-distance shipping, require fuel with high energy density, which current and foreseeable batteries cannot achieve. Those applications will continue to require a liquid hydrocarbon fuel to meet their needs. Currently, our only viable nonfossil option for satisfying that demand is biofuels. If biomass that could be turned into biofuels is instead burned to produce electricity, our ability to reduce petroleum usage in areas that require fuels with high energy density will be greatly diminished.

Our ultimate goal should be to transition completely away from fossil fuels. To do that, we need to look at each type of energy use and assess how best to meet that demand. While producing electricity from biofuels to power electric vehicles may be a more efficient use of that biomass itself, we have many other options available for producing nonfossil electricity (such as nuclear, geothermal, wind, and solar power). However, those options cannot be as easily used to create high energy density fuels. Biomass can meet that need, and therefore would be most wisely used to fill that need, rather than to produce electricity (*1*).

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Reference

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Response

BRIGGS ARGUES THAT ENERGY STRATEGIES should not focus exclusively on efficiency. We agree and explicitly state a wide range of criteria in our Report. Briggs also argues that biomass should be dedicated to liquid fuel production to allow for a complete transition away from fossil fuels, whereas other renewables should be used for electricity. His argument would be compelling if the complete transition away from fossil fuels were within reach in the near term.

Instead, we expect the transition away from fossil fuels to be more gradual. In the meantime, we must find the mix of fossil fuels, biomass energy, and other renewables that best meets the interacting goals of energy independence, climate mitigation, economic competitiveness, and clean air. Multiple forms of biomass energy could contribute to this mix, but much of the current policy focus is limited to liquid fuels (such as ethanol mandates in the United States and other countries). The

CREDIT: ISTOCK

Gasoline from biomass

822

Why mimic?

824

efficiency advantages of bioelectricity (*1*) and bioheat (*2*) provide a strong motivation for broadening the research and evaluating these applications along with liquid fuels.

Support for future research and policy analysis should be broad enough to encourage serious exploration of the prospects for electrifying vehicles, including the potential rate of adoption, cost, range, and the kinds of vehicles compatible with electrification. The situation to avoid is one in which strong starting assumptions about the limited potential of vehicle electrification create so much momentum for liquid fuels from biomass that we forgo the option that makes the most efficient use of the biomass energy.

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Defining Language Boundaries

THE NEWS FOCUS STORY "HOW MANY LANguages? Linguists discover new tongues in China" (M. Erard, 17 April, p. 332) discusses an important challenge: defining accurate language boundaries. I encountered this issue while working on United Nations conflict prevention and resolution initiatives in ethnically diverse regions where success is determined by reliable communication. Although the strict criteria described by Sun Hongkai constitute one approach to delineating language boundaries, the degree to which speakers of one language or dialect can understand speakers of another—mutual intelligibility—remains the ultimate test for defining languages for the purposes of practical application to work such as mine.

Unfortunately, determining mutual intelligibility can be complex and burdensome, and the techniques used to test it are not applied universally. The task of identifying boundaries could benefit from the establishment of a simple and reliable technique that would determine where efforts are needed to ensure communication across language barriers. Assigning definitive language codes is premature until mutual intelligibility criteria and techniques have been applied consistently. This would require a cooperative effort among linguists in all countries, along with the necessary financial support.

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Note

1. The author and Sun Hongkai have published a monograph together.

Plagiarism: Transparency Required

J. COUZIN-FRANKEL AND J. GROM ("PLAGIarism sleuths," News Focus, 22 May, p. 1004) suggest that "[r]epetitious reviews and incremental reports are part of an accepted tradition." Accepted when and by whom? The International Committee of Medical Journal Editors (ICMJE) has for many years included a detailed section on overlapping publications in its "Uniform requirements for manuscripts submitted to biomedical journals" (*1*). The need for transparency, both to editors and readers, is a paramount concern. Nowhere in the ICMJE document is there an exemption for any particular type of manuscript, including reviews and translations. Essentially all journals include in their instructions to authors a statement such as that provided by *Heart Failure Reviews*: "Submission of a manuscript implies that the work described has not been published before and that it is not under consideration for publication anywhere else." Many require a signed declaration. Nevertheless, the authors of many reviews, editorials, and textbook chapters fail to disclose the inclusion of substantial sections of text lifted largely verbatim from previously published or simultaneously submitted material.

One of the authors apparently unhappy about inclusion in the Déjà vu database is quoted as saying "[t]here's going to be redundancy [in review articles], but I don't think that's scientific misconduct." Without appropriate permission from all the relevant editors, as well as the inclusion of an overt notice in the later publication to inform readers, it is deception and therefore is indeed misconduct; copyright may also be a problem if it has been assigned to the original publisher. Moreover, if the second submission occurs with a declaration—signed, implied, or otherwise—that none of the material has been or will be published elsewhere, it amounts to outright fraud.

Authors who have sought editorial permission and been transparent when reusing material have nothing to fear from inclusion in Déjà vu. They may ultimately point to the entry as independent confirmation of their integrity.

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Reference

1. International Committee of Medical Journal Editors, "Uniform requirements for manuscripts submitted to biomedical journals: Writing and editing for biomedical publication" (www.icmje.org).

Plagiarism: Consider the Context

THERE SHOULD BE NO DOUBT THAT ANY form of covert duplication of data represents a serious threat to the integrity of the scientific record ("Plagiarism sleuths," J. Couzin-Frankel and J. Grom, News Focus, 22 May, p. 1004). Duplication and other types of redundancy (such as "salami publication") are a source of great concern for science journal editors (*1*). In that regard, Skip Garner's eTBLAST and his Déjà vu site should be viewed as a welcomed addition in the arsenal to combat and prevent possible scientific misconduct.

The issue of wholesale reuse of an author's previously published text is slightly more nuanced. It is understandable when non-native authors with limited English skills engage in this behavior, particularly when they have received poor relevant guidance. While adhering to a single set of clear, ethical standards equally applicable to all, we also must recognize that each case is unique and should be treated accordingly. In contrast, substantial text reuse by experienced authors who hold a full command of the language is inexcusable and should not be tolerated. An exception might be made for methodology sections because these contain very complex, technical descriptions of materials and procedures that are often difficult to paraphrase (*2*). Even

slight changes to the wording of these sections could potentially lead to subtle misinterpretations of how an experiment was conducted. However, the underlying assumption in this argument—that previously published methods sections are so well written that they cannot possibly benefit from additional clarification or elaboration—is often unwarranted (*3*).

Practices such as patchwriting and authors' recycling of their previously published text should not just be regarded as questionable—they should be unequivocally classified as inappropriate scholarship (*4*). **MIGUEL ROIG**

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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No Paradox for Invasive Plants

THE PERSPECTIVE "AN INVASIVE PLANT PARADOX" by M. E. Rout and R. M. Callaway (8 May, p. 734) overgeneralizes the effect of invasive plants on the nitrogen cycle. An invasive plant's impact on nitrogen cycling is based on plant identity rather than origin. Invasive nitrogen-fixing plants can increase nitrogen cycling in a newly invaded ecosystem, but this does not apply to all functional groups of invasive plants. Mechanistically, it is difficult to imagine how non–nitrogen-fixing plants could enhance total nitrogen pools in the ecosystem, unless they did so by affecting free-living nitrogen-fixing microbes. Furthermore, the effects of invasive plants on nitrogen fluxes are site-dependent (*1*, *2*). To avoid the confounding effects caused by site, we need experimental studies that can unequivocally separate causes from consequences. We agree with Sax and Brown (*3*) that there is no paradox of invasion. Indeed, there are underlying mechanistic explanations for each species in its new environment. A general pattern of enhanced nitrogen cycling does not exist for plant invaders.

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Clarifying Coals

R. A. KERR'S NEWS FOCUS STORY ABOUT THE peak coal controversy, "How much coal remains?" (13 March, p. 1420), did not distinguish between the challenges of mining bituminous compared with sub-bituminous coal. The older mining literature indicates that a large fraction of the bituminous coal resource should be minable to a depth of at least 1200 m. The situation for the globally substantial sub-bituminous and brown coal resource is more complex.

The Royal Commission coal report of 1903 to 1905 (*1*, *2*) showed that for typical U.K. geological conditions, recovery ratios of 80 to 90% were achievable for bituminous coal with the labor-intensive mining technology of the time. Similar arguments apply to the global bituminous resource, given that coal-bearing, post-Devonian strata are too young to have a high probability of being strongly tectonized or metamorphosed. The early- to mid-20th-century mining literature contains numerous examples of very high extraction ratios for thick seams and in multiseam mining (*3*–*5*) to depths of about 1200 m. Later 20th-century mines went deeper [for example, to 1450 m at Monceau-Fontaine (*6*)]. New technologies may enhance productivity of labor-intensive, high extraction percentage, longwall mining. Thus, the ultimate extraction ratios for bituminous coal could well be high.

The situation is less clear for sub-bituminous coal (which forms an important part of the global coal resource). Rock strengths range from similar to those in bituminous coal to an order of magnitude weaker, depending on the basin burial history. The depths at which the rocks become overstressed will be lower. This applies even more strongly to less indurated brown coals. However, the available English literature describing the relationship between pressure temperature history and mechanical properties of such rocks is limited, as is that relating mining problems to overstress and coal/rock mechanical properties. The ultimate extraction depth for brown coal and sub-bituminous coal will depend on the burial history statistics of these materials.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "Bringing hominins back to life" by M. Balter (10 July, p. 136). The name of the *National Geographic* science editor was misspelled. It is James Shreeve.

News Focus: "Private money, public disclosure" by J. Kaiser (3 July, p. 28). The article may have inadvertently given the impression that Stanford psychiatrist Alan Schatzberg failed to report some of his outside financial interests to his university. All income discovered by Senator Grassley had been disclosed to Stanford. Also, the amount of Dr. Schatzberg's equity in Corcept Therapeutics was not initially disclosed to Grassley, but was always disclosed to Stanford.

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MEDICAL GENETICS

Epigenetic Determinism

Michael A. Goldman

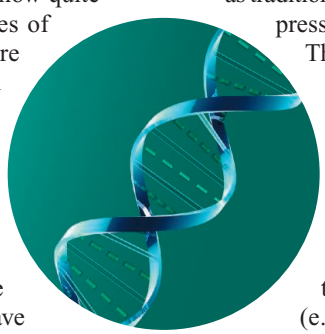
DNA
Promise and Peril

by Linda L. McCabe and Edward R. B. McCabe

University of California Press, Berkeley, 2008. 355 pp. $39.95, £27.95. ISBN 9780520251878.

The juxtaposition of promise and peril appears over and over in books and papers, but in *DNA* Edward McCabe and Linda McCabe actually offer a reasonable view of the true magnitude of each. Although the authors (medical geneticists at the University of California, Los Angeles) devote much of their attention to ethical issues in genome science, they manage to convey the sheer power and potential of genetic technology in medicine.

In an admiring foreword, the late Victor McKusick notes, "The McCabes thoroughly trounce genetic determinism and at the same time reductionism. They make it clear that your genome, or any part of it, is not you." However, I am not sure who the McCabes are describing when they begin their book, "The concept that our DNA sequence—our genome—does not equal or predict our destiny has been extremely difficult for some geneticists to accept." They subsequently comment, "Many of us in the genetics community sincerely believed that DNA analysis would provide us with a molecular crystal ball that would allow us to know quite accurately the clinical futures of our individual patients." There seems to be an assumption here that having completed the human genome project, we have the blueprint, and we still can't explain the organism. But in fact we have not scratched the surface with respect to genome annotation. We don't even have a good idea how many genes there are, let alone how those genes work together (with each other and with the environment) to orchestrate human development.



The remainder of the opening chapter left me questioning the extent to which the McCabes have themselves abandoned genetic determinism, because their reasoning relates entirely to epigenetics. That simply pushes the problem one step back, by suggesting that when we can analyze the methylation and chromatin state or transcriptional activity of every gene, then we will be able to predict the future of each individual. It is not necessarily so. The assumption that every environmental effect upon a phenotype must act through the alteration of gene expression is itself a deterministic and gene-centric view.

Most of the book deals, from a decidedly medical perspective, with rare, relatively predictable single-gene disorders that traditionally come to clinical attention. A physician who sees predictive value in the elevated risk of heart disease in an individual having a total cholesterol level of 230 would understandably think in terms of genetic determinism when dealing with the fate of a newborn with Tay-Sachs disease—or even with a genetic trait that has 80% penetrance by age 65. But the common genetic conditions and traits that profoundly influence medical practice, public health, and the economics of health care involve complex interplay between the action of many genes and the environment. Here, genetic risk factors are only about as powerful as traditional medical tests (such as blood pressure) in predicting the future. The McCabes criticize the film *Gattaca*, but maybe that film offers a more realistic view of genome-based prediction than it seems on the surface. Its protagonist Vincent did not overcome a lethal genetic mutation but rather more subtle, complex disadvantages (e.g., average stature and left-handedness). Stating life expectancy (an average rather than a determined moment of death) in view of genotype will probably be more accurate than the way we do it now—by presence or absence of the Y chromosome.

The McCabes define gene therapy rather narrowly as "the substitution of a normal genetic sequence in place of a disease-causing genetic sequence." That does not accurately describe the examples they then discuss, as those do not rely on the targeted replacement of one stretch of DNA with another. The authors profess an appropriately bleak outlook for this beleaguered field, concluding that "stem cell therapies and regenerative medicine … will have broader clinical benefits." They cleverly admit they have been telling people for ten years that gene therapy would become available in the next five years. Even in the world envisioned in *Gattaca*, technology is limited to embryo selection rather than DNA sequence correction.

Following the academic mainstream, the McCabes vilify Craig Venter, noting that they found identical errors in the public project's and Celera's databases, "suggesting an unacknowledged interdependence." Although they provide a detailed accounting of the simultaneous publication of results in *Science* and *Nature*, they include little acknowledgment of Venter's role in galvanizing the completion of the human genome project ahead of schedule and under budget—a development that profoundly affected my own perception of the role of industry in innovation and research.

Personalized medicine receives relatively little emphasis in the book, with most of that buried in a short chapter on "large population assessments." There, the McCabes consider genetic screening of newborns and others (and related policy issues) in excellent detail. Both authors have thought about and published on this topic for some time. "Newborn screening," they conclude, "was the pioneering system for predictive, preventive, and personalized medicine. … [and] is the model that must be carefully evaluated as health care begins to embrace genomic medicine." But in discussing the broader efforts to identify "those with higher levels of genetic risk," the McCabes are still describing relatively simple disorders such as hemochromatosis rather than complex genetic conditions. It is the latter that afflict the masses and will have enormous impacts on the economy and on public health as personalized medicine comes into its own.

The book is anything but dry reporting. The authors heavily infuse it with their own views, generally identifying them as such. Sometimes to a fault: even the most obvious conclusions are preceded by a modest "we propose." The authors also address such issues as DNA forensics, patenting of tissues and genetic material, genetic discrimination, assisted reproductive technologies, stem cell therapeutics, and reproductive cloning. Well indexed and documented with a chapter-by-chapter bibliography, *DNA: Promise and Peril* will be useful to health professionals, scientists, and students as well as the educated public. Although above I have concentrated on what I think the McCabes may have overlooked, the book offers an enjoyable and stimulating read for specialists in the field and the curious public alike.

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PSYCHOLOGY

Humanizing Conservation

Peter Verbeek

Relating to nature. A Japanese program that provides visually impaired urban children the opportunity to experience nature shows that we relate to nature through all our senses (*7*).

CREDIT: HIROSHI SUGA

Our relationship with nature brings joy and sadness to human existence. We inherited an affinity for life that undoubtedly served our early ancestors well. When young, we delight in the play space that nature affords: trees, shrubs, places to hide and climb, fields to wander, and rivers to swim and fish in. We retain a lifelong propensity to explore and exploit the natural world. Experiencing unspoiled nature can fill people's lives with meaning and joy.

Sadly, human activity has extensively disrupted natural ecosystems and around the globe people are beginning to feel the impact of anthropogenic extinctions and climate change (*1*). Studies from multiple disciplines identify threats to our physical health posed by biodiversity loss (*2*). Our mental health is also at risk (*3*). Our relationship with nature has become dysfunctional.

Aware of the benefits we derive from nature and the threats our activities pose, conservation biologists have asked questions that urgently need answers (*4*): "What is the relationship between individuals learning about environmental problems and their conservation attitudes, knowledge, beliefs, and behaviors?" "What are the impacts of increasing human dissociation from nature on the conservation of biodiversity?" In *Conservation Psychology: Understanding and Promoting Human Care for Nature*, Susan Clayton and Gene Myers tackle these and related questions from multiple psychological angles.

Conservation Psychology
Understanding and Promoting Human Care for Nature

by Susan Clayton and Gene Myers

Wiley-Blackwell, Oxford, 2009. 261 pp. $140, £70, €84. ISBN 9781405194099. Paper, $59.95, £29.99, €36. ISBN 9781405176781.

Clayton (a psychologist at the College of Wooster, Ohio) and Myers (at Western Washington University) have written a timely book. It heralds a new area within psychology that focuses on the "bidirectional relationship between humans and the natural environment" The authors note that the field "arose not in response to a lack of research, but in response to a lack of visibility and identification: both psychologists and non-psychologists are often unaware of the body of psychological research related to sustainability." They see it, like conservation biology, as mission driven: They believe that people must act to minimize or alleviate the environmental threats they are facing. And they argue that "[u]nderstanding the ways in which nature is significant to people enables the construction of initiatives that will promote conservation."

The book covers a wide range of issues. In the first part, "Thinking about nature," Clayton and Myers explore our perceptions, attitudes, values, and moral reasoning about nature. They then discuss our encounters with nature, in our homes, gardens, zoos, aquariums, urban parks, and wilderness. In the third section, they consider environmental education and the promotion of behavior that sustains biodiversity and a livable climate. The authors' judicious choice of findings comes to life through their insightful commentary. Short conclusions at the end of each chapter serve as helpful summaries of the main points.

The final chapter deals with the psychology of hope. Our biological inheritance includes a capacity for empathy and ability to take the perspective of others. We have a natural preference for reciprocity and justice, and we share with other primates a natural ability to keep aggression in check and make peace after a fight (*5*). Basic and applied work in psychology can provide the knowledge and means to draw on these life-sustaining traits as we face the consequences of global environmental crises.

Psychology has been slow in waking up to the realities of our dysfunctional relationship with nature. Ecological psychologist Edward Reed warned that "it may well be a race against time to see whether we can come to understand our way of life before it destroys the only home we have" (*6*). *Conservation Psychology* was written to inspire "a sea-change in the work of psychologists toward addressing sustainability." Hope springs eternal. If editors of key journals across psychology open the gates for reports on the kinds of work discussed by Clayton and Myers, change may come to the discipline. I highly recommend their book to psychologists of all creeds as well as to conservation biologists, environmental scientists, policy-makers, teachers, and anyone concerned about our evolving place in nature.

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RESEARCH ETHICS

Children and Population Biobanks

David Gurwitz,[1]* Isabel Fortier,[2] Jeantine E. Lunshof,[3,4] Bartha Maria Knoppers[5]

Access to samples and individual DNA sequence data from children included in population biobanks should, when feasible, await their consent as adults.

Population biobanks, which store and distribute human DNA, cell lines, and tissue samples collected from large cohorts, are being established and are growing in size (*1*). These population biobanks are often funded wholly or in part by governments and are envisaged as novel resources for national and international biomedical research programs. Such programs include studies on associations between genotypes, environmental exposure measures, socioeconomic parameters, and phenotypes of human health and disease.

Knowledge obtained from such population cohorts as, for example, those in the UK Biobank, with donors' samples prospectively linked to continuously updated electronic health records, is expected to facilitate advances in personalized medicine, including preventive medicine and safer, more effective drug-prescribing (*2*). The UK biobank, launched in 2007, targets half a million adult Britons (*3*); similarly large cohorts are being enlisted in Canada, China, Norway, Netherlands, Denmark, Ireland, and the United States.

Many of these biobanks collect samples and data from children, often along with their parents (see table, p. 819). Some aim to provide longitudinal resources for the study of gene-environment interactions on child growth and development, such as in the Norwegian Mother and Child Cohort (*4*), or to follow specific diseases throughout childhood (*5*).

However, the large-scale collection and use of such data has raised some concerns. Some newborn screening programs launched from the 1960s onward in the United States and elsewhere, with the primary goal of early detection of treatable disorders in newborn children, have recently discovered the research potential of their huge blood-spot card stocks, which has created public outcry over presumed breach of trust and privacy (*6*). Inclusion of children's samples in population biobanks, even with the authorization of parents or guardians, raises specific concerns. Children are a vulnerable research population, in the sense that they lack the capacity for consenting to their participation (*7*). But they are different from other vulnerable populations, such as mentally disabled individuals, or patients with schizophrenia or dementia, or individuals with other conditions affecting their capacity to appreciate the risks and benefits of donating DNA samples and phenotypic data to a biobank. Unlike members of such other populations, children's vulnerability is temporary and does not arise from a disorder; most children will become healthy adult members of society.

DNA remains unique as a permanent identifier throughout an individual's life. Thus, appropriate safeguards are needed when collecting and distributing children's DNA samples and data (*8*). A child whose DNA sample is donated by her parents today and distributed over the next few decades for research projects around the world can potentially be in the public eye decades later. As sequencing of entire genomes becomes a routine procedure, DNA donors' privacy can never be completely ensured within biobanks (*9*). Individuals can be traced even in very large aggregate data sets spanning thousands of donors (*10*, *11*). As a consequence, there is no true "opting out" from biobanks once DNA sequences have been published and deposited with public databases. Even when the DNA sample itself is destroyed, researcher-generated data—including identifying markers or sequences—may not be irrevocably eliminated. By the time children are recontacted for their consent as adults (if one assumes that this approach is taken by the biobank), their identifying DNA sequences along with their phenotypic data sets may already have been shared with other resources around the world. Apart from biobanks, some direct-to-consumer personal genomics providers that share results with clients via protected Web sites are already analyzing children's DNA samples (*12*), creating another avenue by which personal privacy might be compromised.

Kohane and Altman have argued that individuals who donate genotype and phenotype information to research databases must be perceived as "health information altruists" (*13*). Biobanks that collect children's DNA, and possibly phenotype data, do so with the authorization of parents or guardians. Obviously, parents are making many important decisions that affect their children's future, but in the case of biobank participation, the consequences of their nonvoluntary altruism may have unpredicted consequences decades later. For example, although the 2008 U.S. Genetic Information Nondiscrimination Act protects individuals by prohibiting employers or health insurance providers from acquiring or using their genetic information, it does not fully protect them from other modes of discrimination by bankers, life or disability insurance providers, schools, immigration authorities, and so on (*14*).

New policies—not just new consent language—are therefore needed for recruiting participants and for the inclusion of children in population biobanks (*15*). Without such changes, lack of openness about potential risks on the part of researchers may lead to the loss of public trust in population research. Among the adverse effects may be costly delays (in terms of public and individual health) in the realization of personalized medicine—including that for children.

When considering new policies concerning the inclusion of DNA samples and data from children in biobanks, it is crucial to distinguish between disease-specific and population biobanks. Disease-specific biobanks are an integral part of therapeutic research involving children with specific conditions. The decision about storage and future use of samples and data are part of the decision-making process concerning diagnosis and treatment of the affected child and similarly affected family members. The balancing of potential harms and benefits is therefore fundamentally different from that for voluntary participation in population research. We propose that disease-specific biobanks—in particular, when dedicated to childhood diseases and disabilities—should continue to collect and share children's DNA samples and data within the limits authorized by parents and bound by continuous oversight, including ethics review. Records should be kept regarding the use of distributed samples and data.

Ideally, the affected children themselves should be recontacted once they reach the age of consent or maturity to allow contin-

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Biobank Features

Name	Country	Year	Target participants (1000s)
Norwegian Mother and Child Cohort Study	Norway	1999	260 (F, N)
Danish National Birth Cohort	Denmark	1996	100 (M, C)
National Children's Study	U.S.A.	2010	100 (M, C)
European Longitudinal Study of Pregnancy and Childhood	Several	Various	40 (P, C)
Avon Longitudinal Study	U.K.	1991	23 (M, N)
All Babies in Southeast Sweden	Sweden	1997	22 (F)
Northern Finland Birth Cohorts 1966/1986	Finland	1966/86	21 (M, N)
Etude Longitudinale Française depuis l'Enfance	France	2010	20 (N)
Guangzhou Twin Project	China	2005	20 (T 7–15 y)
National Child Development Study	U.K.	1958	19 (N)
Child and Adolescent Twin Study in Sweden	Sweden	2004	15 (F, T 9–12 y)
Generation R Study	Netherlands	2002	10 (F)
Born in Bradford	U.K.	2007	10 (M, N)

Largest biobank projects collecting children's samples (target population of at least 10,000 individuals, arranged by target size). Not all donors are children. The list was prepared by using surveys returned to the P^{3}G and information provided on the biobanks' Web sites. A full catalog of P^{3}G member biobanks and further details are available (*15*). Year, year of recruitment or initial data collection started; numbers of target populations are rounded to the nearest thousands. F, families; N, newborns; C, children; M, mothers; T, twins; P, parents; y, years of age.

ued research on their samples and data. There is evidence that this would be in accord with the desires of the general population. In a recent U.S. survey, 1186 adults were asked about hypothetical samples collected from them as children with their parent's permission; 46% responded that they would want to be contacted as adults for consent to continue research with those samples. Moreover, 33% were concerned or very concerned about research conducted without their consent as adults (*16*). More study is needed regarding the desirability or feasibility of recontact. Moreover, the logistics will be complex and costly. The possibility of recontacting entails the need to use a method for coding that is both secure and allows for reidentification.

In contrast to policies for disease-specific research, we feel that an overhaul is needed for the collection and distribution policies of DNA samples and data from children that have been included in population biobanks. We propose that population biobanks continue to collect, store, and analyze children's DNA and phenotypic data with the appropriate authorization by parents or guardians, but that they may not make these DNA samples (or individual genetic sequence data) accessible outside the biobank until donors are recontacted as adults and give their own informed consent.

Pediatric population biobanks could publish and give access to aggregate phenotypic data and results, including from genetic studies, in order to advance pediatric research. Individual DNA sequence data could not be released. For example, published studies might include reports on genetic deletions or duplications affecting health, giving their chromosomal locations and approximate sizes but not specific sequences, an approach taken by a recent study on the role of chromosome 1q21.1 in mental retardation in children (*17*). Such policies would minimize the risks of revealing children's identifying genetic data, thus protecting their privacy, while still allowing the advancement of pediatric research. Such arrangements could align with current developments in many places, such as the European Union (EU), which has opted against a centralized EU biobank but is striving for harmonization of sample collection and distribution policies (*18*).

There are no perfect solutions: We have to choose the best possible policies even as we evaluate risks and benefits both to the individual donors and to the research community and society at large. Limiting the distribution of children's DNA samples and their individual genetic sequence data by population biobanks while waiting for their own consent as adults may negatively impact research. Some donors whose DNA samples and data were donated by their parents may not be traceable decades later, whereas others may not wish to consent as adults (*16*). Moreover, building in-house genetic capacities within existing population biobanks to minimize delays in research while maximizing privacy protection may be costly. However, we believe that research will only be affected marginally by adopting such a policy and only in the short term, as biobanks are foreseen as long-term projects. Consider that we are still enjoying the fruits of the Framingham Heart Study, six decades after the initial cohort was recruited, and research is ongoing because of the trust that has been built over generations (*19*).

This proposal may seem provocative. However, there are good reasons for devising the best possible strategies for the inclusion of children in population biobanks. For adults, there exists broad consensus on voluntariness, altruism, and consent being essential requirements for morally justifiable participation in population biobanks. Why accept lower standards for children? Children are vulnerable, but unique, for their vulnerability is temporary. The long-term benefits of maintaining public trust in biomedical research by waiting for participating children to consent as adults justify extra governance efforts and added costs.

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PALEONTOLOGY

Fire and Stone

For at least 72,000 years, humans have used heat treatment of stone materials to make more efficient tools.

John Webb and Marian Domanski

Faced with the difficulties of surviving in often hostile surroundings, early humans manufactured various flaked stone tools to increase the success of food gathering and the efficiency of food processing. Special-purpose tools increased the likelihood of obtaining food and were particularly important when it was hard to secure adequate food resources (*1*, *2*). For example, projectile points are more likely to inflict a lethal wound if they have needle-sharp tips and symmetrical, straight, razor-sharp edges that reduce drag on penetration. Such sophisticated tools require not only special skills but also high-quality stone materials. When such materials were unavailable, early humans developed the ability to improve the quality of the available materials through controlled heat treatment by burying selected pieces of stone beneath a fire at a campsite or a specialized workshop, usually for a day or more. On page 859 of this issue, Brown *et al*. show that in coastal South Africa, the deliberate use of this technique dates back at least 72,000 years (and perhaps as long as 164,000 years), predating its use outside Africa (*3*).

This use of fire as an engineering tool is an early step in the evolution of means by which humans could more effectively control their environment. Heat treatment in Africa appears at roughly the same time as widespread evidence for symbolic behavior, signaling the development of increasingly complex cognitive ability. By enabling the manufacture of more efficient tools, heat treatment may have played a key role in allowing early modern humans to spread rapidly from the relatively benign environments of southern Africa into the colder, more hostile environments of Europe. The Neandertals in Europe apparently lacked this technique, perhaps giving the early modern humans an evolutionary advantage as they moved into Eurasia.

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Heat treatment was practiced until recently in the Kimberleys in northwestern Australia (*4*). Kidja aborigines built a large fire in a ~0.5-m-deep pit in sandy soil. When the fire had burnt down, the coals were removed and the bottom of the pit was covered with sand; roughly flaked nodules (blanks) of white chalcedony were placed on the sand and covered with more sand, so that the stone was not exposed to intense heat. Coals and hot sand were shoveled back into the pit and left for 3 to 4 days. When the pit was cold, the blanks were removed and worked by pressure flaking [which involves removal of flakes using pressure rather than percussion (striking)] into long, thin, bifacial "Kimberley points" by a limited number of master craftsmen (see the figure). Heat treatment was crucial in enhancing the flakeability and aesthetic qualities of the stone, and Kimberley points were prized as beautiful objects, given as prestigious gifts, and exported along indigenous trade routes.

Benefits of heat treatment. This Kimberley point from northwestern Australia shows the vitreous luster and finely controlled working typical of heat-treated stone artifacts.

Most flaked stone artifacts are made from microcrystalline siliceous rock types (such as chert, flint, chalcedony, and jasper). Fine pressure-flaking of these materials is extremely difficult in their natural state; their flakeability is substantially improved by controlled heat treatment. As Crabtree and Butler showed in their pioneering replicative studies of stone tool manufacture (*5*), heat treatment increases the length of detached flakes, for example, from a half-inch to 2 inches. Longer, thinner, and sharper flakes can be pressed because heat-treated material can be flaked at angles nearly parallel to the surface; this is not possible on unheated material. Heat treatment also increases homogeneity, allowing greater control, ease, and precision of fracture and resulting in fewer production failures.

Heat treatment markedly reduces a material's fracture toughness (*6*), so that the flaking properties become more like those of the best quality stones, such as obsidian. The reduction in fracture toughness of microcrystalline siliceous materials during heat treatment is due to recrystallization and, to a lesser extent, healing of microcracks (*7*). During heat treatment, strongly interlocking, variably sized quartz crystals recrystallize to become more equidimensional and more similar in size, allowing intergranular fractures (which require less energy than intragranular cracks) to propagate more readily. However, although the heated material is much easier to flake and retouch, it is also less durable and more prone to edge fracturing. Thus, intentional thermal alteration was not desirable in the manufacture of heavy-duty tools.

Heat-treated artifacts are most commonly recognized by a vitreous luster (see the figure) on post-treatment fractures, contrasting with matte flake scars predating heat treatment (*3*). Color changes caused by heat treatment are variable, but generally are toward more reddish hues, and heat treatment was sometimes deliberately used to enhance the beauty of stone materials. Carnelian bead manufacturers in Cambay, western India, have used thermal treatment for at least 4500 years to change the gray to cream colors of agates into vivid red to orange shades (*8*).

Archaeological and ethnographic records document that most stone knappers practiced heat treatment to produce retouched tools, especially projectile points. Heat treatment is particularly beneficial in the manufacture of blades, microblades, and bifacial points by percussion flaking with a soft hammer of wood, bone, or antler, as used by Paleo-Indian cultures of North America to produce the distinctive, leaf-shaped Folsom points, which have wide, shallow grooves running almost the entire length of the point (*9*).

Heat treatment was used to improve the flaking properties of local but poorer quality stone materials and to conserve raw material. Heat-treated stone can be worked by pressure blade techniques, which produce up to four or five times as many blanks from the same volume of material compared with percussion flaking (*10*). This was particularly important in the harsh tundra environments of the Northern Hemisphere, where deep snow cover, frozen ground, and often frost-shattered stone reduced access to suitable raw materials and caused seasonal shortages. Pressure blade techniques may have been invented by reindeer hunters in

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this area as an adaptation to the shortage of raw material (*10*), at the same time producing the specialized, light, and efficient tools needed by the hunting parties.

Pyrotechnology has been practiced regularly through human history and throughout the world (*11*) to increase the quality and efficiency of stone tool manufacturing. Although intentionally heated artifacts are common between 14,000 and 10,000 years ago in Europe, they are extremely rare before that. The discovery by Brown *et al*. of heat-treated artifacts in South Africa dating back perhaps 164,000 years (*3*) suggests that the technique was first discovered in Africa.

The development of heat treatment was a substantial technological advance by early humans. Its continued use up to the present day is a testament to the degree to which it allowed people to more effectively control their environment, and also probably contributed to human cognitive development through the beautification of the artifacts it helped produce.

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ASTRONOMY

Gamma-Ray Pulsars Old and New

The Fermi Gamma-ray Space Telescope is discovering neutron stars that are high-energy gamma-ray emitters.

Jules P. Halpern

A flood of results from the Fermi Gamma-ray Space Telescope, launched on 11 June 2008, is changing the way that we view neutron stars. The Large Area Telescope (LAT), the main instrument on Fermi, detects photons of energy between 0.1 and 100 GeV emitted from spinning neutron stars known as radio pulsars, from supermassive black holes in the "blazar" class of active galactic nuclei, and from other high-energy sources. By timing the arrival of photons from gamma ray–bright points in the Galaxy, Fermi is discovering new pulsars whose existence was only conjectured. Sixteen such "gamma ray–only" pulsars, rotating between 2 and 20 times per second, are reported on page 840 in this issue (*1*). In a companion paper on page 848, the detection of pulsed gamma rays from eight nearby radio "millisecond pulsars" rotating faster than 200 times per second is described (*2*). Complementing these discoveries, Fermi has detected a steady glow of gamma rays from the globular star cluster 47 Tucanae, as described on page 845 (*3*); this region is thought to harbor dozens of millisecond pulsars.

By the early 1960s, considerable theoretical effort had gone into calculating the structure of neutron stars and their x-ray emitting temperatures. A new field of astronomy was born in 1962 when x-ray sources farther than the Sun were discovered. It was not until the 1970s that

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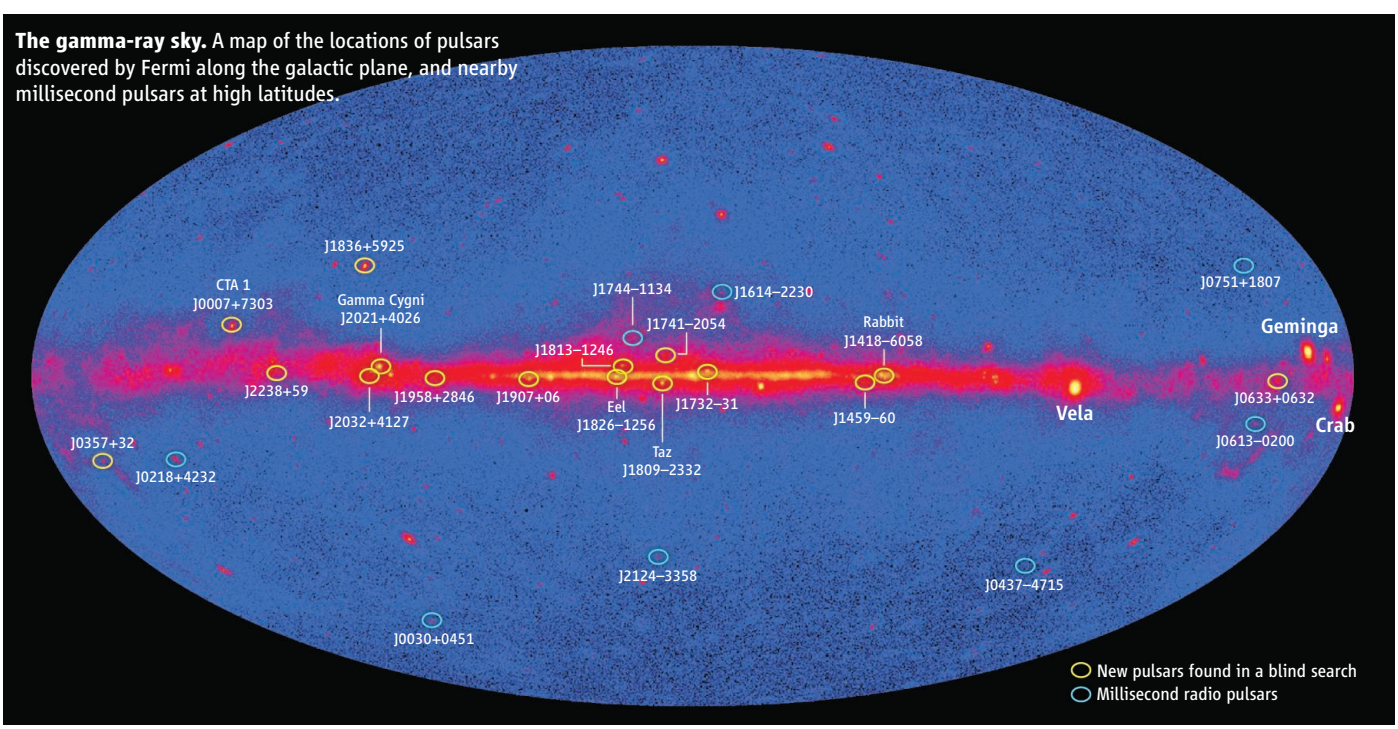

The gamma-ray sky. A map of the locations of pulsars discovered by Fermi along the galactic plane, and nearby millisecond pulsars at high latitudes.

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many x-ray sources were recognized to be pulsars in binary systems, with accretion of matter from a normal stellar companion keeping them hotter and more luminous than was possible for an isolated neutron star.

Although the idea that a neutron star would retain a huge magnetic field by flux conservation during collapse was recognized, the other essential property—that it would be spinning rapidly—seems not to have occurred to anyone until shortly before the discovery of radio pulsars in 1967. Franco Pacini put magnetic field and spin together, and proposed that the rotational energy of a neutron star could be tapped by the emission of electromagnetic waves. The periodic radio blips from pulsars, ever so gradually slowing down, were soon attributed to beams from isolated rotating neutron stars by Thomas Gold in 1968.

The high voltage generated by a spinning magnetic field is what accelerates particles to energies capable of emitting gamma rays. Gamma-ray detections of pulsars began in the 1970s, but only six, including the Crab, were known until last year. The Fermi LAT is the first gamma-ray telescope that is large enough to discover new pulsars on its own.

The LAT is not a telescope in the conventional sense, because gamma-ray photons cannot be focused. Rather, a gamma ray produces an electron-positron pair in one of several layers of tungsten, and alternating silicon planes track those particles, which point back to the direction from which the gamma ray came. Whereas ordinary telescopes can observe only one pulsar at a time, the LAT views 20% of the celestial sphere, and it is rotated in synchrony with its orbit around Earth to scan the entire sky every 3 hours. Recording the direction and arrival time of every pulsar photon, while detecting hundreds of other gamma-ray sources and rejecting unwanted background particles, the LAT accomplishes a remarkable feat of multitasking (see the figure).

Even today, the vast majority of more than 1800 known pulsars are detected only at radio wavelengths, even though gamma-ray luminosities exceed their radio power by several orders of magnitude. With an effective area less than 1 m^2, the LAT can collect only a few hundred photons from a typical source in a few months of operation. In this time, the candidate pulsar may have rotated more than a hundred million times at an unknown frequency that is also slowing down at an unknown rate. The photons from every such promising source were analyzed for periodicity, a computational tour de force that has already revealed 16 new pulsars (*1*) while avoiding false positive detections.

Fermi is discovering mostly young pulsars, including ones inside supernova remnants and synchrotron nebulae, the telltale birth sites of neutron stars that until now had "gone missing." The age of a pulsar is conveniently estimated by dividing its present rotation frequency by its rate of change. The ages of the newly discovered gamma-ray pulsars suggest that the gamma-ray emission mechanism must cease before the radio emission does. As the rotation frequency of a pulsar decreases, so does its spin-down power. But while they are alive, their gamma-ray efficiency, expressed as a percentage of spin-down power, increases up to their death. Although several of the LAT pulsars were born during the latest ice age, the oldest one, PSR J1836+5925, is about 1.8 million years old and is channeling almost all of its power into gamma rays.

Unlike narrow radio beams, gamma rays appear to be emitted in a broad fan that is visible from most directions. This explains why so many pulsars were not detected in radio, and means that they are even more efficient gamma-ray engines than previously thought. The LAT also measures spectra, from which it is inferred that gamma rays are created far above the surface of the neutron star as some models have predicted, where they escape absorption by the strong magnetic field. Millisecond pulsars (*2*) seem to rely on the same physics as ordinary pulsars, even though they are billions of years old. Their spin was "recycled" during a long process of accretion from a companion star, and their weaker magnetic fields allow them to conserve that rotational energy for a longer time. Still, a substantial fraction of their power goes into gamma rays. Dense globular clusters are breeding grounds for millisecond pulsars because of their frequent stellar exchanges. The steady flux of gamma rays detected from the globular cluster 47 Tucanae (*3*) is consistent with dozens of millisecond pulsars having similar gamma-ray efficiencies as the nearby ones.

Fermi has already detected about 20 known young radio pulsars (*2*). Its next goal is to find millisecond pulsars that are radio-quiet, a difficult task because of their rapid rotation. Pulsars detected by Fermi are solving another mystery, the origin of TeV (>10^{12} eV) gamma-ray emission in the galactic plane. Most of the diffuse patches of TeV emission can be associated with Fermi pulsars (*4*), which must release energetic particles into the interstellar medium. The TeV frontier in astrophysics is the latest, but probably not the last, in which pulsars will be implicated. Neutron stars may be small, but they are worth our effort. Unlike black holes, they give a lot back.

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ENGINEERING

Cellulosic Biofuels—Got Gasoline?

John R. Regalbuto

Several routes are being developed to convert biomass into hydrocarbon fuels instead of ethanol.

Most people think of ethanol as the only liquid biofuel, and that the major advances in biofuels will revolve around enzymatic conversion of cellulosic or woody biomass (including nonfood stems and stalks of corn stover or switchgrass, and wood chips) into simple fermentable sugars (*1*). However, in just a few years the commercial scale production of liquid hydrocarbons from biomass will be possible. Hydrocarbons can be made (see the figure) from the sugars of woody biomass through microbial fermentation or liquid-phase catalysis, or directly from woody biomass through pyrolysis or gasification (*2*). Finally, lipids from nonfood crops as well as algae (*3*) can be converted to hydrocarbons. The resulting hydrocarbon biofuels will be drop-in replacements for gasoline, diesel, and jet fuel; will give much higher gas mileage than ethanol; and will work in existing engines and distribution networks.

Ethanol produced from biomass is already used in automotive fuels in the United States as a high-octane, oxygenated additive to improve combustion, which allows clean air standards to be met. The drawback to using ethanol as a complete replacement for gaso-

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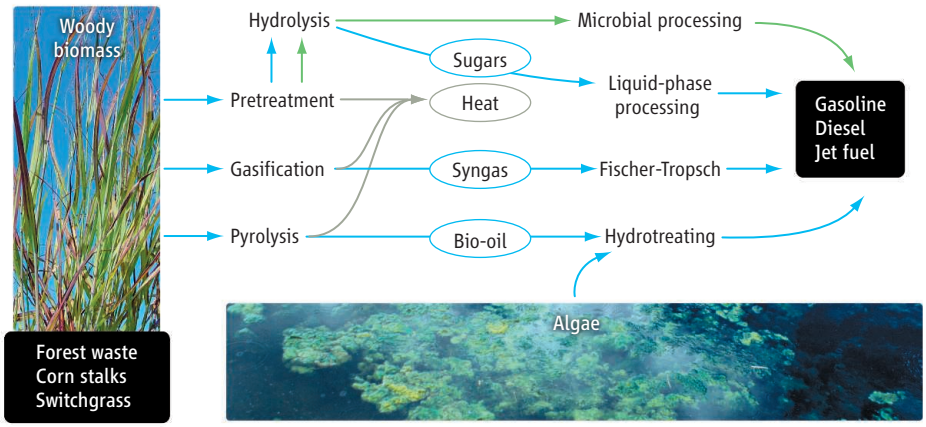

Many paths to biomass hydrocarbons. Woody biomass, or lignocellulose, as well as plant lipids can be converted to hydrocarbon biofuels. The image on the left depicts one lignocellulose source, switchgrass, and the image at the bottom depicts one plant lipid source, algae. Conversion routes may combine a variety of biological, thermal, and catalytic processes (depicted with green, black, and blue arrows, respectively).

line, however, is not only the high cost of its production from cellulose but also its lower energy density. Ethanol has only two-thirds the energy density of gasoline, and cars running on E85 (85% ethanol and 15% gasoline) get about 30% lower gas mileage (*4*).

In the United States, the Energy Independence and Security Act (EISA) of 2007 mandates the production of 16 billion gallons per year of cellulosic (plant-derived) biofuels by 2022. Along with 15 billion gallons/year of corn ethanol and 5 billion gallons/year of other renewable biofuels such as biodiesel, the goal is to replace 20% of current crude oil use in the United States in 15 years. EISA 2007 is not a mandate for cellulosic ethanol but can be met with green gasoline, diesel, and jet fuel as well.

Converting woody biomass to ethanol requires breaking down plant tissues into cellulose, hemicellulose, and lignin. The cellulose and hemicellulose break down further into fermentable sugars. Lignin, which is not readily converted to sugars, constitutes up to 40% of its stored energy, although some of this energy can be recovered to heat the biomass-to-alcohol conversion process. Despite recent developments—including more efficient enzymes, more readily deconstructed plants, and consolidation of processing steps (*1*), production costs remain twice that of fermenting corn starch (*5*).

An alternative is provided by processing sugars with genetically altered microorganisms instead of yeast. Some of these microorganisms can ferment sugars into hydrocarbons instead of alcohols (*6*). The altered microbes produce hydrocarbon products at about the same rate as they would produce ethanol, and because the hydrocarbons spontaneously form a separate organic phase, the microbes are not poisoned by the accumulating fermentation product as occurs with alcohol. Companies such as Amyris and LS9 are near the point of commercializing such routes. Amyris plans to produce a drop-in replacement diesel fuel and specialty chemicals from sugar cane. It is currently scaling up the process to large production volumes in the United States and Brazil and expects to commercialize its first product in 2011 (*7*).

Dissolved sugars can also be converted into hydrocarbons through routes that resemble petroleum processing more than fermentation. Dumesic and co-workers have developed several routes in which dissolved sugars react in the presence of solid-phase catalysts under carefully controlled conditions that avoid unwanted by-products. They can convert carbohydrates into targeted ranges of hydrocarbons (*8*, *9*) for use as fuels or chemical feedstocks. Virent Energy Systems has developed a process known as Bioforming that converts water-soluble sugars into green gasoline, diesel, and jet fuel (*10*). In partnership with Royal Dutch Shell, Virent claims to be 5 to 7 years away from commercial production of hydrocarbon fuels at a capacity of 100 million gallons/year, at a price competitive with petroleum at $60/barrel (*11*).

The routes outlined so far process sugars from biomass. In contrast, pyrolytic methods convert woody biomass, including the lignin fraction, in a spontaneous high-temperature reaction into an intermediate called bio-oil. Traditional versions of pyrolysis simply heated the biomass in the absence of air; the immediate product is very acidic, unstable, and too low in energy content to be a viable fuel. The bio-oil therefore has to be stabilized and upgraded in a subsequent catalytic step. An updated pyrolysis approach developed by Huber and co-workers uses catalysts to convert biomass into high-octane gasoline-range aromatics in a single, simple, inexpensive step (*12*, *13*). These chemical methods produce heat and water, which preserves resources and helps lower cost.

One pyrolysis effort is being spearheaded by two companies: UOP, which has a long history in petroleum refining, and Ensyn, which has expertise in producing bio-oil for use as heating oil. The joint venture, Envergent, has stated its intention to "commercialize viable solutions for converting biomass to drop-in transportation fuels" by 2011 (*14*), also at a capacity of about 100 million gallons/year (*15*).

Another company, KiOR (*16*), is currently developing the "biomass catalytic cracking" process (BCC), which is analogous to fluidized catalytic cracking used in petroleum refineries to convert large hydrocarbons into smaller ones. KiOR intends to commercialize BCC for the production of diesel and gasoline components from forestry or agricultural waste by 2011. The scale of operation is roughly the same as that of the UOP and Virent processes (*17*).

Like pyrolysis, gasification also uses whole biomass but converts it spontaneously at very high temperatures into a mixture of carbon monoxide and hydrogen, or syngas, so named as it is a starting material for processes such as Fischer-Tropsch synthesis (FTS). Schmidt and co-workers (*18*) combined the three reactions of older thermal gasification processes into a single, small reactor in which gasification takes place over a catalyst.

The high transportation costs of biomass feedstocks demands economical operation in smaller-scale processing units. Choren Industries in Germany is in the process of commercializing a biomass-to-liquids operation (*19*) based on gasification and FTS. Diesel will be produced from multiple lignocellulosic feedstocks gathered locally (from a distance of about 50 km).

Cellulosic feedstocks could replace about 30% of petroleum used in the United States (*20*). The need to develop other biomass feedstocks has helped rekindle interest in algae after a decade of dormancy (*3*). Algae can be grown with relatively little land and in brackish water. Algae, as well as unsaturated plant oils, can be converted into fuels by hydrotreating; the main issue is the economical production of the algae feedstock. One company, Sapphire Energy, has developed advanced algae farming technology operating on nonarable land with nonpotable water and plans a production capacity of 100 million gallons of gasoline/year by 2016 (*21*). ExxonMobil has announced a partnership with Synthetic Genomics, Inc., with the goal of producing

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hydrocarbon biofuels from algae in 5 to 10 years (*22*).

Hydrocarbons derived from biomass are attractive because of their high energy density and compatibility with existing energy infrastructure. If recent technological innovations result in competitive production costs, hydrocarbons rather than ethanol will likely be the dominant biofuel.

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BEHAVIOR

Monkeys Like Mimics

Josep Call and Malinda Carpenter

Human and nonhuman primates recognize when they are being imitated. Is there a social advantage to that?

Human adults routinely engage in unconscious (automatic) bodily mimicry of each other, and this has many positive social consequences: When others mimic us, we like them more, empathize with them more, and are more helpful and generous toward them (*1*). On page 880 in this issue, Paukner *et al*. (*2*) show that these social consequences of mimicry may have deeper evolutionary roots than previously thought.

Adding to previous findings that apes (*3*, *4*) and macaque monkeys (*5*) recognize when they are being imitated, Paukner *et al*. demonstrate that imitation recognition has consequences for nonhuman primates in terms of their later affiliative behavior toward the imitator. They found that capuchin monkeys are more likely to sit closer to and exchange tokens with a human who imitated their actions than one who did not.

Most previous experimental work on imitation in nonhuman primates has focused on the instrumental function of imitation: learning new behaviors, typically to extract food from an unfamiliar apparatus. By contrast, Paukner *et al*. look at imitation in nonhuman primates from a more social and interpersonal perspective. In humans, imitation is not only a way of acquiring new behaviors, but also is a way of connecting with others and aligning oneself with them—of communicating one's likeness and affinity to others (*6*). This social function of imitation is apparent from a very early age, when human children copy others closely (see the figure), even in problem-solving tasks when copying the particular actions the demonstrator used often is not necessary to achieve the instrumental goal (*7*). Both adults and children copy others more often when social goals are important (*8*, *9*).

Imitation. Mimicking allows the acquisition of new behaviors and facilitates connections with others.

However, as Paukner *et al*. acknowledge, whereas the capacity to recognize imitation now appears to be widespread among primates, imitation itself is thought to be relatively rare in monkeys and apes, and certainly far less prevalent than in humans (*10*). It is thus unclear whether monkeys actually copy each other enough in their natural social groups for imitation recognition to serve the affiliative functions Paukner *et al*. postulate. Nonhuman primates do show contagious yawning (*11*) and some facial mimicry (*12*), but it is unknown how common and wide-ranging automatic bodily mimicry and imitation are for social functions in the natural social lives of nonhuman primates. Traditionally, researchers have used the distribution of grooming among individuals to assess affiliative networks in apes' and monkeys' social groups. Although it is conceivable that "mimicry networks" could accomplish a similar function, it is also possible that mimicry does not indicate affiliation per se; rather, it could indicate a different type of social information such as dominance. Individuals who copy others might be perceived as subordinate and therefore safer to approach. This could explain why monkeys in the Paukner *et al*. study approached the imitating partner (or avoided the nonimitating partner) to exchange tokens. Future studies will be needed to ascertain precisely what type of social information nonhuman animals extract from social mimicry.

If nonhuman primates do copy others' behaviors for social functions, this raises intriguing questions. Can monkeys mimic tactically, as humans do (*8*), to ingratiate themselves with others? This could be a powerful tool for developing, maintaining, and assessing social bonds with others. And does being mimicked have other, more actively prosocial consequences, as it does in humans (*13*)? For

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example, nonhuman primates who have been mimicked may help or share resources with others more often than those who have not been mimicked.

The finding of a link between imitation recognition and affiliative behavior in nonhuman primates highlights the need for more research into the social functions of imitation in nonhumans. We also need to explain why human imitation goes so far beyond automatic mimicry, in our "overimitation" as children (*14*), our conformity to the majority's way of doing things, our learning of actions conventionally and normatively (*15*), and our faithful transmission of such an extensive variety of cultural artifacts, rituals, and customs. An enhanced motivation to be like others may be what has boosted our imitation to such high levels.

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PHYSIOLOGY

How Much Sleep Do We Need?

Mutations that affect sleep duration are a starting point for understanding sleep regulation and function.

Hyun Hor[1] and Mehdi Tafti[1,2]

Sufficient sleep is necessary for optimal daytime performance and well-being, yet there is a large difference in how much sleep people need, ranging from less than 6 to more than 9 hours. People at all points along this range exhibit no noticeable differences in health and waking performance. Those of us who envy short sleepers would like to reduce sleep duration to the minimum necessary for normal functioning, but do we know what this minimum is? Short sleepers are found in families, as are long sleepers, which suggests a genetic basis for sleep duration. On page 866 of this issue, He *et al*. (*1*) add new evidence by showing that a mutation in a transcriptional factor, DEC2, is associated with short sleep in humans and mice.

Sleep and wakefulness are thought to be controlled by two independently regulated but interacting processes: a circadian program that sets the timing of sleep onset and offset, and a homeostatic process that tracks sleep need (*2*, *3*). The homeostatic process is examined by measuring slow-wave electrical activity in the brain—which is derived from recordings of spontaneous brain activity as an electroencephalogram—to quantify oscillations in the 0.5 to 4.5 Hz frequency range (delta oscillations). These oscillations characterize a phase of sleep called non–rapid eye movement sleep. Slow-wave activity is thought to reflect the intensity of sleep. Although this intensity measure is directly and predictably correlated with the duration of preceding waking, it only marginally predicts sleep duration, which indicates that sleep loss is primarily recovered by increasing sleep intensity and not necessarily by sleep duration.

By sequencing candidate genes in a family in which two individuals (a mother and her daughter) have short sleep durations (average 6.25 hours versus 8.06 hours in nonaffected individuals), He *et al*. identified a point mutation in h*DEC2*. DEC2 is a transcription factor involved in cell proliferation and differentiation, response to hypoxia, and circadian rhythms. The mammalian molecular circadian clock involves a heterodimer of transcriptional regulatory proteins containing BMAL1 and either CLOCK or NPAS2. This complex activates a negative feedback loop involving expression of the *Cryptochrome* (*Cry1* and *-2*) and *Period* (*Per1* and *-2*) genes (*4*). Although DEC1 and -2 were also proposed to act as negative regulators of the circadian clock (*5*), their precise role is still unclear. For example, mice deficient for both *Dec1* and *-2* show intact circadian rhythmicity with only a slightly longer period of their rest-activity cycle and an attenuated entrainment response to light (*6*). By contrast, the gene *Clockwork Orange* (*cwo*) in the fly *Drosophila melanogaster*, which is the closest h*DEC* homolog, cooperates with the clock protein PER to repress *Clock*-mediated transcription of target genes (*7*). Flies lacking *cwo* have a longer circadian period and become arrhythmic in constant darkness, indicating that the fly homolog of h*DEC* is a core clock gene (*7*).

Sleep genes. Sleep amount, like weight and height, is a quantitative phenotype normally distributed in the population. Total daily sleep duration has an estimated heritability of ~50% in humans and mice, suggesting complex underlying genetics with contributions from numerous genes. But mutations in single genes that yield dramatic effects cannot be excluded.

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The mutation identified by He *et al*. causes a proline-to-arginine amino acid change at position 385 of hDEC2 (P385R) and was not found in 250 control subjects. The authors genetically engineered mice to express the P385R mutant form of hDEC2 and found that their activity period was ~1.2 hours longer than in normal mice. This activity period was even longer (~2.5 hours) when the P385R variant was expressed in mice lacking endogenous DEC2 expression, confirming a dominant

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negative effect of this mutation. When they expressed P385R in *Drosophila*, the length of the "sleep-like" state was also reduced.

Human short sleepers experience greater pressure for non–rapid eye movement sleep, as indicated by a fast accumulation of slow-wave electrical activity and a small increase in this activity after sleep deprivation (*8*). Sleep intensity, as measured by these parameters, is a poor indicator of sleep duration, which in most mammals is larger than what can be expected on the basis of the two-process model. On the other hand, long sleepers seem to live under a longer biological night—for example, they have a longer nocturnal duration of high plasma concentration of the hormone melatonin (which regulates the sleep-wake cycle)—suggesting individual differences in the circadian timing program (*9*). Thus, the original assumption of independence between the circadian and the homeostatic processes needs revision. Indeed, recent evidence indicates that mammalian core clock genes such as *Npas2*, *Cry1*, *and Cry2* are involved in sleep homeostasis (*10*) and that behavioral states affect the expression of many circadian genes (*11*). Thus, it becomes evident that the need and intensity of sleep and the circadian timekeeping interact and are co-regulated at the molecular level.

Unfortunately, sleep homeostasis is not defined universally. Changes in slow-wave electrical activity in the brain are widely used in mammals as an index, but the amount of rest (or sleep-like state) is the measure used in invertebrates, even if the biological basis of its regulation might be completely different (*12*). Together with the discovery of several mutations associated with a circadian sleep disorder called familial advanced sleep phase syndrome (*13*), the identification of the P385R mutation in h*DEC2* constitutes a starting point for studying the regulation of timing and organization of sleep. The P385R mutation seems to be very rare (one out of 60 families examined by He *et al*.) and should be replicated in other independent samples of short sleep individuals or families. Although He *et al*. report some preliminary in vitro evidence that P385R causes a reduction in DEC2 repressive activity, the in vivo functional consequences of this mutation are not clear. As opposed to the *Drosophila* homolog *cwo*, DEC1 and DEC2 have no clear circadian role and the proline residue at position 385 is not conserved in invertebrates, which suggests a different interspecies functional contribution. Whether the P385R mutation affects sleep need (homeostasis) or the circadian timing program by, for instance, shortening the biological night, can be evaluated by studying individuals with the P385R mutation in constant conditions or P385R transgenic mice under constant darkness.

The question "How much sleep do we need?" is not only of practical interest for obvious societal reasons, but is also of major importance for understanding sleep function. Recent hypotheses in the field favor a role in memory and/or synaptic plasticity. However, an unbiased approach may turn out to be more efficient. Molecular genetic approaches remain our best hope to find, without a priori assumptions, molecules that regulate the complex phenotype of sleep.

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BIOCHEMISTRY

Friction in Motor Proteins

Single-molecule experiments elucidate the role of friction in the motion of the kinesin motor protein.

Claudia Veigel[1] and Christoph F. Schmidt[2]

What do a violin bow and a motor protein have in common? They both need stick-slip friction to function. On page 870 of this issue, Bormuth *et al*. (*1*) explore just how much friction is involved in the motion of a kinesin motor when it moves along its microtubule track, and how this friction is related to the spontaneous diffusive motion of this motor when it has run out of its fuel.

Friction is important in engineering because it leads to heating, limits the efficiency of machines, and causes surface wear. Friction leads to dissipation of energy: The kinetic energy of a flying golf ball is transmitted to the surrounding air, heating it up. In air or fluids, friction can be described in terms of momentum transfer between the object and successive layers of the surrounding medium (*2*). Velocity changes gradually, at least when the flow of the medium is not turbulent, and calculations are straightforward.

The situation is very different for friction between solids. Here, the action is confined to the atoms and molecules at the surfaces in contact; molecular interactions under locally extremely high pressures and forces thus determine the macroscopic forces of friction. Understanding of such friction is limited (*3*). Some phenomenological laws have been derived. For example, the frictional force is proportional to the normal load pressing the surfaces together and is independent of velocity. Yet microscopic experiments do not always confirm these laws (*4*).

A common phenomenon, where friction rapidly switches between strong and weak, is stick-slip motion (*5*). It is this motion that makes a wine glass sing when rubbed and a violin string vibrate under the moving bow. The reason for this intermittent stickiness is believed to be the alternating formation and rupture of chemical bonds at the surface. This mechanism provides an intuitive picture for the dissipation of energy: Once the bond breaks, the tensed linkage snaps back; the built-up elastic energy is converted to heat when the molecules relax (*4*).

This model, merely postulated for macroscopic friction, can be tested microscopically in single-molecule experiments measuring the forces, for example, between an individual motor protein and the track it moves on. Bonds must be broken and reformed when a continuously moving motor such as kinesin moves along its track. Some fraction of the chemical energy provided by the motor fuel adenosine triphosphate (ATP), needs to be

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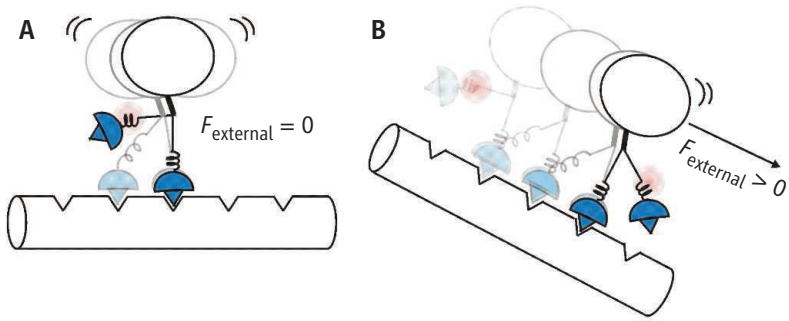
spent on breaking the bonds; this unavoidably leads to friction and dissipation of energy, limiting the motor's efficiency (*4*, *6*).

Bormuth *et al*. now analyze the tradeoffs involved in the motor function from the point of view of friction. If a motor is supposed to carry a load, it must bind strongly enough to withstand the force exerted by that load. Yet strong binding entails strong friction, thus decreasing efficiency. This is a clear optimization problem. But that is not the end of the complexity. Passage through the ATP binding and hydrolysis cycle is commonly assumed to involve switching between states of weak and strong binding to the track (*7*), in turn implying the possibility of different friction coefficients. Some motors appear to use energy-saving diffusive motion to search for their sites of action (*8*, *9*). Even proteins without motor function can switch between diffusive weakly bound states and nondiffusive microtubule-bundling states (*10*).



Microscopic friction. Microscopic models of friction between solids typically involve the formation and rupture of molecular bonds. Dissipation occurs when elastically stretched bridges between the objects rupture and the stored elastic energy is converted to heat. This causes the stick-slip motion of a violin bow that excites the string. For single motor molecules in equilibrium, thermally excited bond rupture leads to diffusion (**A**); the same forces act to oppose small driving forces (**B**).

In the microscopic world of motor proteins, thermal energies are important. Because the activation energy barriers separating the different chemical states of the motor are not very much higher than the amount of energy that can be "borrowed" temporarily from surrounding water, a motor can in the absence of fuel still hop from binding site to binding site as a result of random thermal bond ruptures (see the figure). To comply with the second law of thermodynamics, this results not in directed motion but rather in diffusive, nondirected motion. According to the fluctuation-dissipation theorem (FDT) (*11*), the diffusion coefficient observed in such an equilibrium situation is related to the frictional drag experienced in response to a weak driving force (*12*, *13*).

The FDT opens up new experimental approaches to studying molecular motors. One can measure diffusive motion in different fixed chemical states and from the results draw conclusions on drag coefficients, or one can directly measure drag coefficients and draw conclusions about the energy wells controlling the binding of the motors to their tracks and about diffusive motions.

However, more than one process can lead to dissipation of energy. Kinesin is a homodimer that stays on track while moving by alternating bound heads. One obvious source of friction is the motion of parts of the molecule through the aqueous environment. Friction should also occur when molecules change conformation during the biochemical fuel consumption cycle. Finally, bond rupture friction should accompany the unbinding of the heads. The last two types of friction are difficult to tell apart, but one can generalize the Bormuth *et al*. head-track bond rupture model to include both. After all, internal conformational changes also involve bond rupture events within an individual motor head that dissipate energy. And weakening of the bond really means rupturing a fraction of the collective bonds that bind the motor to the track.

Whether drag in water or bond rupture dominates friction can be guessed at from a combination of various experiments. A fluorescent motor moving without load at saturating ATP concentration gives a maximum speed (v_{max}) that results from the friction force ($F_{friction}$) balancing the driving force (F_{motion}). F_{motion} can in turn be estimated from an experiment in which the motor is prevented from moving (for example, by attaching it to an optically trapped bead). Given $F_{friction} = F_{motion} = \gamma v_{max}$, one can estimate the drag coefficient γ. The result can be compared to an estimated Stokes friction coefficient of the moving motor in water and to the friction coefficient obtained from diffusion measurements of motors on the track.

Bormuth *et al*. conclude that the Stokes drag in water is negligible, and that the drag balancing the driving force for the unloaded motor is too strong to come from rupturing the weakly bound state, but must rather result from rupturing a strongly bound state. In that case, the driving force for the diffusive motion along the track cannot come from the aqueous environment and must thus come from interactions with the track (*6*).

The FDT holds only for small driving forces. Are the forces of a few piconewtons exerted by a motor protein small enough? The results of Bormuth *et al*. show that this is not quite the case. In equilibrium (without ATP), diffusion is direction-independent despite the underlying directed track. Thus, within the validity range of the FDT, the friction coefficient must also be direction-independent. This is seen for forces below ~1 pN, but for larger forces, the friction force depends on the direction of pulling and saturates as a function of speed. This is expected for bond rupture events, in which the rupture rate (which is proportional to speed) depends exponentially on the load, and the rupture force (which equals the friction force) thus depends logarithmically on speed (*4*, *14*).

The study of motor protein performance in the context of bond rupture friction successfully fuses tribology concepts with biophysical single-molecule experiments and with a large body of knowledge from force spectroscopy about intermolecular bonds. Given that more and more proteins in cells, including DNA mechanoenzymes, are found to move in the gray zone between diffusion and directed motion, concepts such as friction and fluctuation-dissipation will become more and more important.

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Strategic Reading, Ontologies, and the Future of Scientific Publishing

Allen H. Renear* and Carole L. Palmer

The revolution in scientific publishing that has been promised since the 1980s is about to take place. Scientists have always read strategically, working with many articles simultaneously to search, filter, scan, link, annotate, and analyze fragments of content. An observed recent increase in strategic reading in the online environment will soon be further intensified by two current trends: (i) the widespread use of digital indexing, retrieval, and navigation resources and (ii) the emergence within many scientific disciplines of interoperable ontologies. Accelerated and enhanced by reading tools that take advantage of ontologies, reading practices will become even more rapid and indirect, transforming the ways in which scientists engage the literature and shaping the evolution of scientific publishing.

The 1980s abounded in descriptions of a coming new world of scholarly communication, predicting functionality that we knew was possible and would soon be technologically feasible. This imagined world, which was never fully realized, predicted advanced navigation; discipline-specific intelligent tools for searching, browsing, and analysis; reader-initiated hypertext linking; "live" data-driven diagrams; computationally available information objects; searchable indexed annotations; thorough-going interoperability; and so on. Substantial improvements in hardware and software and an infrastructure of networked communications now make this anticipated functionality possible. Lying at the heart of the changes taking place is an escalation of strategic reading practices.

Scientists have always read strategically, working with many articles simultaneously to search, filter, compare, arrange, link, annotate, and analyze fragments of content. Now, however, two important trends are interacting to support and intensify the effectiveness of these practices. The first is the wide-scale use by scientists of digital indexing, retrieval, and navigation resources (such as PubMed, Web of Science, the ACM Digital Library, NASA's Astrophysics Data System, CiteSeer, Scopus, and Google Scholar) to exploit large quantities of relevant information without reading individual articles. The second is the emergence within many scientific disciplines of ontologies for representing and linking scientific data. This convergence of digital resources and data-linking ontologies will result in even more rapid and indirect use of the literature, supported not only by text mining (*1*) and literature-based discovery applications (*2*), but by "ontology-aware" strategic reading tools as well.

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Why Will the Revolution Happen Now?

When it was launched in 1992, the *Online Journal of Current Clinical Trials*, jointly designed by the American Association for the Advancement of Science and the OCLC Online Computer Library Center, was seen by some as the beginning of the long-awaited world of advanced digital publishing. However, the journal failed to flourish, and the new world did not materialize. In retrospect, we can see that in the early 1990s, none of the basic conditions required for an advanced scientific publishing system existed. Not only was the basic technology and infrastructure inadequate, but the entire publishing system also would have required extensive coordinated changes.

Although there was no revolution, an important transformation did take place in the 1990s. In 1993, very few scientific, technical, and medical (STM) journals had an electronic version, and yet by 2003, virtually all of them did. For the daily work routines of most scientists, that new format had already become more important than print. The system of digital publishing that emerged from 1993 to 2003 was impressive in some respects, but was still largely another case of new technology compromised by imitation of the old.

The reasons a more radical change failed to occur are understandable in retrospect, and they also suggest why we are now on the cusp of a larger change. None of the developments during this period required costly or uncertain changes in workflow and production processes, software tools, user behavior, or business models. STM publishers were already creating Adobe PostScript files for print production. These could be automatically converted to the Adobe page description language format (PDF), which was suitable for distribution over the existing Internet and could be browsed with existing free software applications. Users, in turn, were presented with a printlike experience that was at once familiar and yet had additional advantages, including Internet delivery, digital storage, full-text searching, and local printing, all of which was easily realized with existing technologies. Hence, as its value became apparent, PDF-based digital STM publishing emerged relatively quickly, with few changes in production or existing infrastructure.

Since 1992, processor speeds, memory, storage, and bandwidth to the desktop have undergone enormous improvements, as have connectivity and costs. Standard protocols for network communication have been adopted, new software tools and software engineering strategies have emerged, and there is now a supporting infrastructure of information professions and institutions. The pervasive use of the World Wide Web via intuitive Web browsers is an especially visible change, and the widespread use of Extensible Markup Language (XML), with its associated standards and technologies, provides a foundational framework for storing, processing, and presenting information on the Web. In addition, an important recent development is the convergence within the STM publishing community on a single XML schema for the representation of scientific articles: the National Library of Medicine (NLM)'s Journal Archiving and Interchange Tag Suite (*3*).

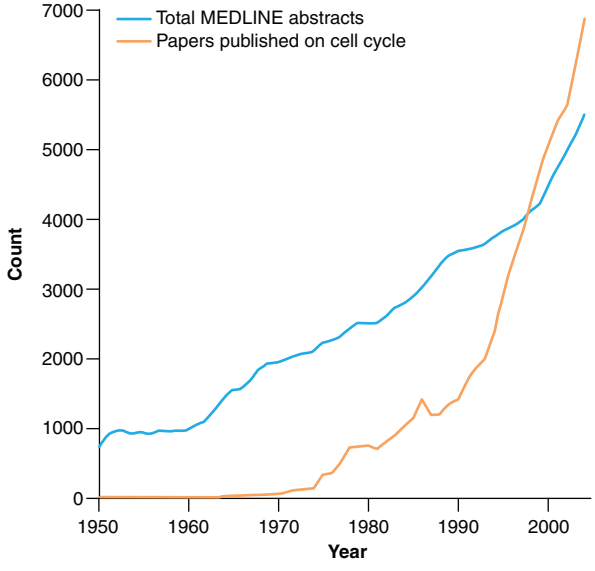

Fig. 1. Increase in number of papers published each year in biomedicine and in one specialized topic, the cell cycle. [Adapted with permission from MacMillan Publishers, *Nature* (*5*), copyright 2006]

The driving force for change remains the same: the growing quantity and complexity of information in combination with limited time for reading. But in some disciplines, we seem to be past the point where any further specialization of research focus or elaboration of collaborative relationships are effective (*4*, *5*) (Fig. 1). Just as the increased quantity of information and general intensity of scientific activity is reaching the point where it cannot be sustained with current practices, technology and user behavior are making new practices feasible, and research scenarios that a decade ago were utopian are now widely anticipated by practicing scientists. P. Bourne, a Public Library of Science journal editor, offers this vision of the near future

> the scientific literature will seamlessly provide annotation of records in the biological databases. Imagine reading a description of an active site of a biological molecule in a paper, being able to access immediately the atomic coordinates specifically for that active site, and then using a tool to explore the intricate set of hydrogen-bonding interactions described in the paper.… Alternatively, if you are starting with the data … viewing the chromosome location of a human single-nucleotide polymorphism associated with a neurological disorder, … immediately access a variety of papers ranked in order of relevance to your profile … pinpointing the reference to the single-nucleotide polymorphism in the full-text article (*6*), p. 179.

This sort of information gathering goes well beyond conventional digital publishing and reveals why the current state of affairs has failed to meet some expectations. As chemists P. Murray-Rust and H. S. Rzepa remarked in 2004, "The current transition to [PDF-based] e-journals seems to be welcomed by many—but not us … a cultural change in our approach to information is needed" (*7*).

How Are Scientists Working with the Literature?

Scientists have always strived to avoid unnecessary reading. Like all researchers, they use indexing and citations as indicators of relevance, abstracts and literature reviews as surrogates for full papers, and social networks of colleagues and graduate students as personal alerting services. The aim is to move rapidly through the literature to assess and exploit content with as little actual reading as possible. As indexing, recommending, and navigation has become more sophisticated in the online environment, these strategic reading practices have intensified.

Now, as scientists search and browse, they are making queries and selecting information in much tighter iterations and with many different kinds of objectives in mind, almost as if they were playing a fast-paced video game. They sweep through resources, changing search strings, chaining references backward and citations forward, dodging integrator and publisher sites to find open-access copies, continually working to reduce the number of clicks required for access. By note-taking or cutting and pasting, scientists often extract and accumulate bits of specific information, such as findings, equations, protocols, and data. In this process, rapid judgments are made—such as assessments of relevance, impact, and quality—while search queries are being formulated and refined. (Fig. 3). The goal often seems to be undifferentiated assimilation of information about a domain or a problem at hand, and the online experience may be highly valuable, even though no clear aim is met and no articles to read are located. In a compelling analogy, Nicholas *et al*. (*8*) describe a "slightly irritated" father watching his young daughter flick from channel to channel while watching television

> [the] father asks … why she cannot make up her mind and she answers that she is not attempting to make up her mind but is watching all the channels. … gathering information horizontally, not vertically (*8*), p. 40.

And they conclude

> Now we see what the migration from traditional to electronic sources has meant in information seeking terms. We are all bouncers and flickers, and the success of Google is a testament to that, with its marvelous ability to enhance and amplify this flicking and bouncing (like a really good remote).… In the past, information seeking was seen to be the first step to creating knowledge. Now … it is a continuous process (*8*), pp. 41–42.

Just as the aim of channel surfing is not to find a program to watch, the goal of literature surfing, is not to find an article to read, but rather to find, assess, and exploit a range of information by scanning portions of many articles. This behavior is common among scientists (*9*).

Longitudinal studies of e-journal use confirm that scientists are indeed "reading" more papers at a faster pace (*10*). That is, the total time spent reading journal articles has risen only a little, whereas the number of journal articles read per year has gone up much faster and appears to be growing still. The number of articles read (as distinguished from those merely browsed) by scientists was ~50% higher in 2005 than in the mid-1990s. Furthermore, though the average reading time per article did not change much from 1977 to the mid-1990s (48 versus 47 min), it started falling in the mid-1990s and is now just over 30 min per article (Fig. 2). At the same time, identifying papers by searching online increased more than fourfold between 1977 and 2005. These changes in journal use are far greater in STM disciplines than the averages over all disciplines, suggesting that as work with the literature has moved online, scientists are scanning more and reading less.

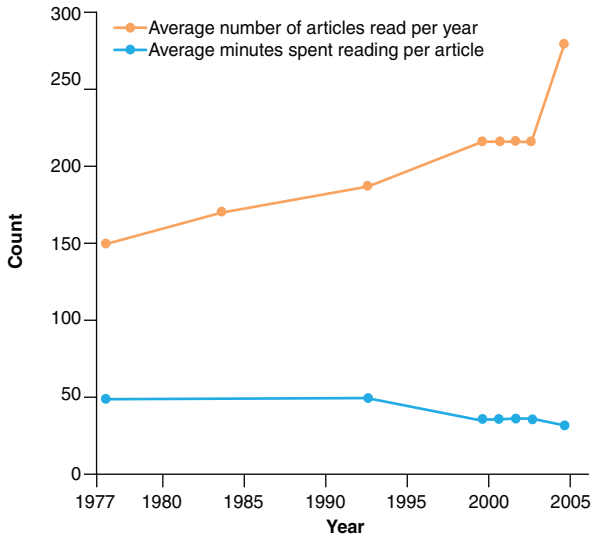

Fig. 2. Increase in the number of papers read by scientists per year and decrease in minutes spent reading each paper, trends based on a series of survey studies conducted by Tenopir *et al*. between 1977 and 2005 (*10*, *34*, *35*).

Early digital library research also showed how scientists scan individual printed journal articles to identify key components—such as tables of contents, references, figures, formatted lists, equations, and scientific names—for quick review and absorption of information (*11*, *12*). More recent studies of the research process have emphasized the varied ways in which scientists work with information (*13*, *14*). The literature is scanned not only to position new findings in cognate fields and learn about collaborators' domains, but also to monitor the progress of peers and competitors. Information is collated to compare measurement and instrumentation details; it is also used to compile personal collections in evolving areas of interest and to extract the facts and evidence needed to build databases. These are all aspects of strategic reading, a robust, well-entrenched behavior that is vastly more efficient in the digital realm and is thus a promising target for digital support.

How Is Scientific Information Being Represented?

Structured terminologies for representing scientific data, along with standard XML-based techniques for defining and using these terminologies, are forming the basis for new types of scientific publishing. Although computer-processible scientific terminologies range from simple standardized

vocabularies to sophisticated formal systems with logical axioms, we have called all of them ontologies.

Ontologies are particularly prominent in the biological sciences (*15*, *16*). One example of rapid adoption is the Gene Ontology (GO) (*17*), which started in 1998 to support the annotation of genes and gene products and is now very widely used, containing more than 25,000 terms and 3.3 million annotations. Although many biological ontologies were originally developed independently, the need for interoperability has driven collaboration, a good example being the Open Biomedical Ontologies (OBO), which currently has 54 participating projects (*18*), including Microarray Gene Expression Data (MGED), BioPAX, for biological pathways data, and Foundational Model of Anatomy (FMA).

Although the size, complexity, and logical design of scientific ontologies may vary, a partial description of GO, drawing on examples from the GO introductory material, will illustrate some of their general features (*19*, *20*). GO consists of three separate ontologies: (i) molecular function, (ii) biological process, and (iii) cellular component. Within each of these, terms are uniquely identified, defined, and related in a network of "is a" relationships (e.g., a nuclear chromosome is a chromosome). GO also contains the relationship "part of" (e.g., periplasmic flagellum is part of periplasmic space), and recently, the relationship "regulates" and subtype relationships "positively regulates" and "negatively regulates" were added. These relationships have logical features; for instance, "is a" and "part of" are transitive (e.g., if X is part of Y and Y is part of Z, then X is part of Z). It is easy to see not only how the controlled vocabulary of a shared ontology can facilitate the integration of data from multiple sources, but also how relationships such as "is a", "part of", and "regulates" can support other information management tasks as well, including information retrieval and text mining, error checking, and automated inferencing.

Neither controlled vocabularies nor even logic-based ontologies are entirely new, although the enormous increase in the amount and complexity of biological data makes such organizational strategies increasingly urgent. Now, however, we can make ontologies and their applications computationally available and interoperable through well-supported standards associated with the Internet and World Wide Web.

In 1998, as work began on GO, the World Wide Web Consortium (W3C) released XML (*21*), a metalanguage for defining markup languages (*22*) for representing information on the World Wide Web. Originally designed for document-oriented languages, XML was soon used for other kinds of information as well. XML languages are defined by a computer-readable schema, which specifies, among other things, the terms of the markup language and the ways those terms can be arranged in valid documents. XML organizes information as a hierarchical structure (an "ordered tree") of labeled nodes and attribute/value pairs and represents that structure in a linear format readable by both humans and computers. Software can read data in this format and construct the correct tree structure, even without the schema that defines the language (this is a virtue of XML). If a schema is available, additional processing is possible, such as verifying that the data are complete and correctly organized; a schema can also configure editing tools so that human coders are only offered legal coding options, making coding easier and syntax errors impossible. In just 10 years, XML and related supporting software and standards have come to dominate information representation in networked environments—all popular Web browsers support XML, and most major database systems import and export XML-formatted data.

Although using XML to declare and apply a terminological vocabulary improves interoperability and access to software applications, it does have some limitations. XML schemas specify syntax, not semantics (*23*). An XML schema does not itself indicate how to interpret portions of a particular XML tree structure in terms of scientific assertions, nor is it, alone, suitable for defining logical relationships among terms. That information must be recorded in the natural language documentation for the schema, but then it is unavailable for computer processing. To address this problem, the semantic Web languages Resource Description Framework (RDF), Resource Description Framework Schema (RDFS), Web Ontology Language (OWL), and Semantic Web Rule Language (SWRL) were developed (*24*). These are computer-processible knowledge representation languages that provide a standard technique for defining ontologies and expressing assertions that use terms from those ontologies. Although technically independent of any particular computer-encoding format, RDFS and OWL each have a standard XML syntax that is now well-supported by software applications and widely used for ontology representation.

Today, an emerging infrastructure of education, research, conferences, organizations, and software tools is sustaining the development and adoption of scientific ontologies and providing opportunities for coordination to improve interoperability and share best practices. Particularly important for biology are the National Center for Biomedical Ontology, OBO, and the International Society for Biocuration, as well as more broadly defined organizations such as the National Center for Biotechnology Information and the European Bioinformatics Institute. One notable software application for ontology development is the widely used and well-supported Protégé ontology editor.

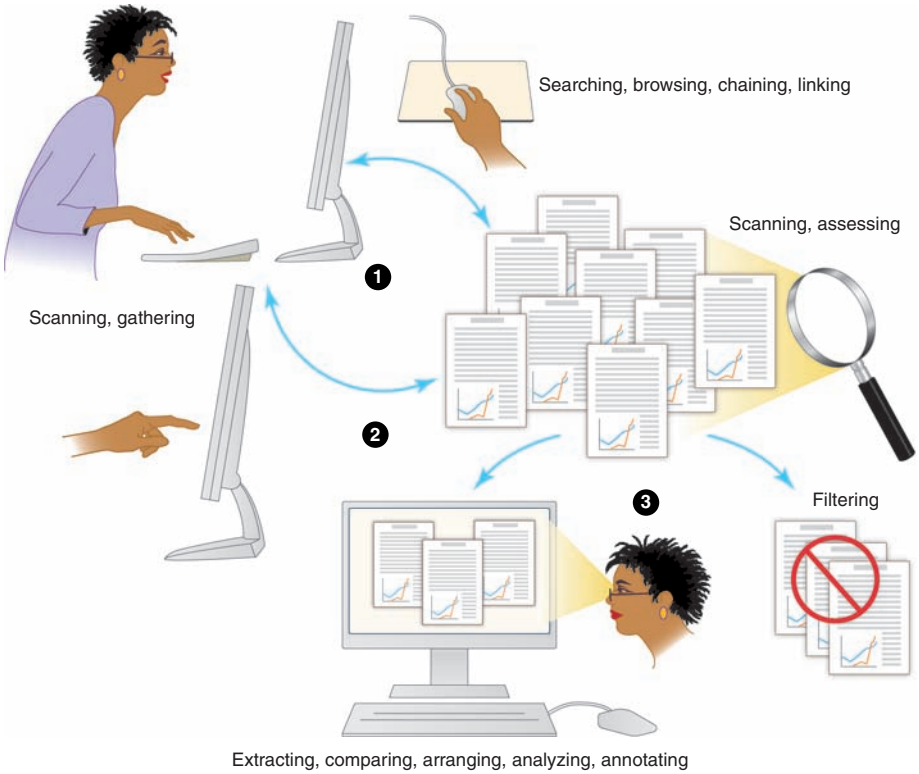

Fig. 3. Current work with digital resources.

How Can Ontologies Help Scientific Publishing?

Originally motivated by the need for data integration, scientific ontologies are now being explored for STM publishing to support information retrieval and text mining, with applications for hypothesis generation and knowledge discovery well underway. Nevertheless, reading-like engagement with scientific articles is not likely to disappear entirely: The natural language prose of scientific articles provides too much valuable

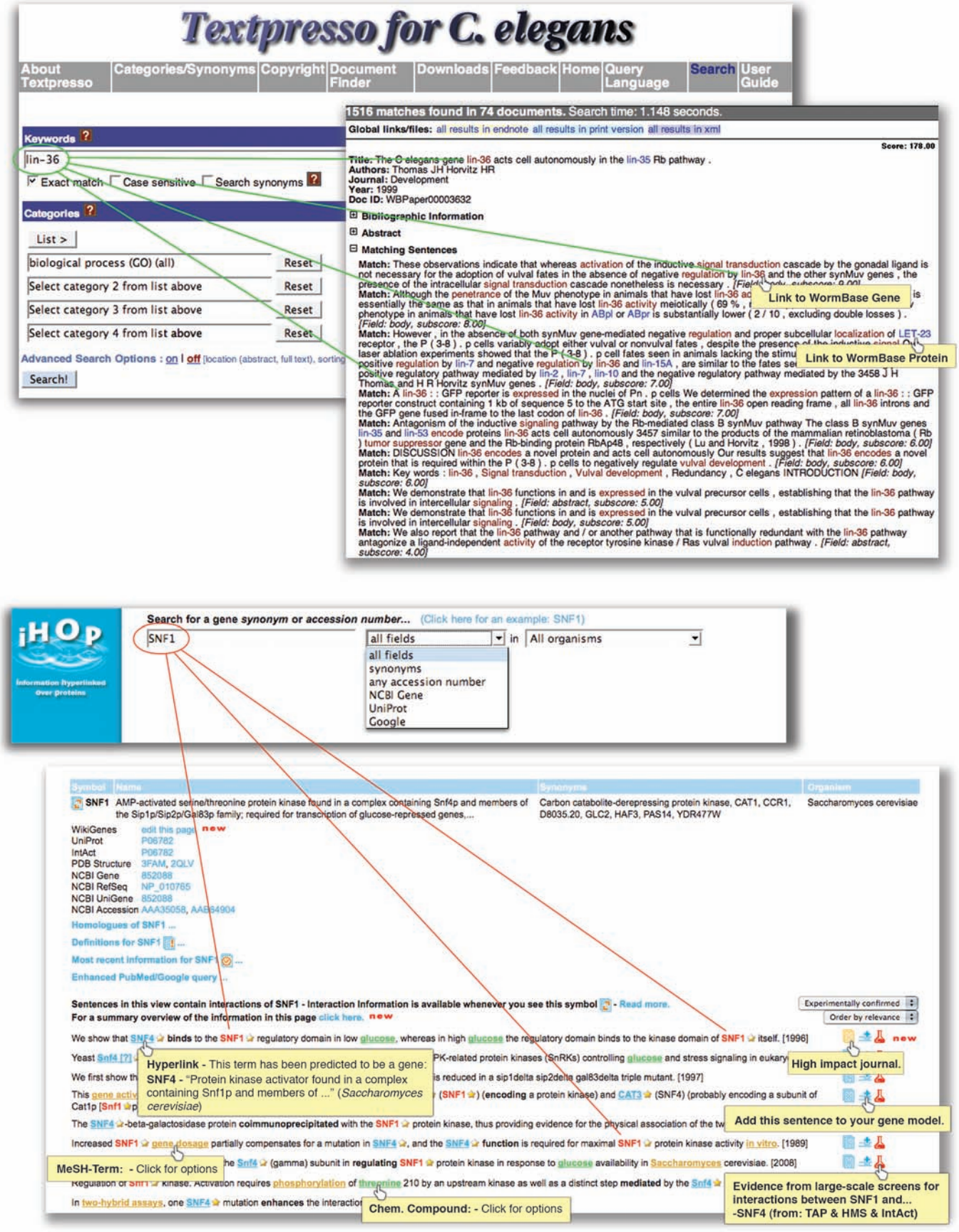

Fig. 4. Two examples of ontology-aware text mining/retrieval systems that support strategic reading. (**Top**) Textpresso (www.textpresso.org/) and (**Bottom**) iHOP (www.ihop-net.org/).

nuance and context to be treated only as data (*25*). Scientists may have moved well beyond traditional reading, but they still remain engaged with the narrative of scientific articles and need tools to help them read, and not only mine, that narrative.

The integration of ontologies into the scientific literature has been recommended by leading scientists (*26*–*28*), and the current generation of ontology-based text mining and retrieval tools in the biomedical sciences is already taking advantage of natural language processing and databases of annotations (*5*, *29*, *30*). One example is Textpresso, an ontology-based mining and retrieval system that works with prepared collections of articles, split into sentences and annotated with terms from 33 ontology categories, three of which correspond to the GO ontologies (*31*). Results screens present a ranked list of sentences within a ranked list of articles, with term highlighting, and links to articles and external databases (Fig. 4, top). Reading the sentences of an article in relevance order rather than narrative order is an example of strategic reading within an article. An example of strategic reading across a collection is provided by Information Hyperlinked over Proteins (iHOP), which uses genes and proteins to create a network of sentences and abstracts for searching and navigating MEDLINE abstracts (*32*). The iHOP database processes abstract sentences using National Center for Biotechnology Information taxonomy identifiers and the Medical Subject Headings (MeSH) thesaurus and supplies pages of configurable results, in ranked lists of sentences retrieved from many abstracts (Fig. 4, bottom).

Unlike similar explorations in the 1980s and 1990s, these are not computer science experiments or pilot projects requiring substantial investment and large upfront changes in infrastructure and practices to scale them up for general use. These are projects that are already producing practical and widely used tools.

How Do We Support and Shape These Changes?

The infrastructures and services to support strategic reading practices will no doubt be promoted by open access and alternative publishing models, which are already being widely discussed in the academic community. However, research on information behavior and the use of ontologies is also needed.

Traditional approaches to evaluating information systems, such as precision, recall, and satisfaction measures, offer limited guidance for further development of strategic reading technologies. Finer-grained methods that analyze what scientists actually do and value are required if we want to understand the nearly subconscious tactics that govern second-by-second interactions with the literature and the nuances of intention and use. We know, for instance, that scientists often have trouble locating very problem-specific information (on methods and protocols, for instance) and that the occasional exploration of results from another discipline can have considerable impact on progress or the direction of research. These are the kinds of information behaviors that we need to understand more fully to design tools that go beyond search and retrieval to support creative strategic reading.

For ontology-aware reading tools to function well, terminological annotations must be included in, or mapped to, the XML encoding of articles during the publishing production process, to connect names and phrases in narrative text with appropriate standard terminology. The emergence of the NLM schema as a standard XML encoding for scientific articles provides a promising shared context for terminological annotation; however, we also need specific strategies that are economically sustainable within the current context of STM publishing workflows, as well as remedies for "legacy data," the articles already published and stored in repositories. To exploit terminological annotations across the Internet, reading tools will have to operate in real time to take advantage of the ontologies that define and relate terms and connect terms with relevant databases with the use of "service-oriented architectures" (*33*). Finally, the development of ontology languages with additional expressive power is needed, as well as continued support for evolving, coordinating, and harmonizing ontologies.

How Will Scientists Work with the Literature in 2019?

Scientists will still read narrative prose, even as text mining and automated processing become common; however, these reading practices will become increasingly strategic, supported by enhanced literature and ontology-aware tools. As part of the publishing workflow, scientific terminology will be indexed routinely against rich ontologies. More importantly, formalized assertions, perhaps maintained in specialized "structured abstracts" (*27*), will provide indexing and browsing tools with computational access to causal and ontological relationships. Hypertext linking will be extensive, generated both automatically and by readers providing commentary on blogs and through shared annotation databases. At the same time, more tools for enhanced searching, scanning, and analyzing will appear and exploit the increasingly rich layer of indexing, linking, and annotation information.

There are no technical obstacles to this trajectory, and it is already under way. The changes, as always, will be incremental: Scientists, who today already make extensive use of existing indexing and retrieval services, will encounter a steady stream of new enhancements and adopt those that allow rapid and productive engagement with the literature. The new functionality will sometimes be provided as part of the application interface (new features in PubMed, for instance) or as shared external tools that users can add to their Web browsers. These developments chart a middle course between the already obsolete activity of finding an article to read on the one hand, and the narrower objectives of text mining on the other, responding directly to the entrenched necessity and value of strategic reading in the daily work of today's scientists.

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Local Adaptation of Bacteriophages to Their Bacterial Hosts in Soil

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Adaptation to local environmental conditions is crucial to the evolution and distribution of biodiversity. Parasites are thought to be responsible for intense and spatially varying selection pressures on their hosts and vice versa. Despite evidence for host and parasite local adaptation in a range of taxa (*1*), very little is known about this process in natural communities of some of the most abundant organisms on the planet: bacteria and their parasitic viruses (bacteriophages, phages). Lytic phages bind to the bacterial cell surface, inject their genetic material, and use the host cellular machinery to replicate. Understanding bacteria and bacteriophage population structure is of particular importance because microbial diversity ultimately determines how ecosystems function and respond to environmental change (*2*). Consequently there has been much recent interest in determining the relative importance of biotic and abiotic factors in driving microbial diversity in natural ecosystems (*3*).

We measured local adaptation of one focal group of bacteria and their associated phages in soil by collecting soil samples from two 25-cm-by-25-cm grids on a grazed flood plain (Port Meadow, Oxford, UK), taking five-by-five evenly spaced soil cores (1-cm diameter) from each grid. We isolated 24 isomorphic bacterial colonies as well as a suspension of the total bacteriophage population from every sample (*4*). Sequencing of 16*S* ribosomal RNA in randomly chosen clones demonstrated that the majority of isolates (75%) were *Stenotrophomonas*.

To first determine the proportion of bacterial clones that were susceptible to phage from the same 25-cm-by-25-cm population, we plated out each of the 600 bacterial clones from a sampling grid with a mixture of all 25 phage suspensions to score phage plaques after overnight incubation (plaques indicate that bacteria have been successfully infected and lysed). A total of 199 and 241 clones displayed plaques for the respective grids. A large proportion of bacteria (33 and 40%) thus could be lysed by phage originating from the same 625-cm^2 area.

We next paired all sensitive clones to all 25 individual bacteriophage suspensions in each grid to test whether local phages (from the same sample) were more or less infective than foreign phages (from other samples) (Fig. 1A). Phages were on average 9% more infective to local than to foreign bacteria (Fig. 1B). No correlation existed between the degree of local adaptation and distance between samples (5 to 28 cm; $R^2 < 0.01$, $P > 0.4$), indicating that local adaptation occurs at a scale of centimeters or less. This finding is particularly striking because the site is grazed and regularly flooded, which presumably would increase soil homogenization over such small spatial scales.

In the absence of selection, local adaptation could only result by chance (*5*), which we have statistically ruled out. Phage local adaptation must therefore result from divergent selection imposed by bacteria, because this is the phage's one essential resource. We are unable to unequivocally establish whether phage-imposed selection is similarly responsible for variation in phage resistance between samples of bacteria because bacteria are likely to experience additional selection pressures from both the abiotic and other aspects of the biotic environment. However, the relatively high degree of lysis by phages suggests that they may impose very strong selection. Moreover, reciprocal selection of bacterial resistance and phage infectivity (antagonistic coevolution) has been inferred from sequence data (*6*, *7*) and has been shown to drive rapid between-population divergence of both bacteria and phage in laboratory studies [e.g., (*8*)].

Phage local adaptation suggests that phages are ahead of the bacteria in the coevolutionary arms race. We propose two non–mutually exclusive reasons (*8*). First, selection acting on phages to overcome bacterial resistance is likely to be stronger than phage-imposed selection for bacterial resistance, simply because phages have to infect bacteria to replicate. Second, the genetic diversity, and hence evolutionary potential, of the sampled phages (the whole community) is likely to be greater than that of the bacteria, which largely belong to a single taxonomic group.

Our study emphasizes the importance of biotic interactions, in addition to variation in the physical environment, in shaping natural microbial communities. More generally, selection, and not just migration, clearly plays a crucial role in the small-scale spatial structuring of microbial genetic diversity in soil.

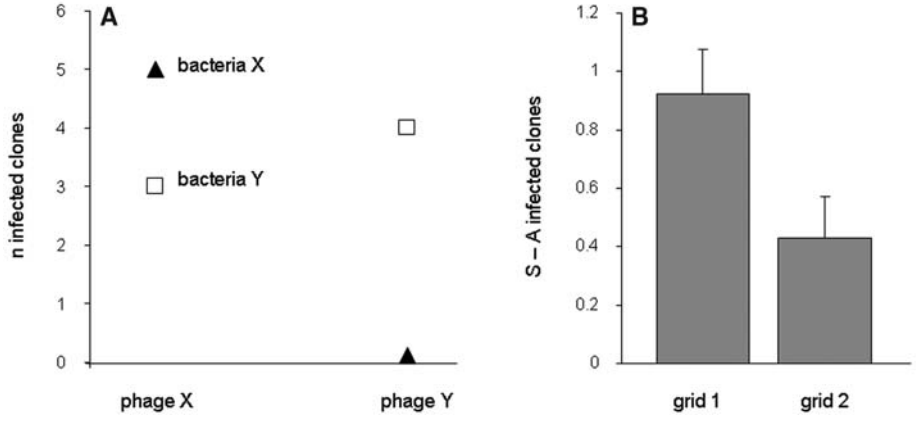

Fig. 1. (**A**) An example of how local adaptation was measured for every pair of soil samples (here termed X and Y) in each grid. We scored the number of clones in sample X that were infected by phages from samples X and Y and vice versa. Local adaptation was calculated for each sample pair as the mean difference in the proportion of local and foreign bacterial clones infected by the two phage populations. When phages on average infect local and foreign bacteria equally well, this measure is zero; when bacteria are locally adapted, this measure is negative; and when phages are locally adapted, this measure is positive. (**B**) Phages are significantly locally adapted, on average infecting 0.92 and 0.43 more clones (out of 24) in sympatric (S) populations than in allopatric (A) populations (one-sample *t* test $P < 0.0001$, $n = 300$; bars display one SEM).

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Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions

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Lysine acetylation is a reversible posttranslational modification of proteins and plays a key role in regulating gene expression. Technological limitations have so far prevented a global analysis of lysine acetylation's cellular roles. We used high-resolution mass spectrometry to identify 3600 lysine acetylation sites on 1750 proteins and quantified acetylation changes in response to the deacetylase inhibitors suberoylanilide hydroxamic acid and MS-275. Lysine acetylation preferentially targets large macromolecular complexes involved in diverse cellular processes, such as chromatin remodeling, cell cycle, splicing, nuclear transport, and actin nucleation. Acetylation impaired phosphorylation-dependent interactions of 14-3-3 and regulated the yeast cyclin-dependent kinase Cdc28. Our data demonstrate that the regulatory scope of lysine acetylation is broad and comparable with that of other major posttranslational modifications.

Acetylation of lysine is a reversible posttranslational modification (PTM), which neutralizes the positive charge of this amino acid, changing protein function in diverse ways (*1*, *2*). It has a key role in the regulation of gene expression through the modification of core histone tails by histone acetyltransferases (HATs) or histone deacetylases (HDACs) (*3*, *4*). Some modified lysines specifically bind bromodomain-containing proteins, which are part of large complexes that modulate chromatin architecture. Lysine acetylation is also important for p53 functions and interactions and for microtubule stabilization (*5*). There are many individual reports of acetylation sites on proteins involved in diverse biological processes, suggesting that lysine acetylation has broad regulatory functions in addition to the few that are actively studied.

HDACs [or lysine deacetylases (KDACs)] are important drug targets in cancer and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases, and there are more than 80 clinical trials currently underway (*6*–*9*). Two KDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) and valproic acid, are in clinical use. Furthermore, KDAC inhibitors are potent reprogramming agents for the generation of induced pluripotent stem cells (*10*). However, the specific mode of action of KDAC inhibitors has remained enigmatic and may involve acetylation on histones and other proteins.

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Despite great biological and clinical interest in lysine acetylation, our knowledge of in vivo acetylation sites is limited. However, advances in quantitative mass spectrometry (MS)–based proteomics (*11*–*13*) allow PTMs to be studied at a proteomic scale (*14*, *15*). In the case of phosphorylation, affinity purification of modified peptides makes it possible to quantify phosphorylation at thousands of sites (*16*, *17*). Peptides containing acetylated lysines can also be partially enriched by an antibody directed against this modification. This strategy was used to identify 388 lysine acetylation sites, mainly in mitochondria, a cellular compartment where this modification had hardly been described before (*18*). We reasoned that high-resolution high-accuracy MS, when coupled to an efficient quantitation strategy, should enable a global view of the "lysine acetylome" and its changes upon KDAC inhibition.

Sequencing and quantifying the acetylome. We enriched acetylated peptides from trypsin-digested whole-cell lysates of MV4-11 cells, a human acute myeloid leukemia cell line with an antibody against acetyl-lysine (*19*). We identified approximately 1000 acetylation sites by means of high-resolution high-accuracy MS (*20*). However, biologically important acetylation sites of low abundance remained undetected because of a large background of nonacetylated peptides. We therefore further separated peptides from immunoaffinity purifications by means of isoelectric focusing into 12 fractions (*21*). We used stable-isotope labeling with amino acids in cell culture (SILAC) (*22*, *23*) so as to determine global acetylation changes in response to two KDAC inhibitors, SAHA and MS-275. Lastly, to assess reproducibility and depth of acetylome coverage, we performed similar analyses in two additional human cell lines of epithelial (A549) and lymphoid origin (Jurkat) (fig. S1A) (*24*). The resulting raw data were processed with the MaxQuant suite of algorithms (*25*).

Overall mass accuracy was in the parts-per-billion range; high resolution was achieved for all peptides (fig. S1B), which facilitated accurate quantitation. We detected more than 3600 acetylation sites on 1750 proteins (table S1) at an overall false discovery rate (FDR) for peptides of less than 1%. The FDR for acetylated lysine-containing peptides, as opposed to all peptides, was even lower (between 0.1 and 0.3%) because SILAC determines the number of arginines and lysines directly, greatly aiding specific identification. We precisely pinpointed the site of modification for more than 95% of the peptides (table S1).

In a separate experiment without affinity enrichment, the number of acetylation sites was 60-fold lower (0.058% as compared with 3.44%). Percentage of acetylation sites C-terminal to the peptide—an in vitro artifact—was 72% as compared with 2.5% in the enriched samples. We used D_3C_1-labeled acetic acid in the sample preparation so as to independently show that C-terminal lysine acetylation was mainly caused by this chemical. We selected nine proteins for which acetylation was not previously described and one known acetylated protein and independently confirmed their acetylation by means of immunostaining (Fig. 1A). Proteins from a human cervical cancer cell line (Hela) or a human osteosarcoma cell line (U2OS) that were expressed as fusions with green fluorescent protein (GFP) under the endogenous promoter (*26*) were immunoprecipitated, and all showed clear lysine-acetylation signals (Fig. 1A). CBX3 was immunoprecipitated from transiently transfected human embryonic kidney 293 cells and also showed clear acetylation. Together, this demonstrates that our large-scale acetylome is of in vivo origin.

The in vivo acetylome. We first assessed the coverage of our data set for histone acetylation sites and found that we detected all sites that we were able to extract from the literature (table S1). The known in vivo acetylation sites of proteins of low abundance, such as the tumor suppressor p53, Ku70, nuclear factor κB subunit RelA, and protooncogene protein c-Myc, were also present. A large number of acetylation sites on proteins exclusively annotated as mitochondrial (381 different sites) (*18*), but these did not constitute the largest category. We compared the acetylomes of the three different cell types (Fig. 1B). For any one cell line, 60 to 80% of the acetylated proteins and 60 to 75% of the acetylated sites were also found in the other two. This indicates that our experimental methodology is accurate and reproducible and that acetylation patterns are similar in cells derived from different tissue types. Our experiment covers the majority of known in vivo sites and expands by at least sixfold the number of such sites reported in UniProt (*27*). The large overlap (fig. S2), even on proteins of low abundance, also implies sub-

stantial coverage of the core acetylome. However, it is likely that many more sites are still to be discovered because they may only be acetylated in specific cell types or at particular developmental or cell-cycle stages.

Lysine acetylation is an ancient PTM that is conserved from prokaryotes to humans. We evaluated phylogenetic conservation of lysine-acetylated proteins and compared it with that of the entire proteome (*28*). Lysine-acetylated proteins are significantly more conserved across the evolutionary tree (fig. S3), indicating additional selective pressure. Acetylated proteins were as conserved as phosphorylated proteins across vertebrates. For more distantly related species such as yeast (fig. S3A) and especially in prokaryotes, the acetylome was much more conserved than the phosphoproteome ($P < 10^{-20}$) (fig. S3B).

Lysine acetyltransferases (KATs) and KDACs are thought to be predominantly nuclear or mitochondrial, and indeed we did find a large number of acetylated proteins in these compartments. However, proteins with exclusive cytoplasmic annotation were also highly represented in the acetylome (Fig. 2A). HDAC6 is the only known cytoplasmic KDAC, but our results make it unlikely that it is the sole deacetylase with cytoplasmic activity.

Phosphorylation mainly occurs in unstructured regions of proteins, such as hinges and loops (*28*, *29*). In contrast, and in concordance with an earlier report (*18*), acetylation sites were frequently located in regions with ordered secondary structure. Furthermore, compared with all lysines, acetylated lysines were significantly enriched in structured regions and depleted in unstructured regions (Fig. 2B).

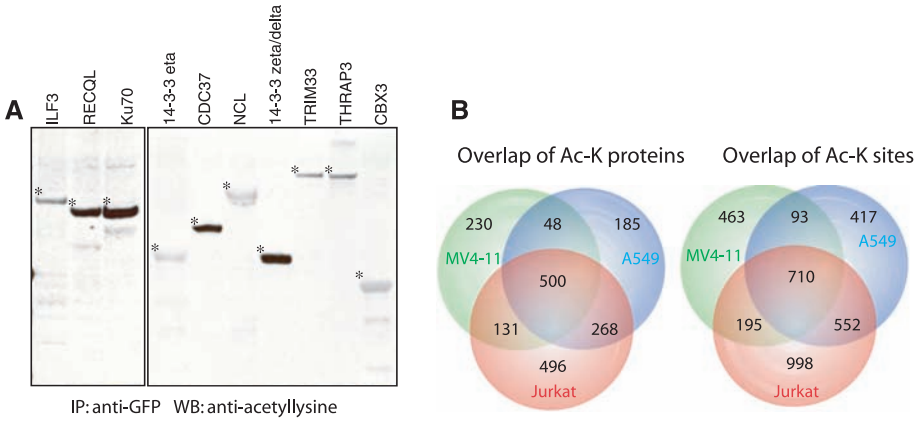

Fig. 1. Overview of in vivo acetylome analysis. (**A**) Independent validation of lysine acetylation of proteins. (**B**) Overlap of acetylated proteins and sites in three different cell lines. Ten different proteins from the acetylome data set were immunoprecipitated from GFP-tagged BAC transgenic cell lines and stained with antibody to acetyl-lysine. The bands marked with an asterisk indicate acetylated proteins.

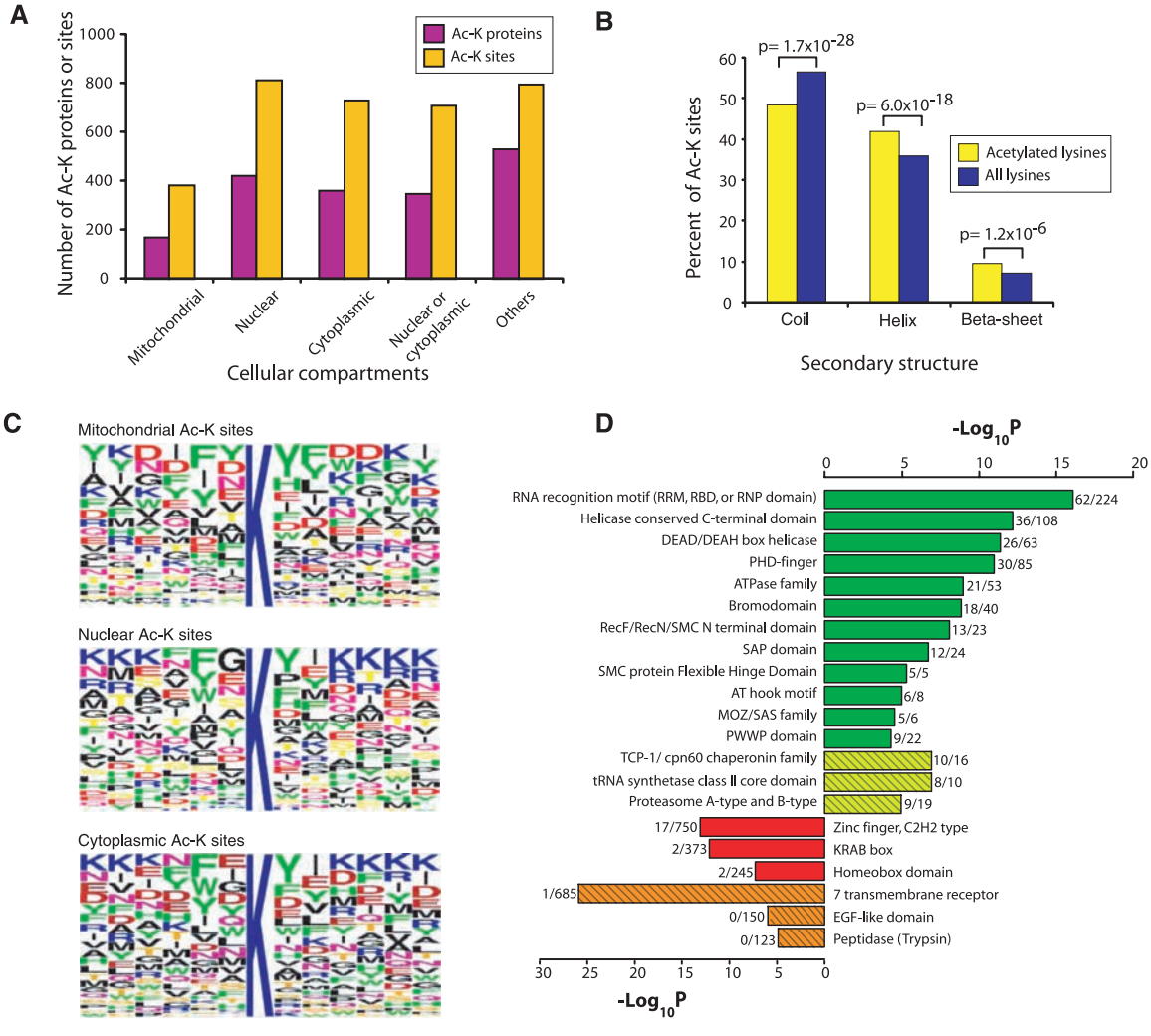

Fig. 2. Properties of acetylated proteins and sites. (**A**) Cellular distribution of acetylated proteins and sites. Proteins were assigned based on exclusive Gene Ontology (GO) annotations. (**B**) Distribution of all lysines and acetylated lysines in structured and unstructured regions of the proteins. (**C**) Sequence logo plots represent normalized amino acid frequencies for ±6 amino acids from the lysine acetylation site. (**D**) Domain architecture of acetylated proteins. The green bars indicate Pfam protein families and domains that are significantly overrepresented, and the red bars indicate underrepresented domains in the acetylome as compared with those in the entire proteome. The light green and orange striped bars represent cytoplasmic domains.

We analyzed local sequence context around the acetylation sites. Amino acids with a bulky side chain—mainly tyrosine and phenylalanine—were enriched in the –2 and +1 positions. This observation agrees with the frequent occurrence of acetylated lysines in ordered regions of proteins. Positively charged amino acids were almost completely excluded from the –1 position. Nuclear and cytoplasmic acetylation motifs are similar but different from mitochondrial motifs (Fig. 2C). For example, lysines were the preferred amino acids beyond the +2 and –3 positions for both nuclear and cytosolic proteins. In mitochondrial proteins, there was a preference for tyrosine (and histidine to a lesser degree) in the +1 position, and hydrophobic amino acids were enriched. Glycine at –1 was observed in vivo here and has been demonstrated on specific proteins (the GK motif) (*1*, *2*).

Cellular localization and function of proteins is often dictated by their domains. Pfam domains (*30*) associated with nuclear functions were most overrepresented in our acetylome, including RNA-binding motifs, various helicases, the PWWP domain, the PHD finger, and bromodomains ($P < 10^{-5}$) (Fig. 2D). Among the most underrepresented domains were those associated with membranes or extracellular space and peptidases.

Acetylation targets macromolecular complexes. We were intrigued by the presence of a large number of acetylated proteins in large macromolecular complexes and classified acetylated proteins according to known chromatin-associated processes (fig. S4). Known nuclear lysine acetyltransferase complexes (*3*) were themselves highly acetylated on multiple subunits (fig. S4A). Three subunits were known to be acetylated in these nine KAT complexes; our analysis detected more than 40. Autoacetylation of p300 is a well-described example of KAT acetylation that regulates its own enzymatic activity (*31*). Our data demonstrate that extensive acetylation of KAT complex subunits is a general property. ATAC is a newly described KAT complex in *Drosophila melanogaster* described after our data were acquired (*32*). Of the human homologs of ATAC subunits, seven were acetylated. SIN3A, the main deacetylase complex, contains at least eight subunits modified on 18 distinct sites (fig. S4B).

Almost no acetylation sites are currently known on chromatin-remodeling complexes, but we found that SWI/SNF, NURD, INO80, and NURF are heavily acetylated, usually on multiple lysines (fig. S4C). Two complexes involved in regulating transcription, FACT (facilitates chromatin transcription) and TAF [TATA-box binder protein (TBP)–associated factor], were also targets of acetylation (fig. S4D).

Histones and p53 are regulated by several different PTMs, suggesting extensive crosstalk between different PTMs. Major methyltransferase complexes that contain catalytic subunits encoded by the mixed lineage leukemia (MLL) genes were extensively modified by acetylation, suggesting crosstalk between KATs and methyltransferases directly at the level of the modifying enzymes (fig. S4E). Histone demethylases such as JARIDs (Jumonji/ARID domain–containing proteins) were also acetylated, further substantiating the regulatory link. Lastly, a large number of nuclear ubiquitin-modifying enzymes were also subject to this modification (fig. S4F). These data suggest a direct and intricate crosstalk between different enzymes involved in modulating chromatin-associated functions via PTMs.

To investigate acetylation-targeting of many macromolecular complexes in an unbiased way, we analyzed the complete acetylome data set using Comprehensive Resource of Mammalian protein complexes (CORUM), a database of manually curated and validated mammalian protein complexes (*33*). A total of 39 nuclear and five cytosolic complexes were enriched for acetylated proteins at $P < 0.05$ (Fisher's exact test) (table S2), including all of the complexes described above.

To quantify protein-interaction properties of the acetylome, we used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database of physical and functional interactions (*34*). Compared with similarly sized randomly selected protein data sets, the acetylome has significantly higher network connectivity ($P < 10^{-6}$) with six interactions per node as compared with less than three for random data sets (fig. S5). This further corroborates the notion of high connectivity among acetylated proteins. In the global STRING-generated protein-protein network, several complexes and cellular functions formed prominent, tightly connected clusters as assessed by means of molecular complex detection (fig. S6A) (*35*). In addition to the physical complexes found manually and by CORUM, cellular processes such as RNA splicing, nuclear transport, protein folding, and cytoskeletal regulation were highlighted (fig. S6B).

Cellular processes regulated by acetylation. With the exception of histone modification and DNA repair, in which acetylation has well-established roles, there is little evidence for a broad role of acetylation in other nuclear processes. Our data reveal that a large number of acetylation sites are present on proteins involved in all major nuclear processes, such as splicing, cell cycle, chromatin remodeling, DNA replication, transcription, and nuclear transport, which strongly suggests that these processes may be influenced by this modification (Table 1). Previously, only a small number of well-studied proteins were known to be acetylated in these processes. For example, there are only three known acetylation sites for proteins implicated in splicing, whereas we found 130 acetylation sites on proteins involved in this process. These acetylated proteins are connected in a dense protein-protein interaction network (Fig. 3A).

Acetylation of a few proteins such as p53, Ku70, FEN1, and WRN is known to be important in the repair of damaged DNA. Acetylation of p53 regulates its stability through crosstalk with the ubiquitination machinery, modulates interactions with TAF1, and regulates its transcriptional activity (*5*). We confirmed acetylation of these proteins and found further sites, which may also be functional. For example, Ku70 and Ku80, which form heterodimers and regulate the activity of DNA-dependent protein kinase (DNAPK), a key kinase involved in DNA damage repair, are both acetylated. Acetylation of Ku70 is already

Table 1. Acetylation regulates diverse protein classes and biological categories. Acetylated proteins were grouped into major cellular functional categories or into protein classes on the basis of their known functions or function predicted by functional domains.

Cellular functional categories and protein classes	Number of acetylated proteins	Number of acetylation sites
DNA replication	52	98
DNA damage and repair	72	167
Chromatin remodeling	26	46
Cell cycle	132	243
RNA transcription	31	71
RNA splicing	109	206
Nuclear hormone signaling	9	22
Nuclear transport	17	41
Cytoskeleton reorganization	50	137
Nucleotide exchange factors	55	92
Endocytosis and vesicular trafficking	39	62
DNA/RNA helicases	46	105
Ubiquitin ligases and deubiquitylases	46	70
Protein kinases	47	71
Acetyltransferases and deacetylases	21	61
Methyltransferases and demethylases	12	34
Transcription factors	29	40
Histones	15	61
Adaptor proteins	14	40
Chaperones	40	127
Ribosomal proteins	75	136

known to be important in this process. Many other proteins that are essential for DNA damage repair, such as MDC1, DDB1/2, RAD50, PCNA, WRNIP1, MSH2, BLM, and RAD54L, are acetylated (Fig. 3B). The human genome encodes six phosphoinositide-3-kinase–related protein kinases (PIKKs) that contain a conserved C-terminal FAT [FKBP12-rapamycin–associated protein (FRAP), ataxia telangiectasia mutated (ATM), transformation/transcription domain-associated protein (TRRAP)], a kinase domain, and FATC (FAT C terminus) domain structure: ATM, ATR, SGK1, DNAPK, TRRAP, and FRAP1. PIKKs play key roles in DNA damage repair, and we found acetylation sites in SGK1, DNAPK, TRRAP, and FRAP1. Acetylation of ATM has been reported (*36*). The FATC domains of these kinases mediate an interaction with Tip60, an acetyltransferase that has a critical role in DNA damage repair and, in the case of ATM and DNAPK, regulates their kinase activity (*36*, *37*). Thus, acetylation may represent a common regulatory mechanism of activation for FATC domain–containing PIKKs.

Acetylation is a widespread modification for nuclear ubiquitin ligases and deubiquitylases including UHRF1, HUWE, BRE1A, and BRE1B, which have roles in the modification of histones and chromatin-bound proteins (table S1). Similarly, HAUSP, USP22, and USP11 deubiquitylases, which have roles in chromatin biology, and Ubch37 and USP14, the two major proteasome-bound deubiquitylases, are acetylated.

The p53 interaction network controls its functions with several mechanisms. We discovered that not only p53 but several proteins that are critical for

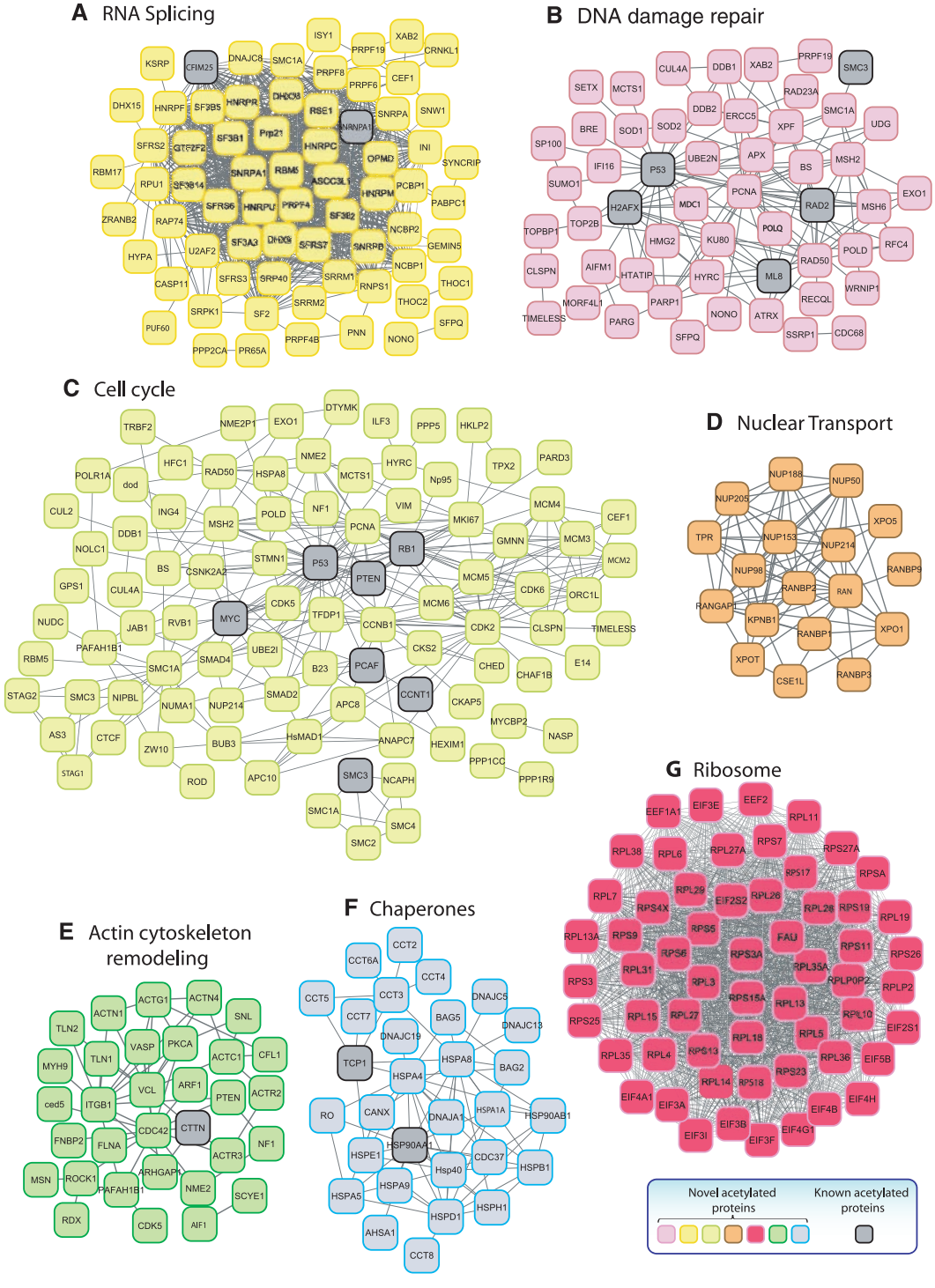

Fig. 3. Acetylation-modulated functional networks. (**A** to **G**) Interaction networks of acetylated proteins in different cellular functions from STRING analysis of the acetylome. Individual networks were generated for each specific functional category (table S1). Gray nodes indicate proteins previously reported to be acetylated.

its functions, such as DAXX, PML, PTEN, and HAUSP (*38*), are acetylated (table S1). HAUSP regulates the stability of p53 and MDM2, but less is known about its own regulation. The five HAUSP acetylation sites reported here represent a possible mechanism with which to modulate its activity. Collectively, these results indicate that not only p53 but many of the core proteins of the p53 circuitry also probably are regulated by acetylation.

A large number of proteins involved in chromatin remodeling and cell cycle are acetylated (table S1). For example, CDC2, a major cyclin-dependent kinase and regulator of S-phase progression and mitosis, was acetylated at two different lysines: K6 and K33. K6 is conserved between CDC2 and CDK2 and was acetylated on both kinases. The K33 acetylation site is located within the kinase domain and is important for the coordination of adenosine 5′-triphosphate (ATP) binding. This lysine is conserved even in the budding yeast homolog. Indeed, acetylation of yeast Cdc28 was confirmed by means of protein immunoblotting (fig. S7A). MS data furthermore confirmed that Cdc28 is acetylated on K40 (fig. S7B), a residue analogous to K33 of CDC2. To address functional consequences of altering this residue, we mutated K40 in yeast Cdc28 to arginine, which is charge-conserving, or to glutamine, which is noncharged and may mimic acetylated lysine. Both point mutants failed to rescue *cdc28*Δ yeast strains (Fig. 4A). This lysine therefore appears to be critical to the proper function of Cdc28 in yeast. CDK9, a non–cell-cycle kinase, is acetylated on the analogous site, and acetylation inhibits its kinase activity (*39*). CDK6, another cell-cycle kinase, and PRPF4B (PRP4 pre-mRNA–processing factor 4 homolog B), a kinase involved in mRNA splicing, were also acetylated on the ATP-binding lysine. Thus, lysine acetylation may have a wider role in the regulation of kinase activity. Acetylation of SMC3 (structural maintenance of chromosomes 3) in yeast and human is important for the separation of sister chromatids (*40*). We found 22 sites, including two recently described ones (*39*), on all major SMC proteins that are part of the cohesion complex (SMC1A, -2, -3, -4, and -5). These data point to a larger role of acetylation in the regulation of the cohesion complex and thus separation of sister chromatids. We also found acetylation of many other cell-cycle regulators, such as TPX2, NUMA1, cyclin T1 and B1, BUB3, CDK6, and STMN1. As shown in Fig. 3C, acetylated cell-cycle proteins are highly connected by functional and physical protein-protein interactions.

Interactions of nuclear receptor–binding proteins to nuclear receptors via LXXLL motifs (where X represents any amino acid) regulate transcriptional activation. Acetylation of nuclear receptor coactivator 3 (NcoA3) near LXXLL motifs regulates these interactions (*41*). We found that both NcoA2 and NcoA3 are acetylated within their LXXLL motifs, which is likely to influence binding properties and therefore the function of these important nuclear receptor coactivators.

Nuclear pores are the gatekeepers of nucleocytoplasmic transport and, together with the RAN guanidine triphosphate cycle, control protein traffic. To our knowledge, acetylation has not been implicated in the regulation of nuclear transport. We discovered that several constituents of this machinery are acetylated (Fig. 3D). RANGAP1 [RAN–guanosine triphosphatase (GTPase)–activating protein 1] is acetylated on K524, the same lysine residue that has an important role in the regulation of nuclear transport when modified by sumoylation (*42*). Acetylation of this lysine abolishes sumoylation and thus may regulate nuclear transport.

Cytoplasmic complexes regulated by acetylation. Acetylation of tubulin regulates microtubule function (*43*). We found several additional acet-

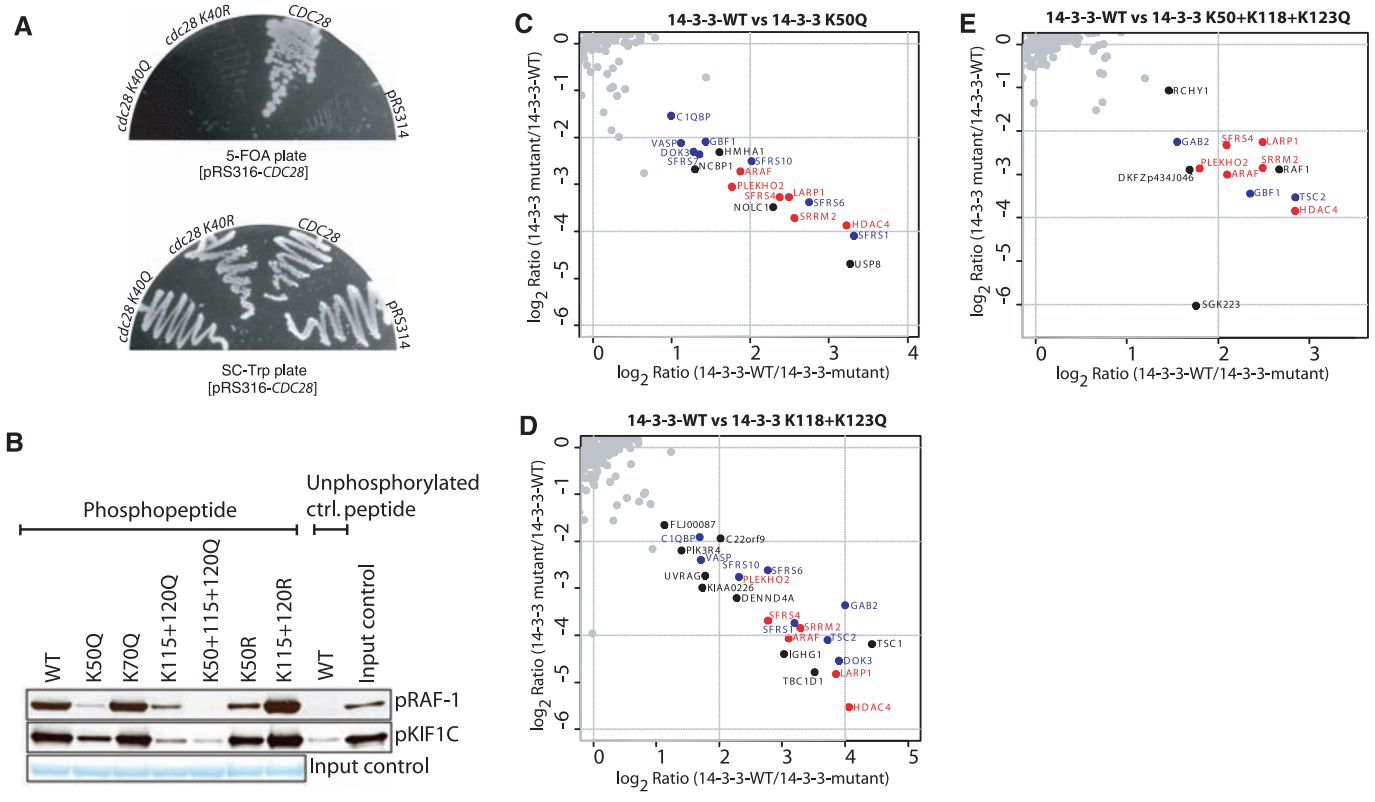

Fig. 4. Acetylation of Cdc28 and 14-3-3 impair their functions. (**A**) Mutation of acetylation site K40 on Cdc28 impairs its function. Growth of haploid yeast strains harboring empty vector or plasmids expressing *CDC28*, *cdc28-K40R*, or *cdc28-K40Q* in a *cdc28*Δ strain were tested for growth on 5-FOA plates, which select against the wild-type copy of *CDC28* on a separate URA3-marked plasmid. SC-Trp plates are shown as control. (**B**) Mutation of acetylated lysine on 14-3-3-ε abolishes binding to RAF1 and KIF1c phosphopeptide ligands. Binding of recombinant GST–14-3-3 proteins to phosphopeptides was analyzed by means of peptide pull-down assays. (**C** to **E**) Mutation of K50Q and K118+K123Q impairs binding of 14-3-3 to full-length proteins. Proteins interacting with 14-3-3 wild type (WT) or mutants were identified and quantified by use of SILAC-based MS as described in fig. S9. Proteins in red are quantified in all six experiments, and proteins that were quantified in four or more experiments are blue. The *x* axis shows the relative binding of 14-3-3–WT compared with the indicated 14-3-3 mutants (log2 SILAC ratio for WT/mutant). The *y* axis shows the relative binding of the indicated 14-3-3 mutants compared with 14-3-3–WT (log2 SILAC ratio for mutant/WT).

ylation sites on various tubulin isoforms. The machinery controlling actin-based cell motility is also highly acetylated (Fig. 3E). All but one of the subunits of the ARP2/3 complex, the major actin nucleation complex, are acetylated, as are cortactin, coffilin, and coronin, which interact with the ARP2/3 complex (table S1). Of the nine acetylation sites identified on purified cortactin (*44*), we confirmed five and identified two others. Thus, regulation of cytoskeleton reorganization and cell motility by lysine acetylation may involve many more proteins than tubulin and cortactin.

The function of chaperone protein HSP90 is inhibited by acetylation (*45*). Hyperacetylation of HSP90 is thought to be a major effector through which KDAC inhibitors interfere with protein folding, and one acetylation site on HSP90-α is known (*45*). We could not confirm this site, but we found HSP90-α to be acetylated on at least 14 lysines and HSP90-β on a minimum of four. Likewise, all the eight subunits of TCP1 or TriC chaperone complex (CCT1 to -8) were also heavily acetylated (Fig. 3F).

14-3-3 proteins are widely expressed proteins that specifically bind to phosphoserine or phosphothreonine and regulate a diverse set of cellular processes, such as signal transduction, cell cycle, and DNA damage repair (*46*). 14-3-3 proteins also interact with HDAC1, HDAC4, and HDAC5. Multiple isoforms of 14-3-3 were acetylated in different cell types (table S1 and fig. S8A). Moreover, analogous sites were acetylated in multiple isoforms (fig. S8B).

To understand the role of 14-3-3 acetylation and its impact on the interaction of 14-3-3 with phosphorylated peptides, we mutated four lysines (K50, K69, and K118+K123) that are acetylated in vivo and are highly conserved among 14-3-3 proteins (*47*). Acetylated lysines in 14-3-3-ε were mutated either to glutamine (Q), so as to mimic acetylated lysine, or arginine (R), so as to prevent acetylation but conserve a positive charge. Mutation of K50 to glutamine reduced binding to two model phosphopeptides, and mutation of both K118 and K123 severely impaired its binding (Fig. 4B). In contrast, K118+K123R showed no such binding deficiency. A triple acetylation mimetic mutant (K50+K118+K123Q) did not bind phosphopeptides. To further confirm the importance of these sites, we performed pull-downs using glutathione *S*-transferase (GST)–13-3-3-ε fusion protein or its mutants and quantified proteins that specifically bound to 14-3-3 (fig. S9). The acetylation mimetic mutants of 14-3-3 (K50Q, K118+K123Q, and K50+K118+K123Q) showed impaired binding to synthetic peptides as well as to full-length proteins from whole-cell lysates (Fig. 4, C to E). Among the proteins that showed decreased binding to acetylation, mimetic mutants of 14-3-3 in these five are known to bind (RAF1, HDAC4, TSC22, GAB2, and ARAF), which independently validates the results obtained with peptide associations. The crystal structure of 14-3-3 bound to its phosphopeptide ligand confirms the importance of K50 and K123 in binding to its ligand (*48*). Thus, our results uncover a mechanism that modulates phosphorylation-dependent interactions on the side of the phosphopeptide-binding domain and suggest crosstalk between phosphorylation and acetylation.

Our data also contain many further interesting leads for functional studies in a wide variety of biological areas. For example, an unexpected role of acetylation was recently reported for the activation of signaling from the interferon-α receptor (*49*). We identified acetylation sites in mediators of interferon actions such as DDX58, IRAK4, OAS2, and TRIM25. These data further strengthen the notion that acetylation may regulate innate immune responses.

Quantitative acetylation analysis upon KDAC inhibition. The clinical success of KDAC inhibitors for specific cancers as well as their promise for treating other diseases make it important to identify their targets at a site-specific level. We used SILAC to quantify downstream targets of SAHA and MS-275 in three different cell lines (fig. S1A). SAHA is reported to have broad inhibitory activity and to target many KDACs, with the exception of sirtuins (*6*). In contrast, MS-275 is thought to inhibit only three class I KDACs. Both inhibitors increased acetylation of specific histone sites, but neither changed acetylation of mitochondrial proteins, which are thought to be deacetylated by sirtuins. The inhibitors only upregulated about 10% of all acetylation sites by at least a factor of two (table S4). Thus, both inhibitors were unexpectedly specific in vivo. Acetylation sites that were regulated more than twofold by both of these inhibitors in the triple-SILAC experiment in MV4-11 cells are listed in table S5. SAHA is a more potent inducer of histone acetylation than MS-275 (fig. S6). For example, a number of sites on the core histones H3 and H4 are several times more highly regulated in response to SAHA than by MS-275 (table S5). Acetylation of the variant histone H2AZ—a mark for DNA damage sites—was upregulated almost 20-fold by SAHA. The fact that acetylation of all histone sites is not equally increased suggests that these drugs target the histone deacetylases differently. Important cytoplasmic targets, such as HSP90α and -β, were highly acetylated in the presence of SAHA but not in cells treated with MS-275 (fig. S1B). Conversely, the acetylation levels of four out of five sites on p53 were increased by MS-275 but not at all by SAHA.

Discussion. We describe a streamlined methodology for the proteome-wide identification and quantitation of acetylation sites and provide an in-depth view of the in vivo acetylome. We identified 3600 acetylation sites and implicated acetylation in the regulation of a diverse set of cellular functions. The data are available to the scientific community via Phosida (www.phosida.com) (*28*), a public database that is a member of ProteinExchange and provides detailed information about each acetylation site, such as evolutionary conservation and local secondary structure prediction of the protein sequence around the acetylation site.

The overall picture of lysine acetylation resulting from our experiments is that it contributes to regulation of almost all nuclear functions and to the control of a surprisingly large array of cytoplasmic functions as well. The relatively high overlap of the measured acetylome in three cell lines and the fact that we covered most of the in vivo sites described in the literature indicate that we sampled a substantial part of all acetylation sites. The several thousand sites detected here place the size of the measured acetylome between that of the phosphoproteome, in which tens of thousands of sites are now quantifiable, and that of the spectrum of ubiquitinated proteins, for which only a few hundred sites are currently known. In common with these major PTMs, acetylation seems to have been adopted in the regulation of a wide variety of dissimilar cellular functions. A striking feature of acetylation is that it tends to occur in large macromolecular complexes.

The application of quantitative proteomics enabled us to assess the effects of perturbing the acetylation network in a global and unbiased manner. KDAC inhibitors were much more specific than generally thought, potentially shedding new light on their unexpected clinical specificity.

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Detection of 16 Gamma-Ray Pulsars Through Blind Frequency Searches Using the Fermi LAT

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Pulsars are rapidly rotating, highly magnetized neutron stars emitting radiation across the electromagnetic spectrum. Although there are more than 1800 known radio pulsars, until recently only seven were observed to pulse in gamma rays, and these were all discovered at other wavelengths. The Fermi Large Area Telescope (LAT) makes it possible to pinpoint neutron stars through their gamma-ray pulsations. We report the detection of 16 gamma-ray pulsars in blind frequency searches using the LAT. Most of these pulsars are coincident with previously unidentified gamma-ray sources, and many are associated with supernova remnants. Direct detection of gamma-ray pulsars enables studies of emission mechanisms, population statistics, and the energetics of pulsar wind nebulae and supernova remnants.

A wide variety of astrophysical phenomena, such as black holes, active galactic nuclei, gamma-ray bursts, and pulsars, are known to produce photons with energies exceeding tens of megaelectron volts. Detection and accurate localization of sources at these energies is challenging because of the low fluxes involved and the limitations of the detection techniques. The sky above 100 MeV was surveyed more than 30 years ago by the COS-B satellite (*1*) and more recently by the Energetic Gamma Ray Experiment Telescope (EGRET) (*2*) onboard the Compton Gamma Ray Observatory. One of the main legacies of EGRET was the detection of ~300 gamma-ray sources, many of which have remained unidentified despite searches at a wide variety of wavelengths (*3*). Many EGRET (and COS-B) unidentified sources are thought to be of galactic origin because of their lack of variability and concentration along the galactic plane. A large fraction of these have been suspected to be pulsars [e.g., (*4*–*6*)] despite deep radio and x-ray searches often failing to uncover pulsed emission, even when the gamma-ray sources were coincident with supernova remnants (SNRs) or pulsar wind nebulae (PWNs). The lack of radio pulsations has usually been explained as the narrow radio beams missing the line of sight toward Earth (*7*). We refer to such pulsars as "radio-quiet"; even though they may emit radio waves, these cannot be detected at Earth. Before the launch of the Fermi Gamma-ray Space Telescope, Geminga (*8*) was the only known radio-quiet gamma-ray pulsar. Current models of pulsar gamma-ray emission predict that gamma-ray beams are much wider than radio beams (*9*), thus suggesting that there may be a large population of radio-quiet gamma-ray pulsars.

Soon after launch on 11 June 2008, the Large Area Telescope (LAT) onboard Fermi began surveying the sky at energies above 20 MeV. A companion paper describes the LAT detection of a population of gamma-ray millisecond pulsars (MSPs) (*10*). Here we report the detection of 16 pulsars found in blind frequency searches using the LAT. Previously, gamma-ray pulsars had been detected only with the use of a radio (or, in the case of Geminga, an x-ray) ephemeris.

Observations and data analysis. The LAT is a high-energy gamma-ray telescope sensitive to photon energies from 20 MeV to >300 GeV, featuring a solid-state silicon tracker, a cesium-iodide calorimeter, and an anticoincidence detector (*11*). Gamma-ray events recorded in the LAT have time stamps derived from a GPS clock on the Fermi satellite with an accuracy of <1 μs (*12*). The LAT operates in continuous sky survey mode, covering the entire sky every 3 hours. Relative to EGRET, the LAT has a larger effective area (9500 cm^2 at normal incidence), larger field of view (2.4 sr), more efficient use of time on orbit for photon collection, and a finer point spread function (5° at 100 MeV, 0.8° at 1 GeV). The first three factors result in more rapid photon accumulation, and the fourth increases the signal-to-noise ratio by improving the background rejection.

Table 1. Names and locations of the gamma-ray pulsars discovered in our blind searches. The "assumed counterpart" column is the x-ray source that provided the position for the timing model, which in some cases may not be the true counterpart; *l* and *b* are galactic longitude and latitude, respectively.

LAT PSR	LAT source 0FGL	EGRET source	Assumed counterpart	RA (°)	Dec (°)	*l* (°)	*b* (°)
J0007+7303	J0007.4+7303	3EG J0010+7309	RX J0007.0+7303 in G119.5+10.3 (*37*)	1.7565	73.0523	119.7	10.5
J0357+32	J0357.5+3205			59.445	32.105	162.7	−16.0
J0633+0632	J0633.5+0634	3EG J0631+0642	Swift J063343.8+063223	98.4333	6.5403	205.1	−0.9
J1418−6058	J1418.8−6058	3EG J1420−6038	R1 in G313.3+0.1 (*24*)	214.6779	−60.9675	313.3	0.1
J1459−60	J1459.4−6056			224.874	−60.878	317.9	−1.8
J1732−31	J1732.8−3135	3EG J1734−3232		263.169	−31.610	356.2	0.9
J1741−2054	J1742.1−2054	3EG J1741−2050	Swift J174157.8−205412	265.4908	−20.9033	6.4	4.9
J1809−2332	J1809.5−2331	3EG J1809−2328	CXOU J180950.2−233223 in G7.4−2.0 (*38*)	272.4592	−23.5397	7.4	−2.0
J1813−1246	J1813.5−1248	GeV J1814−1228	Swift J181323.4−124600	273.3475	−12.7668	17.2	2.4
J1826−1256	J1825.9−1256	3EG J1826−1302	AXJ1826.1−1257 in G18.5−0.4 (*26*)	276.5356	−12.9429	18.6	−0.4
J1836+5925	J1836.2+5924	3EG J1835+5918	RX J1836.2+5925 (*30*)	279.0570	59.4250	88.9	25.0
J1907+06	J1907.5+0602	GeV J1907+0557		286.965	6.022	40.2	−0.9
J1958+2846	J1958.1+2848	3EG J1958+2909	Swift J195846.1+284602	299.6921	28.7674	65.9	−0.4
J2021+4026	J2021.5+4026	3EG J2020+4017	S21 in G78.2+2.1 (*33*)	305.3777	40.4462	78.2	2.1
J2032+4127	J2032.2+4122	3EG J2033+4118	MT91 221 in Cygnus OB2 (*32*)	308.0612	41.4610	80.2	1.0
J2238+59	—			339.561	59.080	106.5	0.5

Even with the improvements of the LAT, gamma-ray data remain extremely sparse. The brightest steady gamma-ray source in the sky, the Vela pulsar, spins 11 times per second; however, it provides fewer than 100 (>30 MeV) photons during every two orbits of the LAT (*13*). As a result, detection of gamma-ray pulsations from sources with more typical brightness requires weeks or months of data. A standard technique for finding a periodic signal in a data set is a fast Fourier transform (FFT). A fully coherent FFT becomes memory-intensive because the number of frequency bins in the FFT increases with the length of the observational time: $N_{\mathrm{bins}} = 2Tf_{\mathrm{max}}$, where T is the duration of the observation and f_{max} is the maximum frequency. Furthermore, pulsars gradually spin down as they radiate away energy, requiring the computation of many tens of thousands of FFTs to scan a realistic frequency and frequency derivative (f and $\dot{f}$) parameter space; this makes FFT searches computationally intensive, if not prohibitive [e.g., (*14*)]. We thus used the time-differencing technique (*15*) and a fixed window of $T = 2^{19}$ s (~1 week). This markedly reduces the number of FFT bins and frequency derivative trials. This method was successfully applied to EGRET data (*16*).

We applied the time-differencing technique to photons from a small region of interest around selected target positions. We used two sets of target positions. The first was a list of ~100 promising locations derived from multiwavelength observations. This list was selected on the basis of location in the galactic plane, spectra, lack of long-term variability, and presence of an SNR, a PWN, or a likely neutron star identified from x-ray observations. Many of these locations were unidentified gamma-ray sources from EGRET. The second list, compiled after the LAT started collecting data, included locations of gamma-ray sources from a preliminary version of the LAT catalog. After eliminating sources with probable active galactic nuclei associations, we ended up with ~200 locations, of which 36 were consistent with previously unidentified EGRET sources.

We analyzed data collected from sky survey observations beginning on 4 August 2008 and ending on 25 December 2008. We applied a barycenter correction using the JPL DE405 solar system ephemeris (*17*), assuming that the source position was either a likely candidate counterpart, found through multiwavelength studies, or our best estimate based on LAT data. This translation into the nearly inertial reference frame of the solar system barycenter corrects

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for classical and relativistic effects of the motion of Earth. We accepted all highest-quality ["diffuse" class (*11*)] photons falling within a 0.8° radius of our source location with energies greater than 300 MeV. For each target, we searched a set of trial frequency derivatives from zero to the spin-down of the young Crab pulsar ($\dot{f} = -3.7 \times 10^{-10}$ Hz s^{-1}). For each trial, we corrected the photon arrival times using the assumed frequency derivative, calculated the time differences, computed the FFT sampled with a Nyquist frequency of 64 Hz, and searched the spectrum for significant peaks.

We refined candidate signals by performing an epoch-folding search over a narrow region of frequency and frequency-derivative space using PRESTO (*18*). Using the parameters from the time-differencing search as an initial timing model, we split the data into intervals (~2 to 4 weeks, depending on signal-to-noise ratio), and measured pulse times of arrival (TOAs) in each segment. These TOAs were fit to a timing model with the Tempo2 pulsar timing package (*19*). We selected the reference epoch for all timing models to be MJD (modified Julian date) 54754.0, roughly in the middle of the observation interval.

Table 2. Rotational ephemerides for the new pulsars. For all timing solutions, the reference epoch is MJD 54754. The given frequency and frequency derivative are barycentric. Numbers in parentheses indicate error in the last significant digit(s). For LAT PSR J0357+32, marked with (†), the measured frequency derivative, and thus the derived parameters, may be significantly affected by positional error (see text). We also list the number of photons, n_γ, obtained with the cuts used in this work (see text) over the 5-month observational period. The 1- to 100-GeV photon number flux, F_{35}, is given, as presented in the Fermi Bright Source List (*39*), except for LAT PSR J2238+59, which was not in the Bright Source List but whose flux was derived from the same data set and analysis approach.

LAT PSR	n_γ	F_{35} (10^{-8} cm^{-2} s^{-1})	f (Hz)	$\dot{f}$ (-10^{-12} Hz s^{-1})	τ (years)	$\dot{E}$ (10^{34} ergs s^{-1})	B (10^{12} G)
J0007+7303	1509	6.14(27)	3.1658891845(5)	3.6133(3)	13,900	45.2	10.8
J0357+32	294	0.64(10)	2.251723430(1)	0.0610(9)†	585,000	0.5	2.3
J0633+0632	648	1.60(17)	3.3625440117(3)	0.8992(2)	59,300	11.9	4.9
J1418–6058	3160	5.42(38)	9.0440257591(8)	13.8687(5)	10,300	495.2	4.4
J1459–60	1089	1.26(19)	9.694596648(2)	2.401(1)	64,000	91.9	1.6
J1732–31	2843	3.89(33)	5.087952372(2)	0.677(1)	119,000	13.6	2.3
J1741–2054	889	1.31(17)	2.417211371(1)	0.0977(7)	392,000	0.9	2.7
J1809–2332	2606	5.63(31)	6.8125455291(4)	1.5975(3)	67,600	43.0	2.3
J1813–1246	1832	2.79(24)	20.802108713(5)	7.615(4)	43,300	625.7	0.9
J1826–1256	4102	5.76(37)	9.0726142968(4)	9.9996(3)	14,400	358.2	3.7
J1836+5925	2076	8.36(31)	5.7715516964(9)	0.0508(6)	1,800,000	1.2	0.5
J1907+06	2869	3.74(29)	9.378101746(2)	7.682(1)	19,400	284.4	3.1
J1958+2846	1355	1.29(18)	3.443663690(2)	2.493(1)	21,900	33.9	7.9
J2021+4026	4136	10.60(40)	3.769079109(1)	0.7780(7)	76,800	11.6	3.9
J2032+4127	2371	3.07(26)	6.9809351235(8)	0.9560(4)	115,800	26.3	1.7
J2238+59	811	0.96(11)	6.145017519(3)	3.722(2)	26,200	90.3	4.1

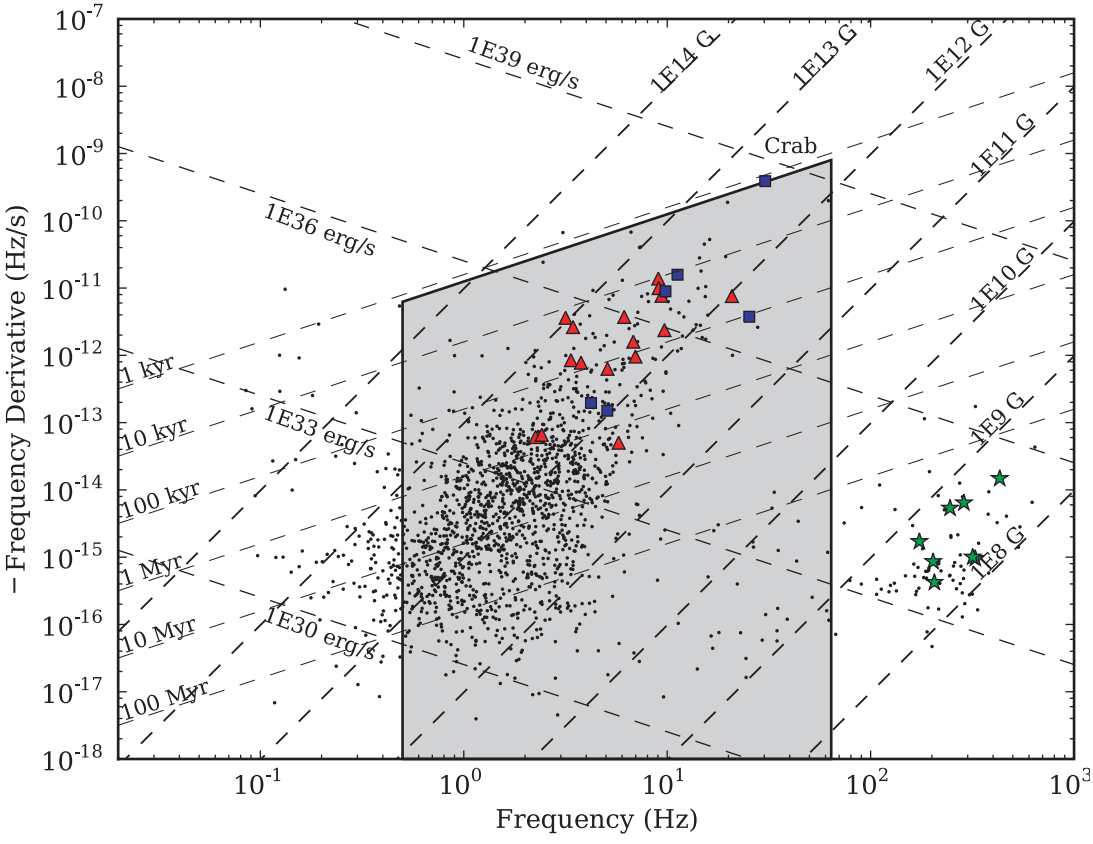

Fig. 1. Frequency (f) and frequency derivative ($\dot{f}$) distribution of the new pulsars. The dots represent the ~1800 pulsars in the ATNF catalog (*20*), the triangles represent the new pulsars reported here, and the squares represent the six previously known EGRET gamma-ray pulsars, including the Crab. The stars represent the new population of gamma-ray millisecond pulsars detected by the Fermi LAT and reported in (*10*). The shaded region shows the parameter space covered by our blind search and includes ~86% of the ATNF pulsars. The three sets of lines illustrate the characteristic age $\tau = -f/2\dot{f}$, inferred surface magnetic field strength $B = 3.2 \times 10^{19}\sqrt{-\dot{f}/f^3}$ G, and spin-down power given by $\dot{E} = 4\pi^2 I f \dot{f}$ ergs s^{-1}, where I is the neutron star moment of inertia ($I = 10^{45}$ g·cm^2). This figure shows that the blind-search gamma-ray pulsars are drawn from the portion of the general population with the highest spin-down luminosity.

This choice minimized the covariances between the timing model parameters. We identified 16 pulsars within the first 4 months of data, and confirmed these with the use of an independent data set of at least 1 additional month (Figs. 1 and 2; Tables 1 and 2).

Six of the 16 pulsars were found using the positions of well-localized counterparts at other wavelengths, whereas 10 were found using LAT source positions. The LAT positions were uncertain by several arc minutes, and in many cases the initial timing models showed very large nonwhite residuals indicative of a significant distance between the true position and the position being used to barycenter the data. We refined the positions in several ways (see below). The number of digits used in the declination of the names assigned to the pulsars reflects our confidence in their localization. The discovery frequency may not be the true pulsar rotation frequency, but rather a harmonic or a subharmonic, especially for gamma-ray pulse profiles with two peaks separated by ~180° in phase. We tested for this effect, but for certain cases (e.g., J0357+32) the results remain inconclusive because of the low photon counts.

The gamma-ray pulsars. The 16 pulsars are representative of the highest spin-down luminosity portion of the general population, which includes 1800 pulsars in the Australia Telescope National Facility (ATNF) database (*20*), including the six gamma-ray pulsars detected with EGRET, as well as eight gamma-ray MSPs detected by the Fermi LAT (*10*).

Thirteen LAT pulsars are associated with unidentified EGRET sources. Indeed, 15 of 36 unidentified EGRET sources searched showed pulsations, although two of them (PSR J1028–5819 and PSR J2021+3651) are known radio pulsars (*21*, *22*). Three pulsars (J0357+32, J1459–60, and J2238+59) correspond to newly seen LAT sources. This result suggests that the blind searches are flux-limited; although many LAT unidentified sources also could be pulsars, most have too low a flux for a pulsation to have been detected in our available data. Five pulsars are likely associated with PWNs and/or SNRs, and an additional one (J1836+5925) is associated with a known isolated neutron star. J0007+7303 (*23*) was long suspected of being a pulsar because of its clear association with SNR CTA 1 containing a PWN. J1418–6058 is in the complex Kookaburra region of the galactic plane and is likely associated with PWN

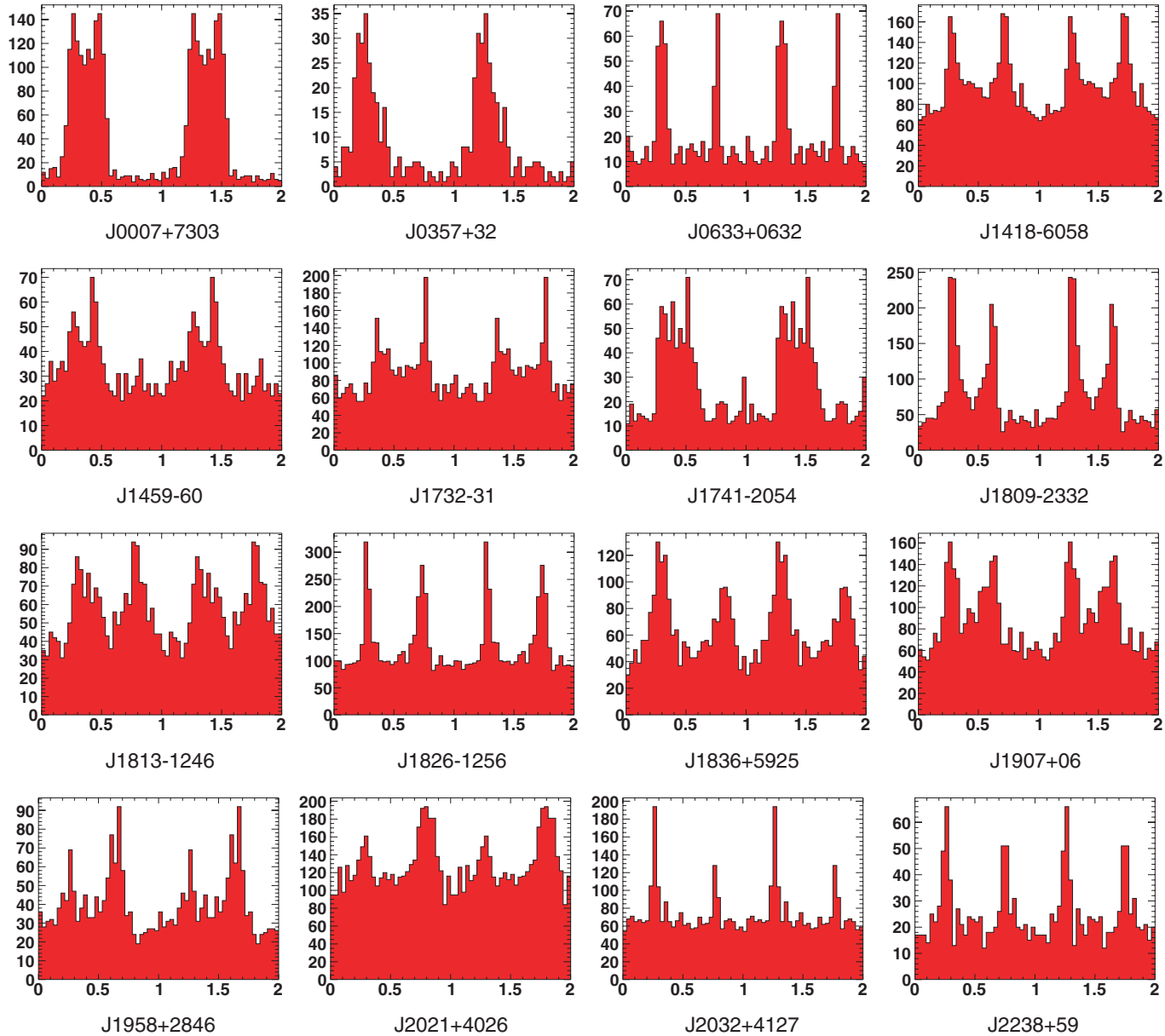

Fig. 2. Folded light curves, with a resolution of 32 phase bins per period, of the 16 pulsars discovered with the Fermi LAT, using 5 months of data with $E >$ 300 MeV, selected from a region of radius 0.8° around the best position for the pulsar. The light curves are not background-subtracted and can include a substantial contribution from the galactic diffuse gamma-ray emission, particularly for the pulsars at low galactic latitude. The *x* axis represents phase; the *y* axis represents counts per phase bin. Two rotations are shown, and the phase of the first peak has been placed at ~0.3 for clarity.

G313.3+0.1, the "Rabbit" (*24*). J1809–2332, likely powering the "Taz" PWN, is also possibly associated with a recently discovered mixed-morphology SNR, G7.5–1.7 (*25*). J1826–1256 is probably powering the "Eel" PWN (*26*), whereas J2021+4026 reveals that a pulsar is present, as long suspected, inside the γ Cygni SNR (*27*). Two more pulsars present plausible associations with SNRs: J0633+0632 is in the fairly old (~30,000 years) complex Monoceros Loop SNR (G205.5+0.5) at a distance of ~1.5 kpc (*28*), and J1907+06 is ~0.3° from SNR G40.5–0.5, but additional evidence is needed before an association can be demonstrated. J1836+5925 is the long-sought pulsar powering 3EG J1835+5918, the brightest and most accurately positioned of the unidentified EGRET sources off the plane (*29*). Our pulsar coincides with RX J1836+5925, an isolated neutron star for which x-ray pulsations were extensively searched but not found (*30*). Deep radio pulsation searches with the Green Bank Telescope at the National Radio Astronomy Observatory placed an upper limit at 1.4 GHz on flux density of 7 μJy for a spin period $P \geq 10$ ms (*30*). The x-ray counterpart of 3EG J1835+5918 was already suspected to be similar to Geminga, just a bit older and slightly farther away [within ~800 pc (*31*)]. Using ~130 ks of publicly available Chandra High Resolution Camera x-ray observations of RX J1836.2+5925 [the same data searched by (*30*)], we searched for x-ray pulsations using our gamma-ray ephemeris, but found none. In addition to J1836+5925, three other pulsars are located off the plane, at $|b| > 3°$ (J0007+7303, J0357+32, and J1741–2054), whereas the remaining 12 are located in the galactic plane.

We imaged the error boxes of nine pulsars with specifically targeted short (~5 ks) Swift observations, and in four cases identified x-ray point sources within the LAT error circle that may be the x-ray counterparts (J0633+0632, J1741–2054, J1813–1246, and J1958+2846). In one other case, J2032+4127, Chandra observations (*32*) have revealed a number of x-ray point sources in the region. The best timing solution was found assuming the position of MT91 221, but this cannot be the true x-ray counterpart because it is known to be a B star. The region around J1418–6058 was mapped in detail by Chandra (*24*), and a promising point source, denoted R1, is consistent with the LAT source position and is likely the x-ray counterpart to the pulsar. Chandra observations of 3EG 2020+4017 show several possible counterparts to J2021+4026, of which S21 (*33*) has the best timing solution and is also most consistent with the LAT source position. Other pulsars were better localized using grid searches (J1459–60 and J1732–31) or on-pulse photon analyses (J0357+32, J1907+0601, and J2238+59). In our grid searches, we scanned 25 equally spaced locations overlapping the 95% LAT error circle, and looked for the position with the best timing solution. In on-pulse photon analyses, we used only the on-pulse photons to improve the source position.

Five pulsars suffer from large (~0.1°) positional uncertainties, which degrade the frequency derivative in the timing solution. The problem is particularly acute in J0357+32 (Table 2), and the ~5′ uncertainty in its position results in an uncertainty in the true value of the frequency derivative of $\sim 6 \times 10^{-14}$. Any parameters derived from its timing solution are not reliable.

Implications. Data from EGRET, COS-B, and SAS-2 demonstrated that rotation-powered pulsars are persistent sources of pulsed gamma rays. EGRET results also showed that the luminosity of detected pulsars over all wavelengths was dominated by the gamma-ray part of the spectrum. EGRET further established a list of persistent sources at low and intermediate galactic latitudes that could not be demonstrated to show pulsations. The Fermi LAT data confirm most of these sources and reveal that many of these persistent galactic gamma-ray sources are pulsars. Of 36 LAT sources with EGRET counterparts that were searched, 15 show pulsations (13 from previously unknown pulsars).

Until the launch of Fermi, the only known radio-quiet pulsar was Geminga, at a distance of ~250 pc (*34*) and with a spin-down luminosity of 3.2×10^{34} ergs s^{-1} and a gamma-ray efficiency of a few percent (for a 1-sr beam) or 50% (for isotropic emission) (*8*). It was already apparent ~30 years ago, as shown by COS-B observations (*1*), that the average unidentified gamma-ray source should have a luminosity greater than 10^{35} ergs s^{-1} to be consistent with the very narrow distribution of low-latitude gamma-ray sources (*4*, *35*). Later studies with EGRET confirmed this (*5*), showing that most unidentified sources were at ~1.2 to 1.6 kpc and had luminosities of 0.7×10^{35} to 16.7×10^{35} ergs s^{-1}. Thus, most unidentified sources could not be like Geminga [e.g., (*8*)]. The detection of mostly high-luminosity, low–galactic latitude, and probably relatively distant pulsars by the LAT confirms the predictions that the unidentified gamma-ray sources could not all be nearby, low-luminosity pulsars such as Geminga.

This new population of gamma-ray selected pulsars helps to reveal the geometry of emission from rotation-powered pulsars. A broad gamma-ray beam is required for at least part of our population of pulsars, as we see the high-energy beam but not the relatively narrow radio beam. Thus, this population favors pulsar emission models in which the high-energy radiation occurs in the outer magnetosphere, nearer to the light cylinder. Outer gap models [e.g., (*6*)], where particles are accelerated and radiate in vacuum gaps in the outer magnetosphere, and slot gap models [e.g., (*36*)], where particles are accelerated and radiate at all altitudes along the open magnetic field boundary, both can reproduce the wide, double-peaked light curves we observed (*9*).

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Fig. S1
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Detection of High-Energy Gamma-Ray Emission from the Globular Cluster 47 Tucanae with Fermi

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We report the detection of gamma-ray emissions above 200 megaelectron volts at a significance level of 17σ from the globular cluster 47 Tucanae, using data obtained with the Large Area Telescope onboard the Fermi Gamma-ray Space Telescope. Globular clusters are expected to emit gamma rays because of the large populations of millisecond pulsars that they contain. The spectral shape of 47 Tucanae is consistent with gamma-ray emission from a population of millisecond pulsars. The observed gamma-ray luminosity implies an upper limit of 60 millisecond pulsars present in 47 Tucanae.

With their typical ages of ~10^{10} years, globular clusters form the most ancient constituents of our Galaxy. They are seen throughout the electromagnetic spectrum, from radio waves up to x-ray energies, revealing their various stellar components. In particular, x-ray observations have shown that globular clusters contain considerably more close binary systems per unit mass than the galactic disc (*1*); this finding is interpreted as a result of frequent stellar encounters in their dense stellar cores (*2*). This scenario is strengthened by the observation that the number of low-mass x-ray binary systems containing neutron stars is directly correlated with the stellar encounter rate (*3*, *4*). These close binary systems may provide a source of internal energy stabilizing the cluster against collapse (*5*). Another consequence of this scenario is the presence of many millisecond pulsars (MSPs, also known as "recycled" pulsars); these are pulsars that were spun up to millisecond periods by mass accretion from a low-mass x-ray binary companion (*6*).

The only domain in which globular clusters have so far eluded detection is gamma rays. Recent observations with the Large Area Telescope (LAT) onboard the Fermi Gamma-ray Space Telescope have revealed gamma-ray pulsations from eight MSPs, establishing these objects as a class of high-energy gamma-ray sources (*7*, *8*). Most of the MSPs detected in gamma rays are within a distance of only a few hundred parsecs from the Sun, which implies that MSPs are rather faint objects with isotropic gamma-ray luminosities that generally do not exceed 10^{33} ergs s^{-1} (*8*). Placed at a distance of several kiloparsecs (the distance to the nearest globular clusters), it is unlikely, although not impossible, that individual MSPs are being detected in gamma rays. Globular clusters, however, may contain tens to several hundreds of MSPs (*9*), and their cumulative mag-

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netospheric emission is probably the first signature that would be picked out in gamma rays.

47 Tucanae (NGC 104) is one of the most promising candidates for high-energy gamma-ray emission because of the large number of known MSPs in the cluster and its relative proximity (4 kpc) (*10*). So far, 23 MSPs have been detected in 47 Tuc by radio and/or x-ray observations, and the total population is estimated to be 30 to 60 (*9*, *11*, *12*), although claims in the past reached up to 200 (*13*). We observed 47 Tuc with the LAT aboard Fermi during the continuous sky survey observations of the satellite. Our data cover the period 8 August 2008 to 3 April 2009 and correspond to 194.3 days of continuous observations, during which a total exposure of ~2 × 10^{10} cm^2·s (at 1 GeV) has been obtained for 47 Tuc. We restricted our analysis (*14*) to photon energies of >200 MeV, where our current knowledge of the instrument response implies systematic uncertainties that are <10% and where the photon energy dispersion caused by incomplete energy measurements becomes negligible.

The LAT image of the region around 47 Tuc shows a bright and isolated gamma-ray source that is consistent with the location of the globular cluster (Fig. 1) (*14*). We determined the gamma-ray spectrum of the LAT source by maximum likelihood fitting of the emission in 10 logarithmically spaced energy bins covering the interval 200 MeV to 10 GeV (Fig. 2). The spectrum reveals a relatively flat spectral energy distribution with a clear cutoff at energies above a few GeV; it is well fitted by an exponentially cut-off power law that provides a 3σ improvement upon a simple power law, with best-fitting spectral index $\Gamma = 1.3 \pm 0.3$ and cutoff energy $E_{cut} = 2.5^{+1.6}_{-0.8}$ GeV. The systematic uncertainty in the spectral index is estimated to be 0.1; that in the cutoff energy is estimated to be 0.3 GeV. By integrating the best-fitting model over the energy range 100 MeV to 10 GeV, we determined the integral photon flux in this band to be 2.6 (±0.8) × 10^{-8} photons cm^{-2} s^{-1}, which is slightly below the upper limit of 5 × 10^{-8} photons cm^{-2} s^{-1} reported by EGRET (*15*–*17*). The photon flux corresponds to an energy flux of 2.5 (±0.4) × 10^{-11} ergs cm^{-2} s^{-1}. The systematic uncertainty in our fluxes is estimated to be <10%. The overall detection significance of the source amounts to 17σ.

We searched for time variability of the gamma-ray signal by dividing our data set into equally sized time bins of durations 1 day, 1 week, 2 weeks, and 1 month. We detected no source at the location of 47 Tuc in any of the daily or weekly time bins, which indicates that the observed emission did not arise from short-duration flares. The LAT source was significantly detected in all 2-week and monthly time bins at a comparable flux level, which suggests that the source was steady over the period of observation. Using ephemerides from (*18*) for 21 MSPs in 47 Tuc, we searched for gamma-ray pulsations in our data without finding any significant detection. The observed gamma-ray signal thus does not appear to be dominated by a single (or a few) known gamma-ray pulsars in 47 Tuc; this is in line with the absence of a single particularly powerful MSP in the cluster (*14*).

Pulsed gamma-ray curvature radiation (and eventually inverse Compton scattering) arising near the polar cap and/or in an outer magnetospheric gap in MSPs has been proposed as a possible source of high-energy photons in globular clusters (*19*–*22*). The main unknowns of this scenario are the exact site of gamma-ray production (polar cap versus slot gap or outer gap), the efficiency η_γ with which the spin-down power is converted into gamma-ray luminosity, and the total number of MSPs in the cluster. The eight galactic MSPs that have so far been detected by Fermi (*8*) have a mean spectral index of $\langle\Gamma\rangle = 1.5 \pm 0.4$ and a mean cutoff energy of $\langle E_{cut}\rangle = 2.8 \pm 1.8$ GeV, similar to what we found for the 47 Tuc source. Cumulative gamma-ray emission from MSPs in 47 Tuc is thus a plausible explanation for the observed signal.

Under this hypothesis, we estimate η_γ from the gamma-ray flux of 47 Tuc by taking the average spin-down power to be $\langle\dot{E}\rangle = 1.8\ (\pm 0.7) \times 10^{34}$ ergs s^{-1} (*14*) and keeping the total number of MSPs in 47 Tuc as a parameter of the solution. For a distance to 47 Tuc of 4.0 ± 0.4 kpc (*10*), the measured energy flux of 2.5 (±0.4) × 10^{-11} ergs cm^{-2} s^{-1} converts into an isotropic gamma-ray luminosity of $L_\gamma = 4.8\ (\pm 1.2) \times 10^{34}$ ergs s^{-1}, which results in $\eta_\gamma = 0.12\ (\pm 0.05)\ \bar{f}_\Omega / N_{23}$, where $\bar{f}_\Omega$ is an average geometrical correction factor that accounts for non-isotropic emission (*23*) and N_{23} is the number of MSPs in 47 Tuc in units of 23. $N_{23} \geq 1$ implies that $0.12\ (\pm 0.05)\ \bar{f}_\Omega$ is an upper limit on the spin-down to gamma-ray luminosity conversion efficiency in 47 Tuc; this is consistent with the predicted conversion efficiency of 6.1% based on a model of the expected gamma-ray emission from a population of MSPs in 47 Tuc in the framework of a fully three-dimensional general-relativistic polar cap pulsar model (*22*). It is also compatible with the estimated conversion efficiency of ~10% of (*24*) that was derived in the framework of a space charge-limited flow acceleration polar cap model.

The conversion efficiencies of the eight galactic MSPs detected by Fermi cover the range from $0.02 f_\Omega$ to $1.0 f_\Omega$ with a mean value of $\langle\eta_\gamma\rangle = 0.14\ \bar{f}_\Omega$ (*8*), which is larger than the upper limit we derive for 47 Tuc. However, the MSPs that have been detected so far by Fermi are faint gamma-ray sources, forming a sample that is likely biased toward intrinsically bright

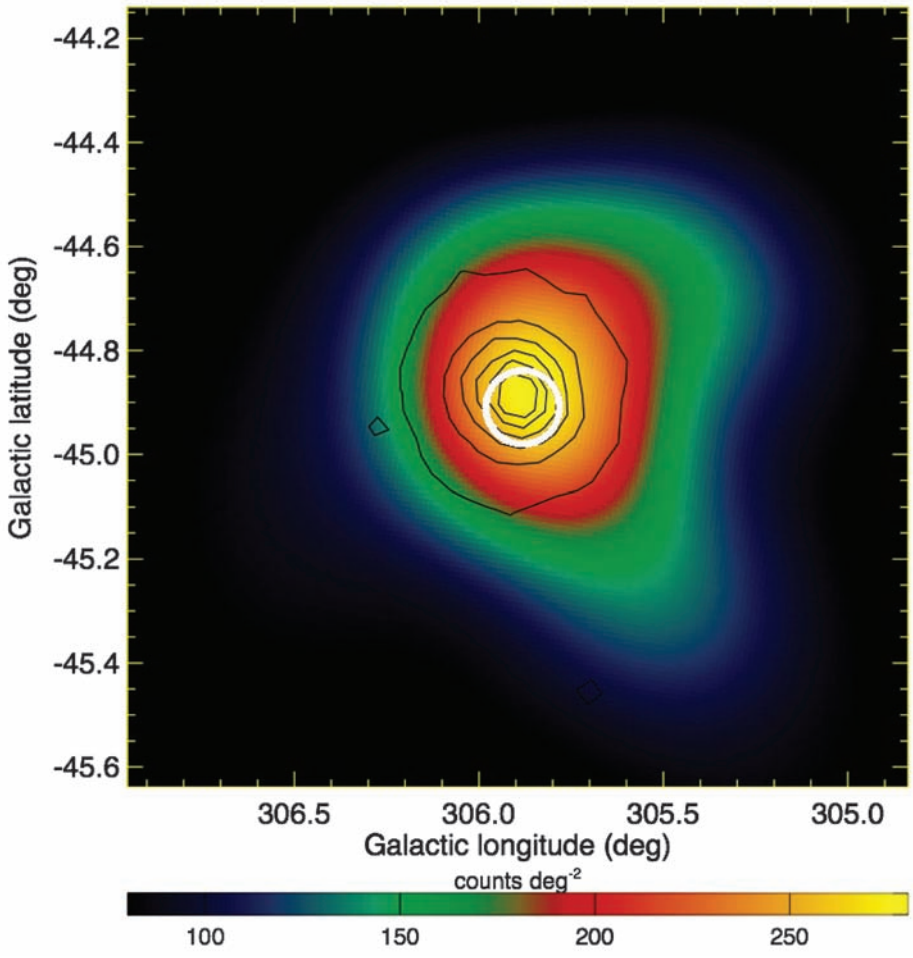

Fig. 1. Fermi LAT gamma-ray image (200 MeV to 10 GeV) of a 1.5° × 1.5° region centered on the position of 47 Tuc. The map was adaptively smoothed by imposing a minimum signal-to-noise ratio of 5. A total of ~290 counts were detected from the gamma-ray source. Black contours indicate the stellar density in 47 Tuc as derived from DSS2 red plates (*30*). The white circle shows the 95% confidence region for the location of the gamma-ray source. The position of the LAT source coincides with the core region of 47 Tuc.

objects or objects for which the beam orientation is favorable. Selecting only the nearest MSPs should reduce this bias, because for close objects a larger fraction of the MSP parameter space is accessible to Fermi. Taking only the three nearest MSPs from the Fermi sample (which also corresponds to the three nearest known MSPs) results in a mean spin-down to gamma-ray luminosity conversion efficiency of $\langle\eta_\gamma\rangle = 0.08\,\overline{f}_\Omega$, considerably lower than the global average. Taking the five nearest MSPs results in $\langle\eta_\gamma\rangle = 0.10\,\overline{f}_\Omega$. Both values are consistent with our upper limit on 47 Tuc. Thus, our data show no evidence for differences in the gamma-ray efficiencies of MSPs in globular clusters with respect to objects observed in the galactic field.

Assuming that the gamma-ray efficiencies of MSPs in 47 Tuc are equal to those of the nearby galactic field sample, and also assuming that their average geometrical correction factors $\overline{f}_\Omega$ are the same, we obtained an estimate of the total number of MSPs in 47 Tuc. Taking the mean $\langle\eta_\gamma\rangle = 0.08\,\overline{f}_\Omega$ that we obtained for the sample of the nearest galactic MSP as the most conservative estimate, we converted the observed gamma-ray efficiency $\eta_\gamma = 0.12\ (\pm 0.05)\ \overline{f}_\Omega / N_{23}$ for 47 Tuc into $N_{23} = 1.5 \pm 0.6$. Formally, this corresponds to a 95% confidence interval of 7 to 62 MSPs in 47 Tuc (assuming that uncertainties are Gaussian), which is consistent with the range of 45 to 60 MSPs estimated on the basis of x-ray observations obtained previously with Chandra (*11*). The x-ray and gamma-ray constraints thus suggest a population of about 50 to 60 MSPs in 47 Tuc. This is a factor of ~2 above the number of known radio MSPs in the cluster and also well above the upper limit of 30 radio MSPs estimated to be present in 47 Tuc (*25*), constraining the radio beaming fraction to >0.5 times that of the gamma-ray beaming. We recall that this result relies on an estimate of the average gamma-ray efficiency of MSPs. We obtained this efficiency from a sample of galactic field MSPs and selected the value that appears to be the least biased and that is the most conservative in the sense that it produces the largest estimate for the number of MSPs in 47 Tuc. The smallness of the gamma-ray sample of MSPs, however, implies that the average gamma-ray efficiency is still uncertain and likely biased.

It has been suggested that MSPs may produce relativistic magnetized winds that, when interacting with stellar winds or winds from other MSPs, create shocks that are capable of accelerating electrons and positrons into the GeV-TeV regime (*26*, *27*). These high-energy particles may eventually undergo inverse Compton scattering on stellar and cosmic microwave background radiation, producing detectable fluxes of GeV-TeV gamma-ray emissions. The expected gamma-ray emission from 47 Tuc has been modeled in this scenario by Bednarek and Sitarek (*26*), who predicted 100 MeV to 10 GeV photon fluxes on the order of 10^{-8} photons cm^{-2} s^{-1} and energy fluxes on the order of ~3×10^{-11} ergs cm^{-2} s^{-1}, which are on the order of the values we observed using the LAT. However, Bednarek and Sitarek assumed for their calculations that the total power injected as relativistic electrons and positrons amounts to 1.2×10^{35} ergs s^{-1}, which corresponds to a mean MSP spin-down power of $\langle\dot{E}\rangle = 5.2 \times 10^{35}$ ergs s^{-1} under the assumptions that the total number of MSPs in 47 Tuc amounts to 23 objects and that the average energy conversion efficiency from the pulsar winds to relativistic electrons and positrons amounts to 1% (*22*, *26*). This spin-down power is about a factor of 30 larger than the average spin-down luminosity of MSPs in 47 Tuc (*14*), which suggests that the gamma-ray flux estimates of (*26*) are overly optimistic and that the contribution of pulsar wind interactions to the gamma-ray emission observed by LAT is probably negligible.

Furthermore, the pulsar wind interaction model of (*26*) predicts gamma-ray spectra that extend well above 1 GeV into the TeV domain, with possible spectral turnovers and breaks above ~100 GeV. These high cutoff energies are at odds with our observed spectral break energy in the GeV range. To explain a GeV spectral break in the pulsar wind interaction model, the maximum energy of the accelerated particles should be limited to a few GeV; this scale is below the injection energies expected for electrons and positrons from the inner pulsar magnetospheres, which may range up to TeV energies (*27*). Consequently, pulsar wind interactions should play a minor role in the acceleration of electrons and positrons in 47 Tuc. This is consistent with the model of (*22*), which suggests that the direct conversion efficiency of spin-down energy into gamma rays, η_γ, is considerably larger than the efficiency $\eta_{e^\pm}$ for electron and positron production. We cannot exclude, however, the possibility that pulsar wind interactions contribute at a low level to the gamma-ray signal we detected from 47 Tuc. Because TeV gamma-ray emission from 47 Tuc has not yet been detected (*28*), we cannot place firm constraints on that contribution.

Until now the study of close binary systems in globular clusters mainly has relied on x-ray observations. Such studies, however, are hampered by the fact that a large variety of binary systems emit x-rays [cataclysmic variables (CVs), low-mass x-ray binaries (LMXBs), chromospherically active main-sequence binaries (BY Dras), and MSPs] and that it is difficult to assess the nature of the sources from x-ray observations alone [however, see (*11*)]. X-ray studies must therefore be backed up by multiwavelength identification programs that help to disentangle these source populations. High-energy gamma-ray observations are unique in that they should be sensitive mainly to the pulsar populations. This is illustrated by Fermi observations of our own Galaxy that have revealed that pulsars form the largest and most luminous point-source population in this energy domain. No CVs, LMXBs, or BY Dras have so far been detected in high-energy gamma rays (*29*). It thus seems rather likely that pulsars (and MSPs in particular) are also the primary population of gamma-ray sources in globular clusters.

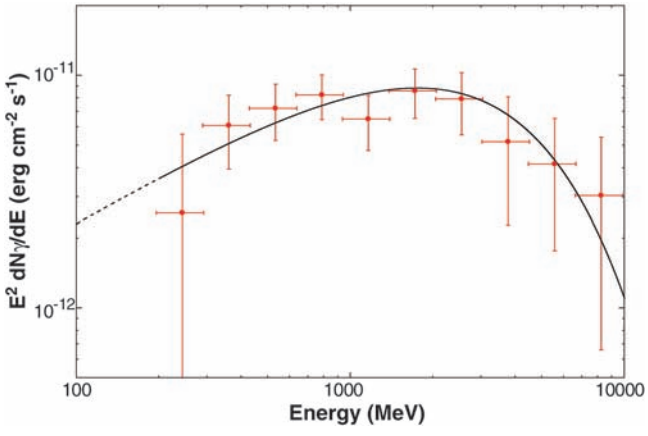

Fig. 2. Spectral energy distribution ($E^2 dN_\gamma/dE$) of the Fermi source seen toward 47 Tuc. The solid line shows the fit of an exponentially cut-off power law obtained for the energy range 200 MeV to 20 GeV. The dashed line indicates the extrapolation of the fit to 100 MeV.

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SOM Text
Figs. S1 and S2
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A Population of Gamma-Ray Millisecond Pulsars Seen with the Fermi Large Area Telescope

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Pulsars are born with subsecond spin periods and slow by electromagnetic braking for several tens of millions of years, when detectable radiation ceases. A second life can occur for neutron stars in binary systems. They can acquire mass and angular momentum from their companions, to be spun up to millisecond periods and begin radiating again. We searched Fermi Large Area Telescope data for pulsations from all known millisecond pulsars (MSPs) outside of globular clusters, using rotation parameters from radio telescopes. Strong gamma-ray pulsations were detected for eight MSPs. The gamma-ray pulse profiles and spectral properties resemble those of young gamma-ray pulsars. The basic emission mechanism seems to be the same for MSPs and young pulsars, with the emission originating in regions far from the neutron star surface.

After the discovery of pulsars, 15 years elapsed before instrumental and computing advances enabled the first radio detections of neutron stars with millisecond spin periods (*1*). Similarly, 17 years after the launch of the Compton Gamma Ray Observatory (CGRO), the Large Area Telescope (LAT) on the Fermi Gamma-ray Space Telescope (formerly GLAST) is now revealing new classes of GeV gamma-ray pulsars. Here, we report LAT detections of pulsed gamma rays from eight galactic millisecond pulsars (MSPs), confirming the marginal detection of PSR J0218+4232 made using the Energetic Gamma Ray Experiment Telescope (EGRET) detector on CGRO (*2*), and including the first MSP seen with the LAT, PSR J0030+0451 (*3*). A companion article (*4*) describes the discovery of 16 young pulsars on the basis of their gamma-ray emission alone. In addition, the LAT has detected about 20 young, radio-loud pulsars (*5*–*7*). The AGILE collaboration has recently detected pulsed gamma-ray emission from an MSP in the globular cluster M28 (*8*).

The Fermi LAT measurements of pulsars in all three of these categories will clarify how neutron stars accelerate the charged particles that radiate at gamma-ray and lower energies. Observed pulse profiles depend on the beam shapes and how they sweep across Earth; comparison of the radio, x-ray, and gamma-ray profiles constrains models of beam formation in pulsar magnetospheres. For gamma-ray pulsars, the high-energy emission dominates the power of the observed electromagnetic radiation (*9*). Consequently, gamma rays provide a probe of these cosmic accelerators. Millisecond pulsars shine for billions of years longer than do normal pulsars. We now know that they can radiate brightly in gamma rays.

The LAT images the entire sky every 3 hours at photon energies from 20 MeV to >300 GeV (*10*). Incident gamma rays convert to electron-positron pairs in tungsten foils, leaving tracks in single-sided silicon strip detectors that provide the photon direction. A hodoscopic CsI calorimeter samples the photon energy, and charged particles are rejected through the use of information from a segmented scintillator array.

MSPs form a distinct class, with small spin periods ($P < 30$ ms) and minuscule braking rates ($\dot{P} < 10^{-17}$). Most are in binary systems. The idea that they have been spun up by the torque resulting from accretion of mass from their companions (*11*) is supported by the recent observations reported in (*12*). MSPs are 10^8 to 10^{10} years old, whereas the young gamma-ray pulsars are 10^3 to 10^5 years old. Their surface magnetic fields are a factor of 10^4 weaker than when the neutron star first formed. However, both the rate of rotational kinetic energy loss, $\dot{E} = 4\pi^2 I\dot{P}/P^3$ (on the assumptions of dipole magnetic fields and a neutron star moment of inertia $I = 10^{45}$ g·cm²), and the magnetic field at the light cylinder, $B_{LC} = 4\pi^2(3I\dot{P}/2c^3P^5)^{1/2}$ (where c is the speed of light), are comparable to those of newly formed pulsars (*13*). On the basis of theoretical models of gamma-ray emission from MSPs, it

was predicted that Fermi would detect roughly 10 pulsed detections in 1 year (*14*, *15*).

The Australia Telescope National Facility (ATNF) pulsar database, V1.35 (*16*, *17*), lists 1794 spin-powered pulsars, of which 168 have $P < 30$ ms and $\dot{P} < 10^{-17}$. Of these, 96 are in globular clusters (*18*). Here, we consider the 72 remaining field MSPs. A radio and x-ray pulsar timing campaign provided rotation ephemerides for Fermi (*19*). MSP timing solutions were obtained from the Nançay Radio Telescope (*20*), the Parkes Radio Telescope (*21*), the Green Bank Telescope (*22*), the Lovell Telescope at Jodrell Bank Observatory (*23*), the Arecibo Observatory radio telescope (*24*), and the Westerbork Synthesis Radio Telescope (*25*). For six of the field MSPs, we used noncontemporaneous ephemerides from the ATNF database. The timing parameters used in this work will be made available on the servers of the Fermi Science Support Center (*26*).

For the gamma-ray timing analysis, we used LAT data acquired from 30 June 2008 to 15 March 2009, selecting events with energy > 0.1 GeV that passed the diffuse gamma-ray selection cuts (*10*). For pulsars with galactic latitude $|b| > 10°$, we selected events within 1° of the radio position; this threshold was reduced to 0.5° for $|b| < 10°$ because of the bright gamma-ray background in the galactic plane resulting from cosmic rays interacting with the interstellar medium. LAT photon arrival times were recorded with an accuracy relative to UTC better than 1 μs (*27*).

Following this analysis, eight MSPs showed strong gamma-ray pulsations with H-test (*28*) values of >25 (Fig. 1 and Table 1). Three are associated with EGRET sources: PSRs J0030+0451, J0218+4232, and J1614–2230. The latter was discovered in a radio search of unidentified EGRET sources (*29*). All of the detected pulsars had con-

Table 1. Properties of the millisecond pulsars detected by Fermi. For each pulsar we give the galactic longitude and latitude (l, b), the rotational period P, the distance d, and the spin-down power $\dot{E}$. Pulsars marked "b" belong to binary systems. The distances come from parallax measurements except for the values marked by an asterisk, which are based on the dispersion measure. The $\dot{E}$ values have been computed using period derivatives corrected for the Shklovskii effect (*36*). The δ parameter gives the phase offset between the maximum of the radio emission and that of the nearest gamma-ray peak, and Δ is the peak separation for two-peaked gamma-ray profiles. Integral photon and energy fluxes over 0.1 GeV are given, as well as spectral indices, exponential cutoff energies, and gamma-ray emission efficiencies η. The systematic uncertainties stemming from the instrument response and the diffuse background are (–0.1, +0.3) for Γ, (–10%, +20%) for E_c, (–10%, +30%) for the photon flux, and (–10%, +20%) for the energy flux.

Pulsar name	l, b	P (ms)	d (pc)	Log $\dot{E}$ (ergs s^{-1})	δ	Δ	Photon flux >0.1 GeV (10^{-8} photons cm^{-2} s^{-1})	Energy flux >0.1 GeV (10^{-11} ergs cm^{-2} s^{-1})	Spectral index	Exponential cutoff energy (GeV)	η (%)
J0030+0451	113.1°, –57.6°	4.865	300 ± 90	33.54	0.16	0.45	5.5 ± 0.7	4.9 ± 0.3	1.3 ± 0.2	1.9 ± 0.4	15 ± 9
J0218+4232 (b)	139.5°, –17.5°	2.323	2700 ± 600*	35.39	0.50	—	5.6 ± 1.3	3.5 ± 0.5	2.0 ± 0.2	7 ± 4	13 ± 6
J0437–4715 (b)	253.4°, –42.0°	5.757	156 ± 2	33.46	0.45	—	4.4 ± 1.0	1.9 ± 0.3	2.1 ± 0.3	2.1 ± 1.1	1.9 ± 0.3
J0613–0200 (b)	210.4°, –9.3°	3.061	480 ± 140	34.10	0.42	—	3.1 ± 0.7	3.1 ± 0.3	1.4 ± 0.2	2.9 ± 0.7	7 ± 4
J0751+1807 (b)	202.7°, 21.1°	3.479	620 ± 310	33.85	0.42	—	2.0 ± 0.7	1.7 ± 0.2	1.6 ± 0.2	3.4 ± 1.2	11 ± 11
J1614–2230 (b)	352.5°, 20.3°	3.151	1300 ± 250*	33.7	0.20	0.48	2.3 ± 2.1	2.5 ± 0.8	1.0 ± 0.3	1.2 ± 0.5	100 ± 80
J1744–1134	14.8°, 9.2°	4.075	470 ± 90	33.60	0.85	—	7.1 ± 1.4	4.0 ± 1.0	1.5 ± 0.2	1.1 ± 0.2	27 ± 12
J2124–3358	10.9°, –45.4°	4.931	250 ± 125	33.6	0.85	—	2.9 ± 0.5	3.4 ± 0.3	1.3 ± 0.2	2.9 ± 0.9	6 ± 6

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temporaneous radio ephemerides with weighted root mean square timing residuals of 10 μs or less. For all eight MSPs, uncertainties in the dispersion measure led to uncertainties of less than 0.005 rotations in the extrapolation of the radio pulse arrival times to infinite frequency; such values are negligible for the gamma-ray light curve bin widths imposed by the photon counts. Analyses for PSRs J0218+4232, J0613–0200, J1614–2230, J1744–1134, and J2124–3358 using ephemerides from different observatories confirmed the absolute phase alignment.

We also searched for steady point-source emission at the locations of the 72 field MSPs. For 13 locations, including those of the eight pulsed detections, emission exceeded the diffuse gamma-ray background by at least 5σ. For the five sources for which only steady emission was seen, the 95%

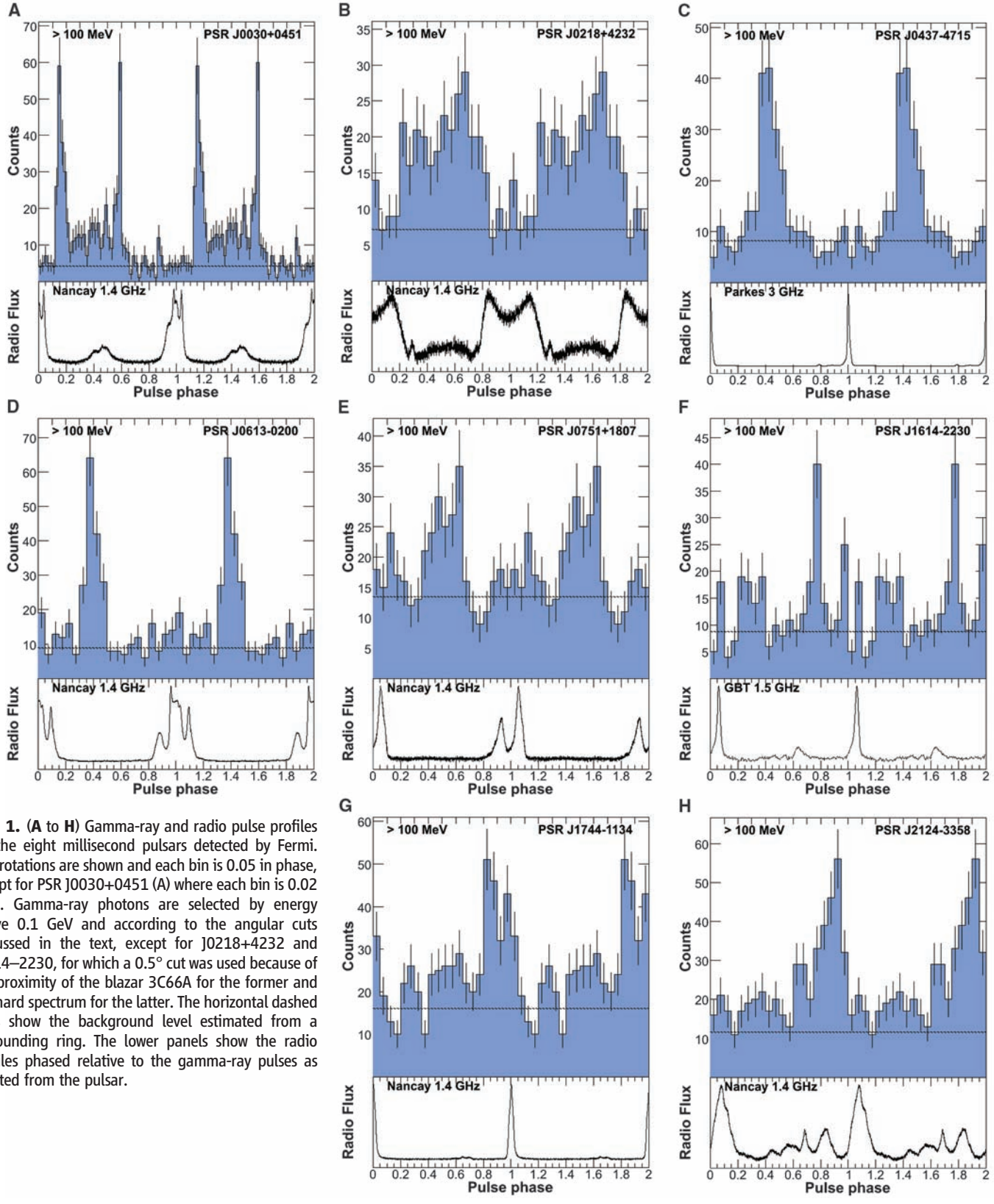

Fig. 1. (**A** to **H**) Gamma-ray and radio pulse profiles for the eight millisecond pulsars detected by Fermi. Two rotations are shown and each bin is 0.05 in phase, except for PSR J0030+0451 (A) where each bin is 0.02 wide. Gamma-ray photons are selected by energy above 0.1 GeV and according to the angular cuts discussed in the text, except for J0218+4232 and J1614–2230, for which a 0.5° cut was used because of the proximity of the blazar 3C66A for the former and the hard spectrum for the latter. The horizontal dashed lines show the background level estimated from a surrounding ring. The lower panels show the radio profiles phased relative to the gamma-ray pulses as emitted from the pulsar.

confidence level radii contained no other candidates besides PSRs J0034–0534, J0610–2100, J1600–3053, J1939+2134, and J1959+2048.

We used the spectral likelihood methods described in (*30*) (see supporting online text). To reduce the background from cosmic-ray interactions in the upper atmosphere, we required photon zenith angles to be less than 105° and excluded time periods when Earth's limb came within 28° of the source. Because of uncertainties in the instrument response, we rejected events with energies below 0.2 GeV. We modeled the gamma-ray spectra with an exponentially cut-off power law of the form $N_0 E^{-\Gamma} \exp[-(E/E_c)]$, where E is the photon energy, E_c is the exponential cutoff energy, Γ is the spectral index, and N_0 is a normalization factor (Table 1). The cutoff energies ranged from 1 GeV to almost 4 GeV (neglecting the J0218+4232 cutoff, which has a large error) and the spectra were hard ($\Gamma < 2$). Overall, the MSP spectral shapes resembled those of young pulsars.

We converted the integral energy fluxes h to luminosities using $L_\gamma = 4\pi h d^2$, where d is the pulsar distance. This corresponds to a flux correction factor $f_\Omega = 1$, appropriate for a fan-like beam as given by outer-magnetosphere emission models (*31*). Six of the pulsars are close and have parallax distance measurements (*32*–*34*), although uncertainties are large in some cases. The distances to PSR J0218+4232 and PSR J1614–2230 are based on the dispersion measures and the NE2001 galactic electron density model (*35*). MSPs have low intrinsic $\dot{P}$ values and are relatively close; hence, the kinematic Shklovskii contribution (*36*) $\dot{P}_s = P\mu^2 d/c$ (where μ is the proper motion) is non-negligible. $\dot{P}_s$ is subtracted from the observed $\dot{P}$ before computing the spin-down power $\dot{E}$ and the corresponding gamma-ray efficiency $\eta = L\gamma/\dot{E}$ (Table 1). Uncertainties in $\dot{E}$ are generally a few percent or less, except for PSRs J1614–2230 and J2124–3358, where they are larger (60% and 32%, respectively) because the large uncertainty in the distance leads to a correspondingly large uncertainty in $\dot{P}_s$. Uncertainties in η are much larger because $L_\gamma \sim d^2$ and hence the effect of the distance uncertainty is doubled. Such a large efficiency for PSR J1614–2230 would indicate that the distance is overestimated. Reducing the distance would both reduce the Shklovskii correction (thereby increasing $\dot{E}$) and decrease L_γ. Other possible systematic uncertainties in the $\dot{E}$ and η values come from the neutron star moment of inertia, which is assumed to be 10^{45} g·cm^2. Measured values of neutron star masses cover a range from about 1.25 to 1.75 solar masses (*37*, *38*), and the estimated moments of inertia vary correspondingly (*39*). Also, the flux correction factor f_Ω may differ from the assumed value of 1 (*31*).

Five of the eight gamma-ray MSPs are in binary systems. An eclipsing orbit, or interactions with the stellar wind of the companion, could affect the gamma-ray flux. We found no flux variability at their orbital periods (<25% of the flux at the 95% confidence level).

The observed MSP gamma-ray profiles and their relation to the radio profiles are similar to those observed for young pulsars. For PSRs J0030+0451 and J1614–2230, the double-peaked profiles with separation $\Delta \sim 0.45$ and first-peak lag $\delta \sim 0.15$ are almost identical to observed profiles for most young pulsars (*5*–*7*, *30*). A higher proportion of MSPs have a dominant single gamma-ray peak at $\delta \sim 0.5$, but the young pulsar PSR J2229+6114 has a similar pulse profile. For both MSPs and young pulsars, the gamma-ray peaks (single or multiple) are centered on phases 0.3 to 0.4 relative to the radio peak. MSP radio profiles tend to be complex with many components, and in these cases it can be difficult to identify the relevant radio phase. Also, the statistics of the gamma-ray profiles are currently relatively poor.

The spin-down powers of all the detected millisecond and normal gamma-ray pulsars lie above a common threshold of ~5×10^{33} ergs s^{-1} kpc^{-2}, another similarity between these two classes (Fig. 2). Pulsars undetected in gamma rays of both classes lie above this threshold, possibly because (i) distance estimates may be in error for individual pulsars; (ii) the gamma-ray emission beam (or at least strong parts of it) may not sweep across Earth; or (iii) neutron star moments of inertia may be less than the assumed 10^{45} g·cm^2 for some pulsars, so that a given $\dot{P}$ corresponds to a smaller $\dot{E}$.

Polar cap MSP models, where the bulk of the emission originates near the surface of the neutron star, predict that the pulsed gamma rays are roughly aligned with the magnetic poles (*40*). In outer gap (OG) (*41*) and slot gap (SG) (*42*) models, the bulk of the emission originates in the outer magnetosphere in narrow gaps along the last open field lines, forming wide fan beams that

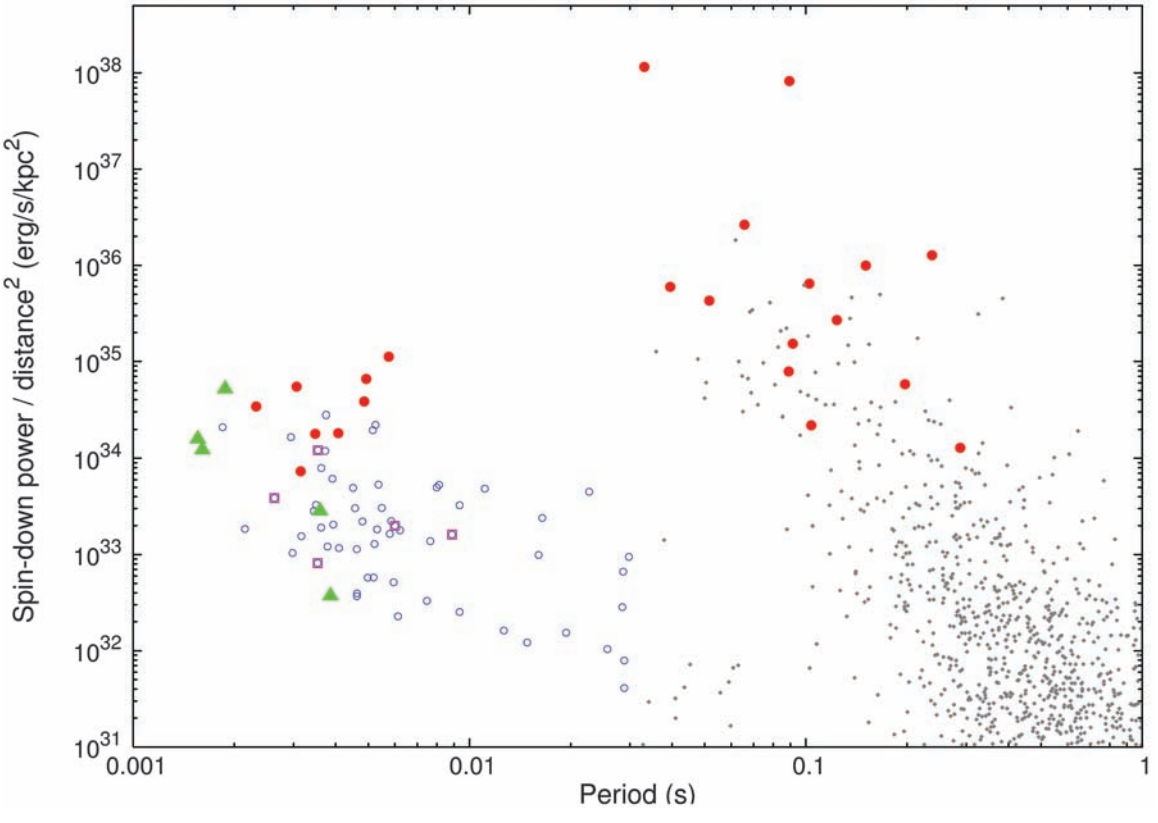

Fig. 2. Spin-down power $\dot{E}$ normalized to the distance squared versus the rotational period for pulsars outside of globular clusters. Where proper motions are available, the $\dot{E}$ values have been corrected for the Shklovskii effect (see text). The eight MSPs reported here are indicated by solid circles, as are young, radio-loud gamma-ray pulsars. The five MSPs likely associated with the nonpulsed point-source detections are indicated by triangles. MSPs for which contemporaneous rotation parameters are unavailable are shown as squares. Undetected MSPs are indicated by open circles, and small dots show undetected normal pulsars. The young radio-loud gamma-ray pulsars are the seven CGRO detections (*9*) and recent Fermi detections (*7*, *44*).

are not aligned with the magnetic poles. In the MSP gamma-ray light curves in Fig. 1, we see that although some of the gamma-ray peaks are aligned with the radio peaks that are thought to be aligned with the magnetic poles, most are not. This favors the outer-magnetosphere model geometry.

The similarities of the gamma-ray pulse profiles, the $\dot{E}$ dependence, and the spectral properties strongly suggest that the same basic emission mechanism is operating in both classes. Magnetic field strengths at the neutron star surface, derived assuming dipole fields, differ by four orders of magnitude between MSPs and young pulsars. On the other hand, B_{LC} is comparable for both. Fermi data for young pulsars (*5*–*7*, *30*) favor outer-magnetosphere emission models over models where the emission comes from close to the polar cap.

The MSP models (*40*–*42*) assume curvature radiation from electrons whose energies arise from a balance between acceleration by the pulsar electric field and the curvature radiation loss in a dipole magnetic field. The cutoff energy thus directly measures the accelerating electric field. The observed values in the range 1 to 4 GeV indicate that the emission is not taking place near the surface—where the electric field is stronger and the cutoff energies for MSPs would reach 10 GeV and could exceed 50 GeV (*43*)—but at some altitude above the neutron star surface.

For current SG and OG models, only MSPs with the highest spin-down power have a high enough electric potential for electron-positron pair production. Most of the MSPs detected by Fermi are below this threshold. Thus, some revision of the outer-magnetosphere models is needed. Surface magnetic fields may be stronger than assumed, perhaps because of magnetic multipoles or more compact neutron stars. Alternatively, the magnetic field at the light cylinder may play a greater role in particle acceleration than has been assumed.

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Impact of Anode Microstructure on Solid Oxide Fuel Cells

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We report a correlation between the microstructure of the anode electrode of a solid oxide fuel cell (SOFC) and its electrochemical performance for a tubular design. It was shown that the electrochemical performance of the cell was extensively improved when the size of constituent particles was reduced so as to yield a highly porous microstructure. The SOFC had a power density of greater than 1 watt per square centimeter at an operating temperature as low as 600°C with a conventional zirconia-based electrolyte, a nickel cermet anode, and a lanthanum ferrite perovskite cathode material. The effect of the hydrogen fuel flow rate (linear velocity) was also examined for the optimization of operating conditions. Higher linear fuel velocity led to better cell performance for the cell with higher anode porosity. A zirconia-based cell could be used for a low-temperature SOFC system under 600°C just by optimizing the microstructure of the anode electrode and operating conditions.

Although solid oxide fuel cells (SOFCs) are available commercially for local and emergency power generation, there are materials challenges that must be overcome for their wider use (*1*–*5*). Some of the features that make them attractive—their high efficiency and use with hydrocarbon fuels—stems from their high operating temperatures (often in excess of 700°C). These high temperatures are also a drawback in that transition metals used in the electrode materials can diffuse into the electrolyte and lower performance and, ultimately, lifetime. Thus, lowering the operation temperature can be beneficial for the commercialization of SOFC systems, since it can offer quick start-up ability, which in turn can allow for their use in applications such as transportable power sources and auxiliary power units for automobiles. Many studies of SOFCs aim at lowering their operating temperature (*6*–*13*). In recent years, much effort has been devoted to the development of SOFCs, especially in the search for new electrode and electrolyte materials. Lanthanum gallate perovskite [e.g., (La, Sr)(Ga, Mg)O_3, or LSGM] is one of the successful materials for a low- or intermediate-temperature SOFC elec-

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trolyte and has been shown to have high ionic conductivity, such that more than 2 W/cm^2 at 600°C could be realized with a 2-μm thick LSGM electrolyte (*6*). A Ba-based perovskite-type oxide was effective as a cathode material using a ceria-based electrolyte (*7*), and a $LaCrO_3$-based anode could be an alternative anode material, with high sulfur tolerance property (*8*).

Another approach for lowering the operating temperature is to develop new cell structures. It has been shown that ~100-nm-thick zirconia-based electrolyte and 80-nm porous Pt electrodes (cathode and anode) could be fabricated with the help of sputtering, lithography, and etching. As a result, a power density as high as 0.4 W/cm^2 at 400°C was demonstrated (*9*). Use of an SOFC with a microtubular design was also proposed for low-temperature operation because it was shown to have high thermal stability under rapid heating, as well as high volumetric power density (*14*–*18*). For a ceria-based electrolyte, it was reported that a microtubular cell with an 0.8-mm diameter could generate >1 W/cm^2 at 550°C (*18*).

Although these approaches are promising, there are also advantages to using the materials in commercial SOFC systems—that is, zirconia-based electrolytes and NiO and (La, Sr)MnO_3 or (La,Sr)(Co,Fe)O_3 as the anode and cathode materials, respectively. These materials remain attractive because of their long-term reliability as well as cost factors; thus, specifically, the use of zirconia and Ni cermet remains interesting. In fact, SOFCs using such materials have been shown to be operable down to 650° to 750°C (*19, 20*). Further lowering the cell operating temperature below 650°C and using zirconia-based electrolytes could be expected to accelerate the commercialization of the SOFC system for a variety of applications.

This study focused on the improvement of SOFC performance by controlling the microstructure of an anode electrode with the conventional materials and fabrication techniques. The SOFCs we report here have a microtubular design and consist of NiO-Sc-stabilized zirconia (ScSZ) and Ce-doped zirconia (10Sc1CeSZ) for the anode, 10Sc1CeSZ for the electrolyte, and (La, Sr)(Fe, Co)O_3 (LSCF)-Gd–doped ceria (GDC) for the cathode, with an interlayer of GDC between the cathode and the electrolyte. All materials used for the SOFC fabrication are commercially available. Details of the sample preparation and characterization are presented in (*21*).

An image of the tubular SOFC and the cross-sectional scanning electron microscope (SEM) image of the cell are shown in Fig. 1A. The size of the cell was 1.9 mm in diameter, and the length of cathode was 5 mm; thus, the electrode area was 0.3 cm^2. A crack-free dense electrolyte with a thickness of ~3 μm has been successfully prepared on the anode-supported tube by a cosintering technique. Three kinds of cells were prepared, corresponding to different cosintering temperatures of the anode/electrolyte: cells A, B, and C, which have different anode microstructures. Table 1 summarizes the properties of each cell. The porosities of the anodes were 54, 47, and 37% for cells A, B, and C, respectively, before reduction. These cells have relatively high porosity, even cell C. The electrical conductivity of the anode (after reduction) was mainly contributed by the Ni particle connections and turns out to be higher for cell C because increased Ni particle size led to better electrical connection.

Figure 1B shows the cumulative volume of the pores in the anode (before reduction) as a function of pore size. As can be seen, all samples showed two major peaks at around 1.5 and 0.5 μm. The differences in the samples were the total volume of the pores below 0.5 μm. The SEM images of the anode microstructure before the cell test and after in-test reduction are shown in Fig. 2 for (A and D) cell A, (B and E) cell B, and (C and F) cell C, respectively. Large pores shown in the images resulted from the use of a 5-μm pore former. The porosity of the microstructure increases because of the reduction of NiO to Ni;

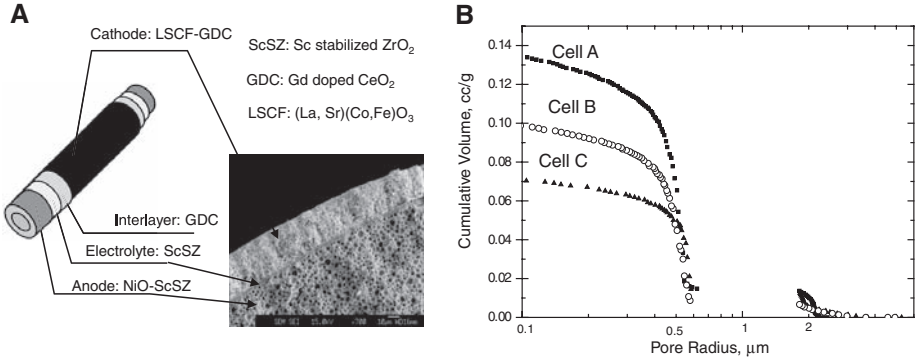

Fig. 1. (**A**) Schematic of the tubular SOFC and cross-sectional SEM image of the cell. The thickness of the electrolyte was 3 μm. (**B**) Cumulative pore volume in the anode (not reduced) as a function of pore size.

Table 1. Summary of the properties for cells A, B, and C. The difference in these cells is in the different cosintering temperature of the anode tube electrolyte. The conductivity of each cell shows that the anode tube itself is in the reducing atmosphere where NiO reduces to Ni. The porosity of each cell indicates that of each anode tube as cosintered in the air.

	Cosintering temperature (°C)	Conductivity of anode tube at 600°C (S/cm)	Porosity of the anode tube (%)
Cell A	1250	613	54
Cell B	1300	795	47
Cell C	1400	837	37

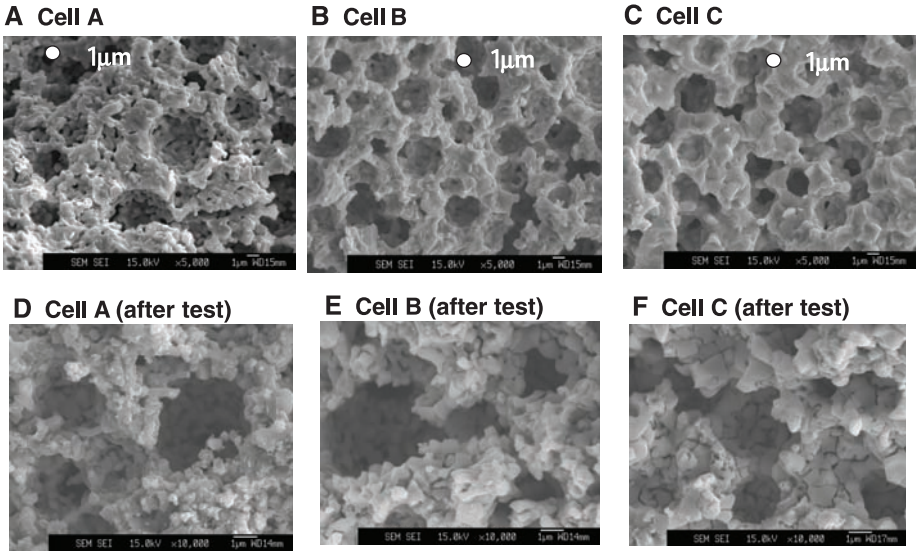

Fig. 2. SEM images of the anode microstructure before the cell test (not reduced) (**A** to **C**) and after the cell test (reduced) (**D** to **F**) for cell A, cell B, and cell C. Because of the reduction of NiO to Ni, which expands the lattice parameter, the porosity preferably increased for SOFC operation.

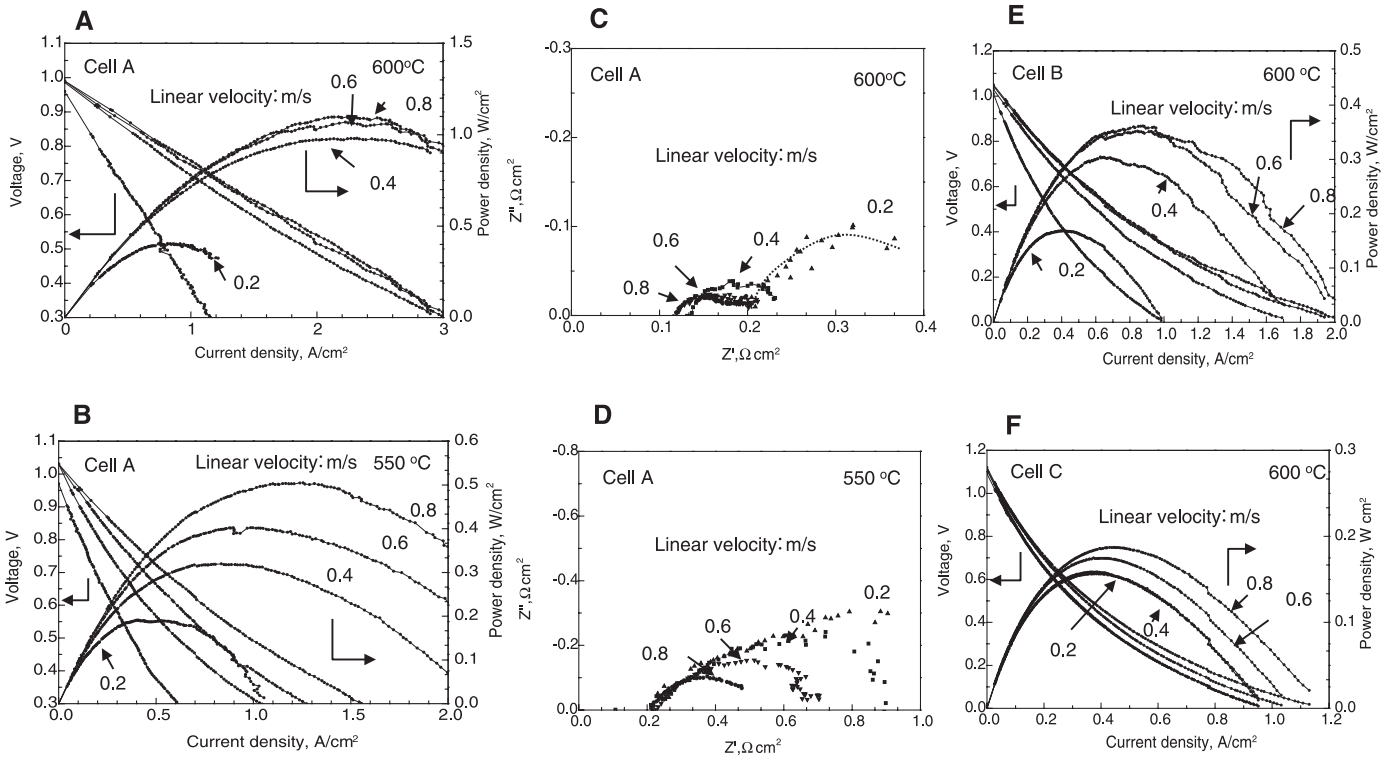

Fig. 3. Performance of cell A. Cell voltage and power as functions of current at (**A**) 600° and (**B**) 550°C and impedance spectra of the cell obtained at (**C**) 600° and (**D**) 550°C. Performance of (**E**) cell B and (**F**) cell C. Cell voltage and power as functions of current at various linear fuel velocities at 600°C.

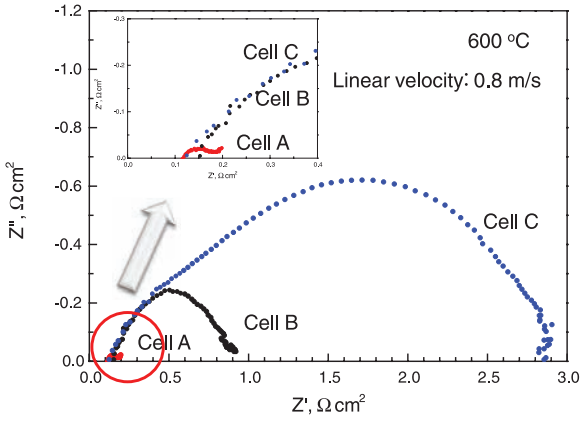

Fig. 4. Comparison of the impedance spectra for cells A, B, and C obtained at 600°C and a linear fuel velocity of 0.8 m/s. Whereas cells B and C overlap in the first semicircle, the second semicircle is effectively decreased for cell B. Cell A showed further reduction of the first semicircle, which is correlated to electrochemical reaction in the anode.

in the reduced samples, Ni particles were <100 nm in size, in the case of cell A, whereas the size of Ni particles in cells B and C was >500 nm.

Figure 3, A and B, shows the performance of cell A for various linear fuel velocities using wet H_2. As can be seen, maximum power densities of 1.1 and 0.5 W/cm^2 at 600° and 550°C were obtained at a linear fuel velocity of 0.8 m/s. Impedance spectra shown in Fig. 3, C and D, shows the effect of linear fuel velocity on the SOFC performance; as the linear fuel velocity increases, the low-frequency semicircle part effectively decreases. The gas diffusion in the anode microstructure is apparently improved by the increase of linear fuel velocity. The linear fuel velocity below 1 m/s is rather low compared with typical operating conditions for planar type SOFCs. Figure 3, A and B, also shows that the influence of linear fuel velocity becomes greater for lower operating temperature.

For comparison, the results of cell performance tests are displayed in Fig. 3, E and F, for cells B and C at 600°C. The maximum power densities of these cells were found to be 0.36 and 0.2 W/cm^2 for cells B and C, respectively, which are comparable to the literature using the same electrolyte (*22*). The positive effect of linear fuel velocity on performance was also observed for cell B; however, in the case of cell C it became smaller with lower porosity. Actually the cell performances of cells A, B, and C obtained at 0.2 m/s linear fuel velocity are almost identical because of the gas diffusion limitation. Thus, the microstructural effect on performance occurs most clearly at higher linear fuel velocity.

Figure 4 shows the impedance spectrum of each cell obtained at 600°C and 0.8 m/s linear fuel velocity. As can be seen in the inset, the high-frequency intercepts of the three cells, corresponding to the ohmic resistances, were almost similar, ~0.1 Ωcm^2. According to previous reports (*23*), the resistance of 3-μm-thick ScSZ electrolyte (σ ~ 0.014 S/cm at 600°C), can be estimated to be 0.02 Ωcm^2. We conclude that the ohmic resistance corresponds to the sum of electrolyte resistance and the anode resistance that arises during current collection. However, the size of the semicircles increases as the porosity of the anode decreases. The semicircle of cell B is identical to the second semicircle of cell C. Thus, the difference between cells B and C is caused by the performance being limited by gas diffusion. Thus, providing sufficient porosity of the anode leads to improvement of the cell performance at 600°C. Several modeling studies of gas transport in SOFC electrodes have been reported that help to understand this effect on the performance (*24, 25*). According to a model shown by Chan *et al.* (*24*), the effect of concentration polarization in the anode is much greater than that in the cathode of an anode-supported cell and is strongly affected by the hydrogen partial pressure at the reaction sites. Thus, such a model may be helpful in understanding the impact of the fuel flow rate on the performance of the cell.

Furthermore, compared with the impedance spectra of cells B and C, the main contribution of the semicircle for cell B seemed to be an overpotential associated with electrochemical reactions.

This overpotential should be affected by the catalytic activity of the electrode and, thus, the size of the Ni particles in the anode should also affect the cell performance, especially at lower operating temperature.

The outstanding performance of cell A seems to arise from the improvement of the anode microstructure (Ni particle size below 100 nm). The open-circuit voltages slightly decreased from 1.1 V for cell C to 1.0 V for cell A as sintering temperature decreased from 1400° to 1250°C. This change possibly relates to the density of the electrolyte. Bundling of the cells, stack design, and modularization are other challenges that need to be overcome for the realization of high-performance SOFC systems.

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Docking in Metal-Organic Frameworks

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The use of metal-organic frameworks (MOFs) so far has largely relied on nonspecific binding interactions to host small molecular guests. We used long organic struts (~2 nanometers) incorporating 34- and 36-membered macrocyclic polyethers as recognition modules in the construction of several crystalline primitive cubic frameworks that engage in specific binding in a way not observed in passive, open reticulated geometries. MOF-1001 is capable of docking paraquat dication (PQT^{2+}) guests within the macrocycles in a stereoelectronically controlled fashion. This act of specific complexation yields quantitatively the corresponding MOF-1001 pseudorotaxanes, as confirmed by x-ray diffraction and by solid- and solution-state nuclear magnetic resonance spectroscopic studies performed on MOF-1001, its pseudorotaxanes, and their molecular strut precursors. A control experiment involving the attempted inclusion of PQT^{2+} inside a framework (MOF-177) devoid of polyether struts showed negligible uptake of PQT^{2+}, indicating the importance of the macrocyclic polyether in PQT^{2+} docking.

The concept of architectural domains that operate independently, yet are interconnected, is common in biology but difficult to achieve in synthetic materials. We believe that this concept offers a useful strategy for achieving materials with higher complexity. We have focused our attention on the design and synthesis of porous crystals composed of several architectural domains, one of which is capable of docking molecules in a manner akin to the well-known docking of drug molecules.

Our design takes advantage of the emerging chemistry of metal-organic frameworks (MOFs) (*1*–*3*), which has been used effectively to assemble components with simple constitutions—specifically, organic struts and inorganic joints—into three-dimensionally ordered structures. The vast majority of porous MOFs prepared thus far can be regarded (Fig. 1) as having two important architectural domains: (i) the pore aperture, which is responsible for the shape- and size-selective binding of incoming molecules, and (ii) the internal surface of the pores, onto which gases or small molecules can be compacted and distributed with simple interaction sites covering the struts and joints; in some cases, the interaction is with open metal sites. These two domains are called the sorting domain (*2*) and the coverage domain (*4*–*8*), respectively.

The synthesis of more complex MOFs, where more than two domains are present, has remained unexplored. Here, we show how molecular recognition components, much used in supramolecular chemistry (*9*, *10*), can be integrated in a modular fashion into struts of MOFs, thereby creating recognition sites into which incoming guests will dock in a highly specific manner with stereoelectronic control. This third architectural domain—the active domain—combines shape, size, and electronic elements in the recognition of incoming guests, and brings order to otherwise highly disordered guests in conventional MOFs. Hence, this chemistry describes a class of MOFs with a level of complexity higher than that of known open reticulated geometries (*1*).

We used the primitive cubic topology of the archetypical MOF-5 (*11*), in which benzene struts are joined by $Zn_4O(CO_2)_6$ cluster joints, as the target for our design. Initially, we demonstrated the feasibility of using the long 1/4DMBDA (**1**) to make MOF-1000 (*12*), which has the MOF-5 topology, albeit quadruply interpenetrated (Fig. 2A). This approach was extended to the more complex struts BPP34C10DA (**2**) and 1/5DNPPP36C10DA (**3**), which are known to act as electron-rich receptors for electron-deficient substrates (*13*), to make the corresponding MOF-1001A, MOF-1001, and MOF-1002 (Fig. 2, B to D). Each of the crown ether receptors in MOF-1001 is accessible, as evidenced by the docking of the paraquat dication (PQT^{2+}) at every one of the receptor sites (see below). In contrast to known MOFs, where the frameworks are used mainly as passive platforms for the adsorption of gases and molecules, MOF-1001 not only has active components in precise recognition sites but also, by virtue of the openness of its structure, allows substrates to diffuse freely from solution, through the pores, and finally dock in these active domains.

Crystals of MOF-1000 (*14*) (Fig. 2A and Fig. 3A) were obtained by mixing a solution of strut **1** (*15*) with $Zn(NO_3)_2{\cdot}4H_2O$ in *N*,*N*-diethylformamide under conditions previously used in the synthesis of MOF-5 (*5*, *11*). Its crystal structure displays the same structural topology as does MOF-5. It is found to be four-fold interpenetrated because of the length and slender nature of the strut; the distance between the two carboxylate carbon atoms is 19.3 Å. The successful crystallization of MOF-1000 confirmed the practicality of creating MOFs with higher complexity by means of this synthetic protocol.

Struts **2** and **3** respectively contain 34- and 36-membered polyether rings, which have been extensively used (*13*) as receptors for a wide range of electron-deficient substrates. These struts are ideally suited as molecular recognition modules for making MOFs. Strut **2** was prepared by means of a convergent synthetic approach (*14*) and was used under conditions similar to those used in the synthesis of MOF-1000 to yield MOF-1001A

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and MOF-1001 (*14*). The crystal structure of MOF-1001A is a triply interpenetrating framework (Fig. 2B and Fig. 3B), whereas that of MOF-1001 is the corresponding noninterpenetrating form (Fig. 2C and Fig. 3C); both have the MOF-5–type topology. The existence of MOF-1001A, despite its occasional appearance as a minor product, validates indirectly the high porosity of MOF-1001. The sheer openness of the structure, however, led us to further optimize the reaction conditions to successfully obtain MOF-1001 as a pure phase (*14*). MOF-1001 has $Fm\bar{3}$m

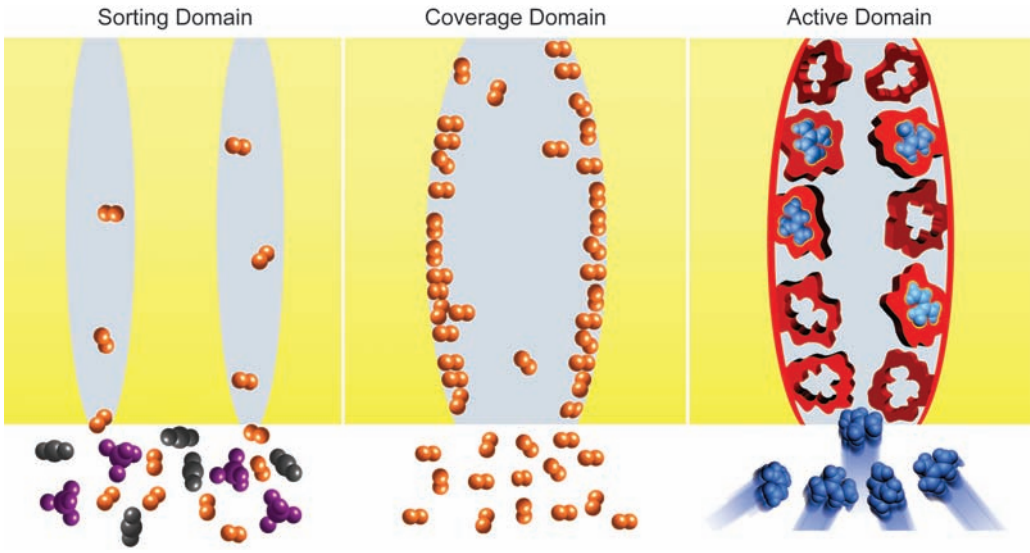

Fig. 1. Classification of the different porous domains in metal-organic frameworks. In the sorting domain, guest molecules are selected according to their size at the orifices of the pores. The entry of H_2 (orange) and concomitant exclusion of CO_2 (black) and CH_4 (purple) reflects the sieve-like action at the entrances to the pores. In the coverage domain, the guest molecules along the walls of the pores are disordered on account of their weak nonspecific interactions with the framework surrounding the pores. By contrast, the active domain has built-in recognition sites that help to maneuver and dock incoming guests in a highly selective and stereoelectronically controlled manner. These recognition sites (red) could be π-electron–rich and, as such, would seek out π-electron–deficient substrates (blue).

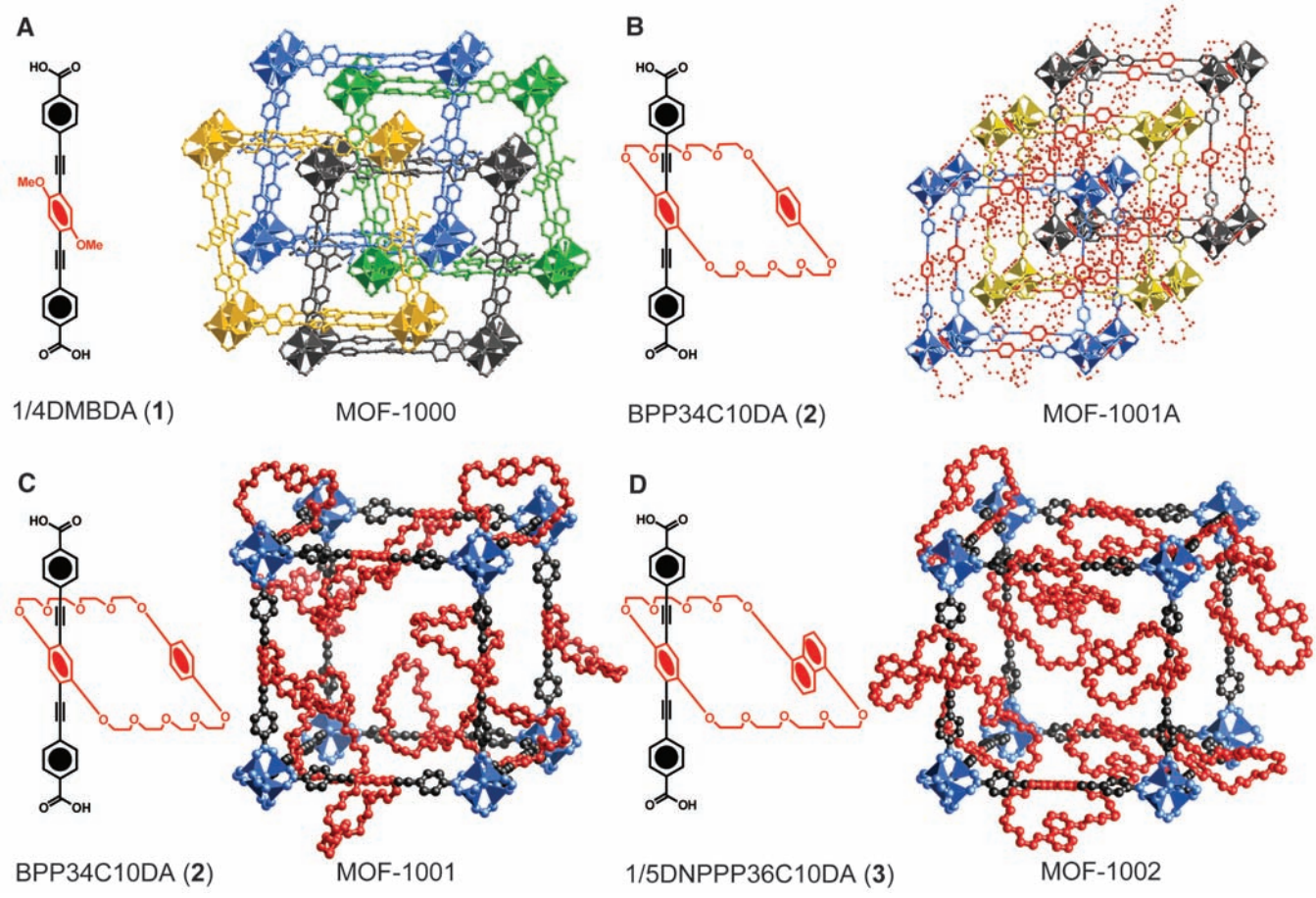

Fig. 2. Ball-and-stick drawings of single-crystal structures of MOF-1000, MOF-1001A, MOF-1001, MOF-1002, and their corresponding organic struts. Strut **1** was used to obtain MOF-1000 (**A**), which has a four-fold interpenetrating structure with different frameworks shown in four different colors. The crystal structure of MOF-1001A from strut **2** (**B**) is a triply interpenetrating cubic structure (shown in blue, gold, and gray), with polyethers represented by red balls and wires. (**C** and **D**) MOF-1001 from strut **2** (C) and MOF-1002 from strut **3** (D) share an identical cubic framework backbone, and crown ethers are placed precisely throughout the whole framework [$Zn_4O(CO_2)_6$ polyhedra, blue; organic struts, gray; crown ethers, red]. Crown ethers in all the structures were modeled by Cerius[2]. All hydrogen atoms have been omitted for clarity.

symmetry, with an exceptionally large unit cell parameter a = 52.93 Å.

We then extended the methodology to the synthesis of MOF-1002 (Fig. 2D and Fig. 3D) by using the 1,5-dioxynaphthalene–containing strut **3,** which was produced by a divergent synthetic route (*14*). Single-crystal x-ray diffraction studies (*14*) indicate that MOF-1002 shares an identical cubic backbone with MOF-1001, affirming the generality of such a methodology for building a variety of crystalline structures with long struts capable of molecular recognition.

Calculations of the volumes of open space within the MOF structures confirmed the highly open nature of these crystals (86.9% space unoccupied by MOF-1001 framework atoms, as assessed by a model using the program Cerius^2, version 4.2). The inherent flexibility of the macrocyclic polyether substructure was evident from the single-crystal x-ray analysis of MOF-1001. The bismethylenedioxy units of the tetraethylene glycol loops in the substructure are found to be highly disordered. Nonetheless, the positions of all the atoms in the inorganic joints and the rigid backbone of the links are unambiguous, as judged by comparison of the resulting bond distances and angles with the model structure (*16*). On the basis of the overall geometry and stoichiometry of the MOF framework, we can conclude that the crown ether receptors—capable of the complexation behavior required (*17*) for molecular recognition—are integrated precisely and periodically inside a robust framework. Thus, the extended framework provides the basis for their strategic placement so that they are exposed to the maximum accessibility to guests in three-dimensional space.

To date, a number of reports (*18*–*20*) have appeared on the synthesis and structure of hybrid organic-inorganic compounds with macrocycles and mechanically interlocking components. The MOFs presented here combine the precise positioning of the active domains with docking as an expression of molecular recognition. This property was revealed by examining the molecular recognition behavior of the macrocyclic polyethers **2** and **3** as docking sites. When MOF-1001 crystals were introduced into a saturated solution of PQT·2PF$_6$ in acetone, the crystals immediately turned red, and the color intensified over 60 min (*21*) (Fig. 4, A to E, and movie S1)—a typical behavior for this binding event that indicates charge-transfer interactions (*22*) between PQT^{2+} and crown ether rings. This observation points to the formation of MOF-1001 pseudorotaxanes (*23*) by threading of PQT^{2+} through the middle of the crown ether. The reversibility of such a process was evidenced by the reappearance of the original light yellow color upon rinsing with acetone, where 60% of PQT^{2+} could be removed after rinsing MOF-1001 pseudorotaxanes (2.8 mg) four times with 1 ml every 30 min (*14*). The complexed MOF-1001 maintained the original high crystallinity of the parent framework, as confirmed by coincident powder x-ray diffraction patterns.

Further evidence of complexation was obtained by examining the ^{1}H nuclear magnetic resonance (NMR) spectrum of the MOF-1001 pseudorotaxanes after dissolution in DCl (*14*). Integration of the peaks appearing at 7.96 ppm (d, 4H, Ar-H^a in **2**, ^3J = 8.5 Hz; Fig. 4F) and 4.60 ppm (s, 6H, N-CH$_3$ in PQT^{2+}) revealed the expected 1:1 ratio of strut **2** and PQT^{2+}, indicating that the docking phenomenon of PQT^{2+} does indeed take place at every crown ether ring throughout the whole MOF framework (fig. S10). Solid-state ^{15}N NMR spectroscopy—a technique that is highly sensitive to the environment of the nitrogen (^{15}N) in PQT^{2+}—provided further evidence for docking in MOF-1001. Isotope-labeled PQT^{2+} (*14*) with 25% abundance of ^{15}N was used to make the MOF-1001 pseudorotaxanes, and the resulting solid was examined by ^{15}N cross-polarization magic angle spinning (CP/MAS) spectroscopy (*24*). The spectrum of the uncomplexed PQT^{2+} has a ^{15}N signal centered on 207.2 ppm, whereas the spectrum of PQT^{2+} bound within the crown ether rings in MOF-1001 shows an upfield shift to 204.6 ppm for the ^{15}N resonance resulting from docking into the macrocyclic polyether units of the struts (Fig. 4G).

Similar studies carried out on strut **2** were used as a molecular analog for comparison with MOF-1001 complexation experiments. Here, addition of PQT·2PF$_6$ to an acetone solution of strut **2** led to the formation of a pseudorotaxane, [PQT⊂**2**]·2PF$_6$. The binding affinity (K_a = 829 M^{-1}) (fig. S2) between PQT^{2+} and strut **2** in solution was obtained from spectrophotometric titrations. Single-crystal x-ray diffraction of the [PQT⊂**2**]·2PF$_6$ (Fig. 4H) clearly shows the insertion of the π-electron–deficient bipyridinium dication through the middle of the macrocyclic polyether. π-π stacking and [C–H···O] interactions are reflected in the interplanar separation of 3.6 Å between the bipyridinium unit of PQT^{2+} and the hydroquinone rings. The same upfield shift trend in the ^{15}N NMR spectra observed for MOF-1001 pseudorotaxanes was also evident in the ^{15}N NMR spectra of [PQT⊂**2**]·2PF$_6$ in the solid state (*14*) (Fig. 4I) as well as in solution (fig. S5). Control experiments were carried out by attempting to introduce PQT·2PF$_6$ into porous MOF-177 crystals (*25*), the pore dimensions (d = 11.8 Å) of which were expected to allow the free movement of PQT^{2+} within the pores. We found that fewer than 0.06 PQT^{2+} molecules per strut of MOF-177 were incorporated in the pores (fig.

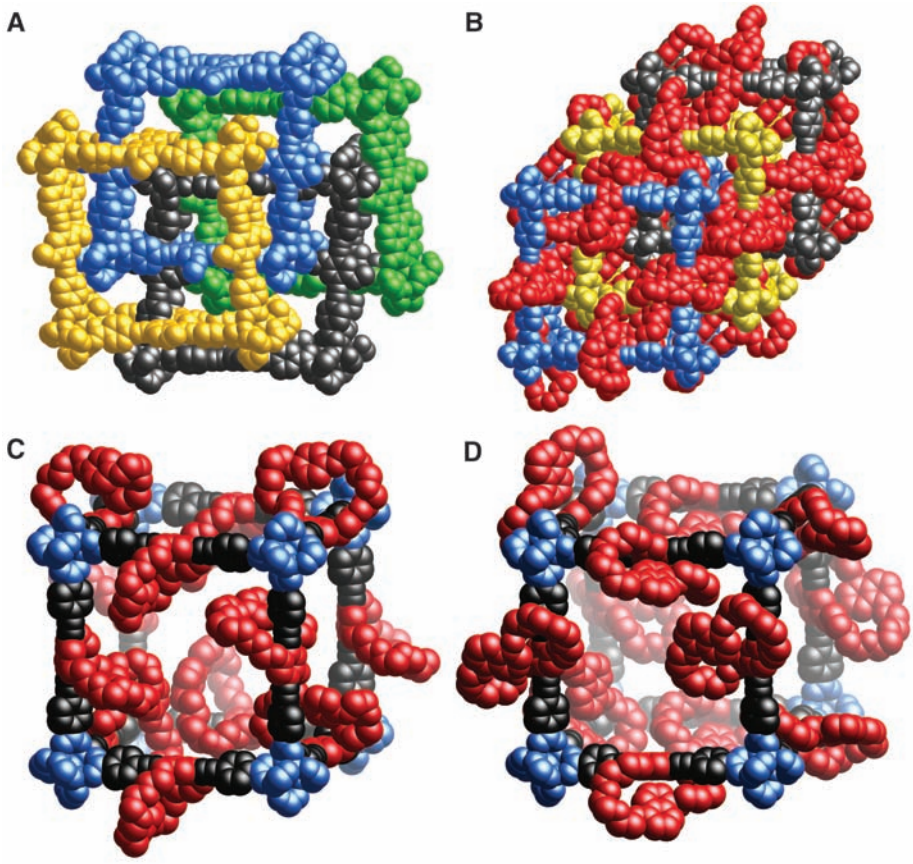

Fig. 3. Space-filling illustration of MOF-1000, MOF-1001A, MOF-1001, and MOF-1002. (**A** and **B**) The length of the struts (19.3 Å) allows the structures of MOF-1000 (A) and MOF-1001A (B) to interpenetrate. (**C** and **D**) In contrast, high volumes of open space were present in the noninterpenetrating MOF-1001 (C) and MOF-1002 (D) synthesized from struts with the same length. This feature ensures the full accessibility of the electron-donating receptors for the incoming substrates within the pores. The same color codes as in Fig. 2 were applied. All hydrogen atoms have been omitted for clarity.

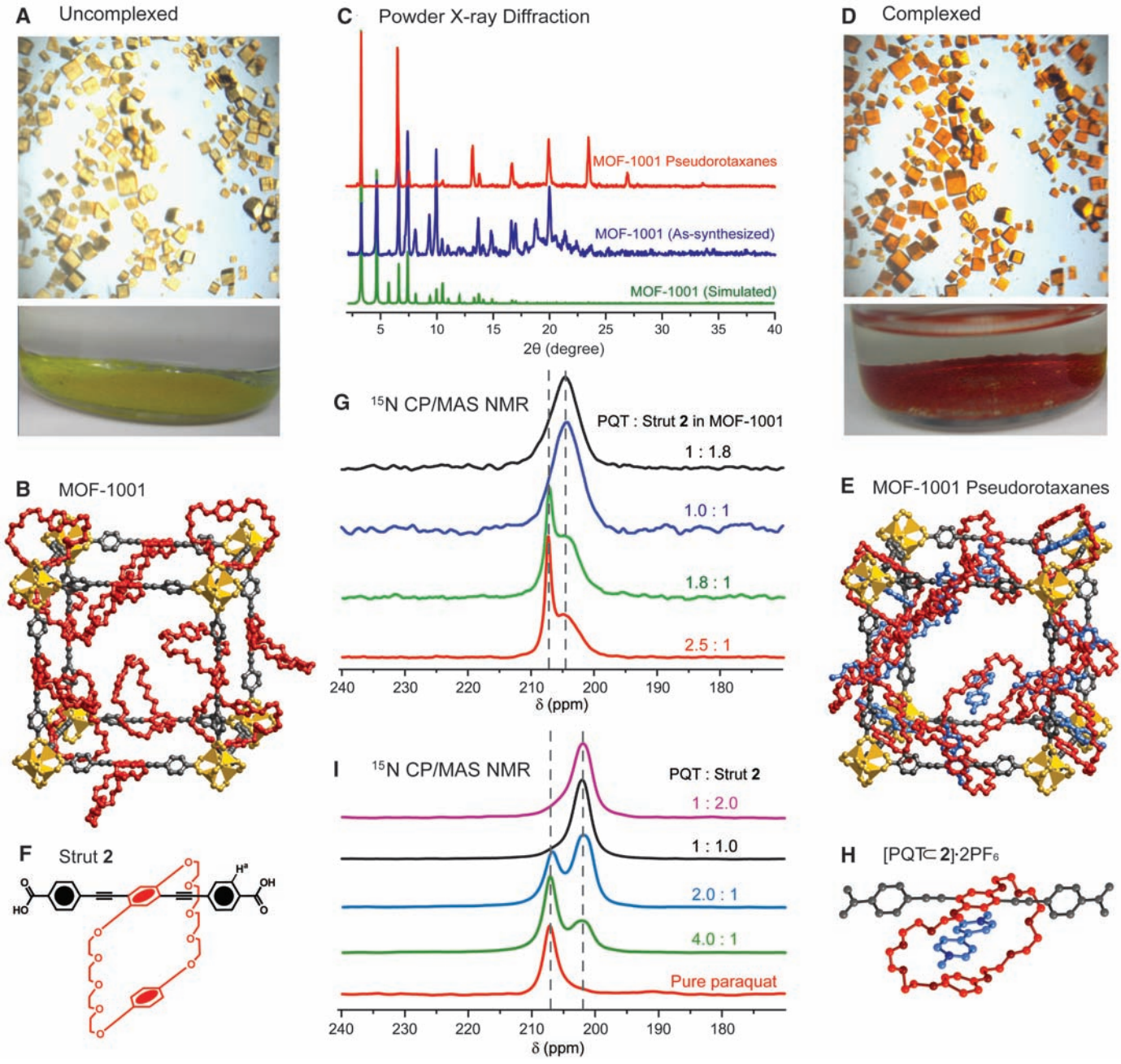

Fig. 4. X-ray diffraction and solid-state NMR spectroscopic studies on MOF-1001, MOF-1001 pseudorotaxanes, and their molecular analogs. (**A** to **C**) MOF-1001 [(A) and (B)] maintained its crystallinity after docking of PQT^{2+}, a single crystal–to–single crystal transformation revealed by the x-ray diffraction pattern (C). Dimensions of the cubic crystals varied from 0.05 to 0.45 mm. (**D** to **F**) This quantitative threading to form MOF-1001 pseudorotaxanes [(D) and (E)] was confirmed by the 1:1 stoichiometry of PQT^{2+} and strut **2** (F). The crystal structure of MOF-1001 and the simulated MOF-1001 pseudorotaxanes structure are illustrated in ball-and-stick models. (**G**) This docking phenomenon resulted in the upfield shifts of the ^{15}N CP/MAS signals (*11*) relative to free PQT^{2+}. (**H** and **I**) The molecular pseudorotaxane analog [PQT⊂**2**]·$2PF_6$ (H) was found to have the same upfield shift trend (I) ($\Delta\delta$ = 4.9 ppm). Color code: $Zn_4O(CO_2)_6$ polyhedra, gold; organic struts, gray; crown ethers, red; PQT^{2+}, blue. All hydrogen atoms and counterions have been omitted for clarity.

S11). These results clearly show that specific stereoelectronic host-guest interactions, rather than simple diffusion and adsorption, are responsible for the all but quantitative formation of the MOF-1001 pseudorotaxanes.

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Supporting Online Material

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Fire As an Engineering Tool of Early Modern Humans

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The controlled use of fire was a breakthrough adaptation in human evolution. It first provided heat and light and later allowed the physical properties of materials to be manipulated for the production of ceramics and metals. The analysis of tools at multiple sites shows that the source stone materials were systematically manipulated with fire to improve their flaking properties. Heat treatment predominates among silcrete tools at ~72 thousand years ago (ka) and appears as early as 164 ka at Pinnacle Point, on the south coast of South Africa. Heat treatment demands a sophisticated knowledge of fire and an elevated cognitive ability and appears at roughly the same time as widespread evidence for symbolic behavior.

There is debate as to when modern human behavior appeared, although there is increasing evidence for symbolic behavior by 80 to 70 thousand years ago (ka) (*1*, *2*) and perhaps earlier (*3*, *4*), during the African Middle Stone Age (MSA, ~280 to 35 ka) (*5*). The MSA also displays tool traits that anticipate technologies occurring later in Eurasia. This includes the regular and sometimes predominant use of blade technology (*4*), the production of unmodified and backed bladelets for probable use in composite tools (*6*), the refinement of bifacial technology, the production of formal and standardized tool types (*7*), and the use of refined bone tools (*8*). Although most raw materials during the MSA came from local nearby sources, early modern humans expanded their use of fine-grained raw materials (exotics) (*4*, *9*) from distant sources (*10*). The Still Bay [~71 to 70 ka (*11*) or earlier (*12*)] and Howiesons Poort (~65 to 60 ka) (*11*) MSA occurrences in South Africa display a preference for fine-grained materials, commonly silcrete [supporting online material (SOM) text] (*13*). The Still Bay occurrence has thin and symmetrical lanceolate and foliate shaped bifacials. The Howiesons Poort occurrence includes small retouched blade tools, along with the prepared-core and flake-and-blade technology typical of the MSA. The focus on silcrete has been argued to reflect functional need (*14*), increased mobility (*9*), trading networks (*15*), and even symbolic behavior (*16*).

Silcrete is traditionally described by archaeologists as a nonlocal fine-grained material that is highly workable in its natural state (*4*, *9*, *17*). However, our experimental replication using silcrete from sources on the south coast near Mossel Bay and Still Bay shows that these silcretes in their raw quarried form are difficult to flake consistently into formal tools. In Australia, indigenous knappers heated silcrete with fire (heat treatment) to improve the flaking quality of the material (*18*). Silcrete responds to heat treatment with significant improvement in workability and has a greater tolerance for high temperatures than do chert and flint (*19*). Following this lead, we found that heated South African silcrete is significantly more workable than unheated materials, and both bladelets and bifaces are easier to flake, with higher success rates. This transformation in workability is remarkably palpable when flaking both heated and unheated silcrete from the same source. Given these results, we undertook a systematic study of silcrete heat treatment and attempted to identify its presence or absence in the MSA.

We collected silcrete samples from sources located within 100 km of Pinnacle Point (*20*) (fig. S1). A witness control sample has been retained at our field laboratory in Mossel Bay, South Africa, for each nodule used in the experimental heat treatment study. Experimental silcrete samples were slowly heated to ~350°C in a scientific furnace or in sand beneath a fire pit (*20*).

The complexities of fracture mechanics make it difficult to quantify the workability of stone in a way that is relevant to human knapping (*21*). For this reason, we applied objective measures of workability and less objective but more realistic systematic flaking experiments. The rebound hardness test (*22*) assesses both the ability of a given rock mass to absorb energy and fracture predictability (SOM text). Rocks with internal flaws or low overall stiffness have lower rebound values (*23*). In these types of stones, the propagation of fracture will follow the internal structure of the rock rather than the direction of applied force. Heat-treated samples had significantly higher rebound hardness values (Wilcoxon matched-pairs test: $z = 2.512$, $P = 0.004$) than their paired untreated samples (fig. S2A and table S1) (*20*).

More carefully crafted bifacial tools have higher width-to-thickness ratios (W/T) (*24*), and variants of the W/T measurement correlate with projectile point function and ballistics (*25*). Timed heat-treated bifacial tool replications conducted by us had significantly higher W/T values [related-samples *t* test: $t(49) = 8.11$, $P < 0.001$] than their paired unheated bifaces (*20*). Using heated silcrete biface blanks, we could produce a significantly thinner biface that maximizes cutting edge, in the same amount of time needed to work the unheated bifaces (fig. S2, B and C). The heated biface samples closely resemble those of actual Still Bay point specimens (fig. S3). The rebound hardness and replication experiments combine to show that heat-treated silcretes consistently display more predictable fracture patterns, allowing

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more efficient production of complex tool forms than do those that are not heat-treated.

Methods for recognizing intentionally heat-treated raw material have only been anecdotally applied to lithic technologies of similar age (SOM text). In some cases, burnt lithics are assumed to be deliberately heat-treated, when they could have been thermally altered unintentionally by bush fires, hearths, and the burning of the organic-rich sediments typical of caves. We suggest that arguments for intentional heat treatment must (i) be documented by at least two independent techniques for recognizing the heating, (ii) be applied to a statistically valid sample, and (iii) be from dated and unburned archaeological contexts where incidental heating is unlikely to have occurred.

We applied three independent methods [archaeomagnetism, thermoluminesence (TL), and maximum gloss (MG)] for recognizing heated silcrete from Pinnacle Point site 5-6 (PP5-6) after developing expectations from our work with experimentally heated and unheated silcrete samples. PP5-6 is one of a series of caves/rock shelters at Pinnacle Point (on the southern coast of South Africa) (*3*). We selected 26 piece-plotted silcrete artifacts for TL and magnetic analysis from five PP5-6 layers dated by optically stimulated luminescence dating to between ~47 and ~72 ka (Fig. 1 and table S2) from contexts showing no evidence for in situ burning as documented by field observations, micromorphology, and magnetic susceptibility of the sediments. Sample size was limited because the TL and magnetic analyses are destructive. An additional Still Bay biface from Blombos Sands was also analyzed (fig. S4). Nondestructive gloss analysis was performed on a larger sample (n = 153) of artifacts (table S3).

Our archaeomagnetic analyses included both mineral magnetic and palaeomagnetic approaches (*20*, *26*). Mineral magnetic analysis identifies a consistent mineralogy for burnt versus unburnt sediment and lithic samples, because heating generally tranforms weaker magnetic minerals to stronger magnetic mineral phases at temperatures below ~600°C. Mineral magnetic analysis was conducted on all the deposits excavated from the site (SOM text). It indicates that the deposits from which the stone tools were recovered were not combustion features and do not contain any significant amount of burnt sediment (Fig. 1). As such, the stone tools are unlikely to have been burnt accidentally in the locations from which they were recovered.

Palaeomagnetic analysis identifies directional components of the natural remanent magnetization caused by different geological and anthropogenic factors (SOM text). An unheated rock will retain a geological remanence formed either during deposition, precipitation, or secondary chemical alteration. Heated rocks acquire a thermoremanent magnetization (TRM) if heated to above the Curie point of the remanence-carrying minerals (575°C for magnetite) (Fig. 2A) or a partial TRM (pTRM) if heated to below the Curie point. The thermomagnetic history of the rock can be identified through stepwise incremental heating in the laboratory. The paleomagnetic vector method shows that all of the tested archaeological samples from PP5-6 (n = 12; some were too small for analysis) and the Still Bay biface from Blombos Sands (Fig. 2B) had been heated. Most samples had a single heating component with maximum temperatures between 300° to 400°C (table S4). This is a typical temperature range for anthropogenically burnt rocks and hearth stones from modern and South African prehistoric sites (*26*).

Electrons trapped in a mineral (such as quartz) under natural or artificial irradiation can be released when the mineral is heated (*27*), producing TL, whose intensity is related to the amount of trapped electrons (*20*). If the heating is high enough (for example, to 450°C for a few seconds), all the trapped electrons are released and the TL signal is zeroed. The TL signal grows again with the amount of applied irradiation. For geological samples, natural irradiation occurred for long enough to fully saturate the sample, as is the case with the unheated experimental samples (Fig. 2C). For the heated samples, the 0 dose signal is at the background level, and the 75- and 150-Gy dose signals increase with the applied dose. Our TL analyses of the archaeological samples confirm the findings from mineral magnetics and show that all 26 samples were heated (Fig. 2D).

An increased reflectance [measured by a Novo Curve Glossmeter as gloss units (GU)] of the flaked surface is an indication of heat treatment in silcretes. This change is only visible on surfaces that are flaked after heat treatment (Fig. 3A). The flaked surfaces of heated experimental samples have significantly higher MG units than do those of unheated control samples [related-samples t test: $t(49) = 14.71$ $P < 0.001$] (*20*). Thus, a higher degree of gloss on archaeological materials documents that heat treatment occurred before tool production (SOM text). A sample of 153 silcrete lithics from PP5-6 and nearby PP13B was analyzed for gloss (table S3). The PP5-6 MG histograms for the SADBS (~71 to 72 ka) (fig. S5D) and DBCS (~60 to 65 ka) (Fig. 3) stratigraphic aggregates closely resemble that of the heated and flaked experimental samples, indicating that the majority of these archaeological samples exhibit gloss consistent with flaking after heat treatment. The sample histograms from the LBSR aggregate at PP5-6 (~79 to 86 ka) (fig. S5E) and LCMSA aggregate of PP13B (~164 ka) (Fig. 3) fall between the unheated and heated experimental data sets but do contain specimens with MG values above the experimental threshold for unheated

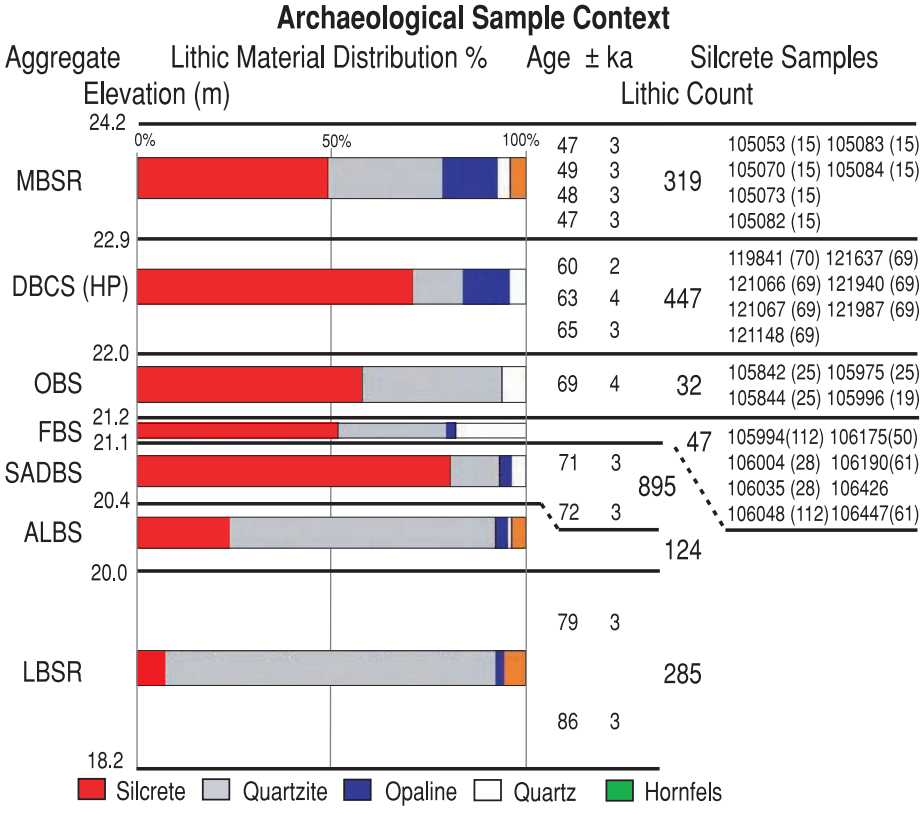

Fig. 1. Age (in thousands of years) and stratigraphic context for archaeological heat-treated samples from PP5-6. Raw material bar graphs are provided for each stratigraphic aggregate (see SOM text for complete description of PP5-6 stratigraphy) to show change in lithic raw materials used through time. Elevation is in meters above mean sea level, and age was determined by Optically Stimulated Luminescence dating (SOM text). Lithic count is the number of piece-plotted artifacts cataloged to date. Magnetic susceptibility values in parentheses after the sample specimen numbers provide an estimate of the potential for incidental burning (SOM text).

silcrete, indicating that some artifacts from these older layers were flaked from heated silcrete (Fig. 3 and fig. S5).

Heat treatment provides the option of exploiting more-local but poorer raw materials and compensating by improving their quality (*28*). Heat treatment front-loads tool production costs by forcing the toolmaker to invest in fire production in order to improve the subsequent knapping process. This practice may only become economically advantageous when fuel is abundant or when social conditions restrict access to preferred raw materials.

The controlled use of fire was a breakthrough invention that allowed cooking, the production of warmth and light, and protection from predators (*29*). Evidence of cooking extends back to 790 ka (*30*), and eventually fire was used for more complex technologies such as firing clay for ceramics and heating ores for metallurgy. However, the technological links between using fire for simple tasks of light and heat production and using it as an engineering tool to alter raw materials remain poorly documented and understood. Heat treatment and its requirements signal an important technological advance, in that fire was now being carefully manipulated as an engineering tool.

Starting around ~71 ka in South Africa, there is recurrent evidence for early symbolic behavior and complex technologies that predate their occurrence outside Africa. Our results at PP5-6 show that at this same time, early modern humans regularly employed pyrotechnology to increase the quality and efficiency of their stone tool manufacture process. This technology required in these early humans a novel association between fire, its heat, and a structural change in stone with consequent flaking benefits that may signal a complex cognition. Our gloss analysis of the PP13B lithics suggests that this technology may have originated by 164 ka. Heat-treatment technology in Africa may explain the presence of advanced tools in the African MSA and their rarity in the Eurasian Middle Paleolithic, where Neanderthals predominated. As these early modern humans moved into Eurasia, the ability to alter and improve available raw materials and increase the quality and efficiency of stone tool manufacture may have been a behavioral advantage.

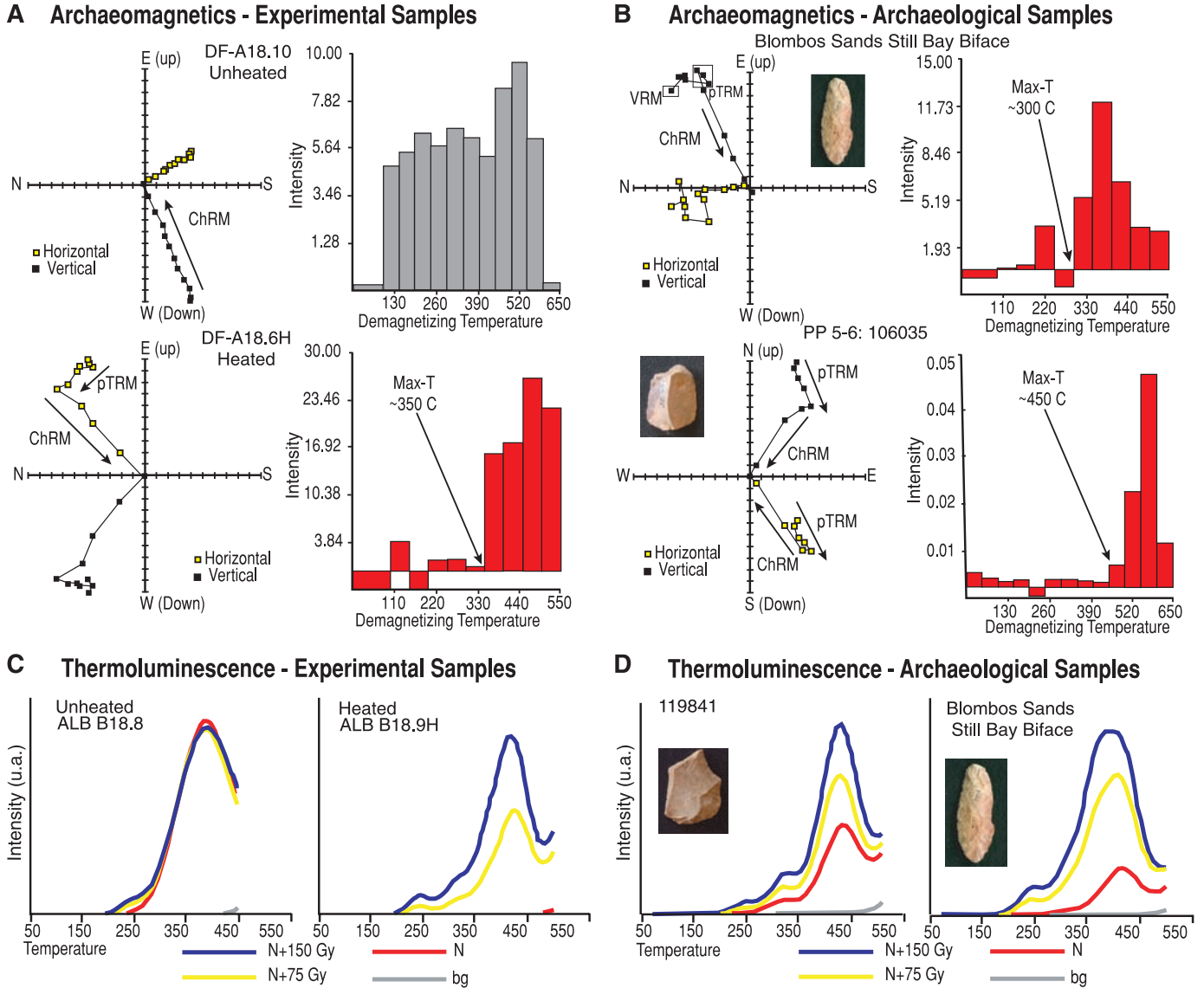

Fig. 2. Archaeomagnetic and thermoluminescence graphs of heated and unheated stone from experimental and archaeological samples. (**A**) The unheated specimen (top) shows a weak single-component geological remanence (ChRM). The heated experimental sample (bottom) shows a two-component signal that includes the remanent ChRM and pTRM from the heating process. The maximum temperature of heating has been reached at ~300°C in this sample. (**B**) The Still Bay point (top) and Howiesons Poort flake (bottom) samples show a multicomponent signal, with the pTRM from the heating event being removed at ~350° and 450°C, respectively. (**C**) TL has not been zeroed on the untreated experimental sample (left) and is fully saturated (no increase of the signal with dose). The TL signal increased with the given dose on the heated experimental sample (right). (**D**) The archaeological samples resemble the unsaturated, heated experimental samples, because the TL signal still increases with the given dose.

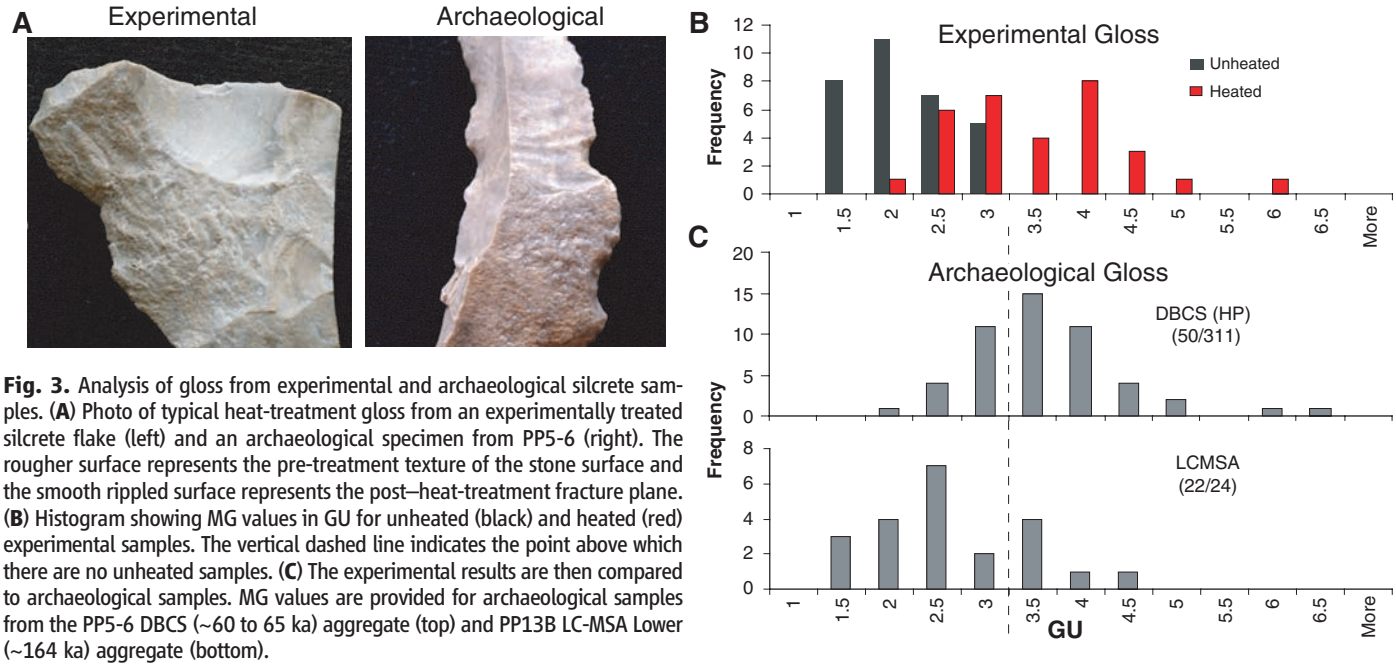

Fig. 3. Analysis of gloss from experimental and archaeological silcrete samples. (**A**) Photo of typical heat-treatment gloss from an experimentally treated silcrete flake (left) and an archaeological specimen from PP5-6 (right). The rougher surface represents the pre-treatment texture of the stone surface and the smooth rippled surface represents the post–heat-treatment fracture plane. (**B**) Histogram showing MG values in GU for unheated (black) and heated (red) experimental samples. The vertical dashed line indicates the point above which there are no unheated samples. (**C**) The experimental results are then compared to archaeological samples. MG values are provided for archaeological samples from the PP5-6 DBCS (~60 to 65 ka) aggregate (top) and PP13B LC-MSA Lower (~164 ka) aggregate (bottom).

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Mesotocin and Nonapeptide Receptors Promote Estrildid Flocking Behavior

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Proximate neural mechanisms that influence preferences for groups of a given size are almost wholly unknown. In the highly gregarious zebra finch (Estrildidae: *Taeniopygia guttata*), blockade of nonapeptide receptors by an oxytocin (OT) antagonist significantly reduced time spent with large groups and familiar social partners independent of time spent in social contact. Opposing effects were produced by central infusions of mesotocin (MT, avian homolog of OT). Most drug effects appeared to be female-specific. Across five estrildid finch species, species-typical group size correlates with nonapeptide receptor distributions in the lateral septum, and sociality in female zebra finches was reduced by OT antagonist infusions into the septum but not a control area. We propose that titration of sociality by MT represents a phylogenetically deep framework for the evolution of OT's female-specific roles in pair bonding and maternal functions.

Sociality, as defined by modal species-typical group size, is a core component of social organization that strongly affects reproductive behavior, disease transmission, resource exploitation, and defense (*1*, *2*). However, the neural mechanisms that titrate sociality and regulate the preference to live as singletons, in large groups, or somewhere in between are largely unknown. This likely reflects limited tractability, partly because the space requirements of large species-typical group sizes may be difficult to accommodate in experimental settings and, more importantly, because the behavioral dimension of sociality is difficult to isolate in comparative studies. For instance, because rodent species that differ in sociality also tend to differ in mating system, patterns of parental care, and other aspects of behavior and ecology that can influence neural and endocrine mechanisms associated with sociality (*3*, *4*), comparative studies are not

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informative with respect to group size. Even in songbirds, which are speciose and socially diverse, only few opportunities exist for controlled comparisons, most notably in the family Estrildidae (finches, waxbills, and munias; about 132 species total). All estrildid species are monogamous, exhibit long-term or life-long pair bonds, and show biparental care. However, even within specific ecological niches, estrildids display an extraordinary diversity in sociality, ranging from territorial male-female pairs to groups containing dozens or hundreds of colonially breeding pairs (*5*). This family includes the zebra finch (*Taeniopygia guttata*), a socially tractable laboratory songbird (*6*).

In mammals, the neuropeptides arginine vasopressin (VP) and oxytocin (OT) influence numerous behavioral processes, including parental care, anxiety, and monogamous pair bonding (*7*–*11*). Behavior may also be influenced in a sex-specific manner [e.g., pair bonding (*12*)] such that VP is required for a given behavior in males, but in females the same behavior is dependent on OT. It has been postulated that separate nonapeptide clades giving rise to VP and OT originated because of a duplication of the arginine vasotocin (VT) gene in fish (*13*) and that sex-specific and affiliation-related nonapeptide functions date back to this duplication (*14*–*16*).

The major OT and VP cell groups in the mammalian brain are found in the preoptic area and hypothalamus, and these populations are strongly conserved across the vertebrate classes, as are their central and hypophyseal projections (*3*, *17*). In amniotes, OT-like peptide neurons are largely restricted to the hypothalamus, whereas homologous cell groups lie within the preoptic area of anamniotes (*3*). VT and VP cell groups are found in these same areas, although tetrapods also produce VT and VP in numerous extrahypothalamic parts of the brain, including the medial extended amygdala (bed nucleus of the stria terminalis, BSTm) (*3*). Most vertebrates (including birds) have multiple VT and VP receptor subtypes but only a single OT-like receptor (*18*).

Given that OT promotes bonding in mammals (*7*), we conducted experiments to test the hypothesis that endogenous MT promotes preferences for familiar conspecifics in birds (*19*). Zebra finches were housed in same-sex groups of six birds and individually tested for novel-familiar preferences by recording the amount of time subjects spent in close proximity to five familiar cagemates versus five novel same-sex conspecifics (see diagram in Fig. 1A). We established behavioral effects by using peripheral injections of the selective OT receptor antagonist desGly–NH_2,d$(CH_2)_5$[Tyr(Me)^2, Thr^4] ornithine vasotocin [OTA; 5 μg subcutaneously (*20*)] or saline, and, on the basis of the positive results of this experiment, we fitted finches with chronic guide cannulae and replicated the experiment by using intracerebroventricular infusions of OTA [250 ng; OTA and peptide dosages were used on the basis of previous experiments (*15*, *21*)]. All tests were conducted by using a within-subjects design, and tests were spaced 2 days apart.

As predicted, peripheral administration of OTA significantly reduced the percent of time that subjects spent in close proximity to familiar cagemates (Fig. 2A). This effect is most pronounced in females, although significance is achieved only for the main effect of treatment [$F_{1,1,21} = 3.833$, $P < 0.05$, repeated-measures analysis of variance (ANOVA)]. Central OTA infusions exerted a similar and significantly sex-specific effect (Fig. 2B; sex by treatment $F_{1,1,20} = 4.019$, $P < 0.05$). In contrast, central administrations of 50 ng MT but not VT tended to increase the time spent with familiar cagemates (Fig. 2C; main effect of treatment $F_{1,2,20} = 3.900$, $P < 0.05$), especially for females, although the main effect of treatment in this experiment appears to come from the difference between VT and MT. Despite weak trends, no treatment effects were observed in any of these experiments for the percent of time spent with novel conspecifics (fig. S1).

We tested the hypothesis that endogenous MT promotes sociality by using the same apparatus as above, but, rather than being exposed to novel and familiar conspecifics, subjects were exposed to a group of two same-sex conspecifics at one end of the testing cage and a group of 10 same-sex conspecifics at the other end (as in Fig. 1B). In the first two experiments, subjects received OTA or saline subcutaneously and were exposed to groups of either novel conspecifics (Fig. 3A) or familiar conspecifics (Fig. 3B). For this latter experiment, stimulus animals were housed in a cage immediately adjacent to the subject's cage for a minimum of 5 days before testing. Significant sex by treatment interactions were obtained in both experiments, with OTA reducing the percent of time that female subjects spent in close proximity to the large group ($F_{1,\ 1,\ 21} = 8.426$, $P < 0.01$ and $F_{1,1,22} = 4.871$, $P < 0.05$, respectively, repeated-measures

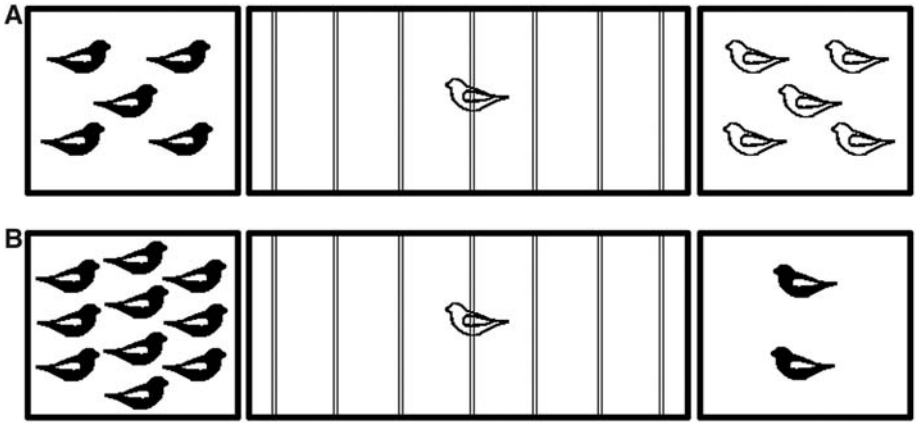

Fig. 1. Choice apparatus design. A 1-m-wide testing cage was subdivided into zones by seven perches (indicated by thin lines). Subjects were considered to be within close proximity when they were within 6 cm of a stimulus cage (corresponding to the perches closest to the sides). For novel-familiar choice tests (**A**), the two stimulus cages contained either five familiar cagemates or five unfamiliar individuals (all of the same sex). For sociality tests (**B**), the stimulus cages contained either 2 or 10 same-sex conspecifics. Sides were counterbalanced across subjects. Tests were 5 min, and subject location was recorded at 15-s intervals.

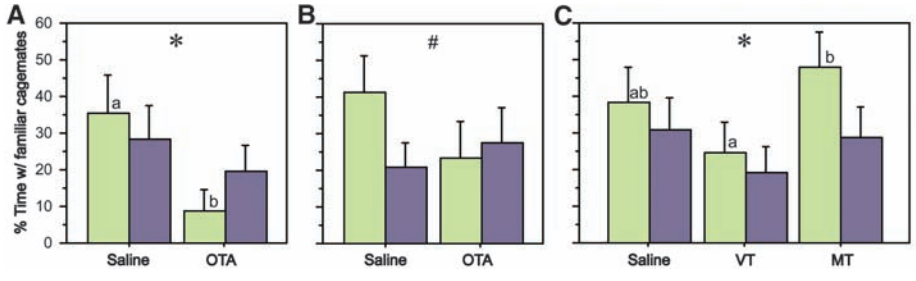

Fig. 2. Zebra finches were tested in a novel-familiar choice paradigm (as in Fig. 1A) after administrations of OTA and nonapeptides. Females and males are shown in green and purple, respectively. (**A**) OTA delivered subcutaneously significantly reduced time spent in close proximity to familiar same-sex cagemates (*$P < 0.05$, main effect of treatment; $n = 12$ males, 11 females). Significant pairwise comparisons within a sex are indicated by different letters above the bars. Data plotted are mean ± SE. (**B**) A similar result is observed after intracerebroventricular infusions of OTA, although a significant sex by treatment interaction is obtained (single pound symbol indicates $P < 0.05$; $n = 11$ males, 11 females). (**C**) Central infusions of MT and VT differentially influence time spent with familiar cagemates (*$P < 0.05$, main effect of treatment; $n = 11$ males, 11 females). Corresponding data for time spent in close proximity to novel conspecifics is shown in fig. S1.

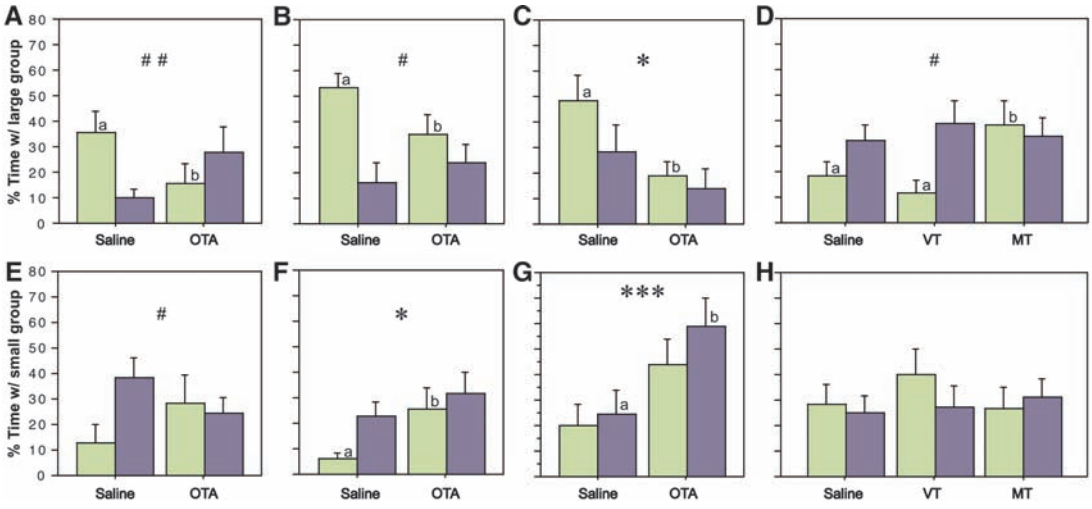

Fig. 3. Zebra finches were tested in a group-size choice paradigm (as in Fig. 1B) after administrations of OTA and nonapeptides. The percent of time spent in close proximity to the large group is shown (**A** to **D**). Corresponding data for time spent in close proximity to the small group are shown directly below (**E** to **H**). Data plotted are mean ± SE. Females and males are shown in green and purple, respectively. Significant pairwise comparisons within a sex are indicated by different letters above the bars. (A) OTA delivered subcutaneously significantly reduces the percent of time spent in close proximity to the large group in sociality tests with unfamiliar social partners but only in females. (E) This pattern is reversed for time spent in close proximity to the small group (single pound symbol, $P < 0.05$; double pound symbol, $P < 0.01$; sex by treatment interaction; $n = 12$ males, 11 females). (B and F) Similar results are obtained with familiar social partners after subcutaneous delivery of OTA (single pound symbol, $P < 0.05$, sex by treatment interaction; $^*P < 0.05$, main effect of treatment; $n = 12$ males, 12 females). (C and G) Central OTA infusions decrease time in close proximity to the large group and increase time in close proximity to the small group (tests conducted with unfamiliar social partners; $^*P < 0.05$, $^{***}P < 0.001$, main effect of treatment; $n = 12$ males, 12 females). (D) Central infusions of MT but not VT produce a female-specific increase in the percent of time in close proximity to the large group (tests conducted with unfamiliar social partners; single pound symbol, $P < 0.05$, sex by treatment interaction; $n = 17$ males, 16 females).

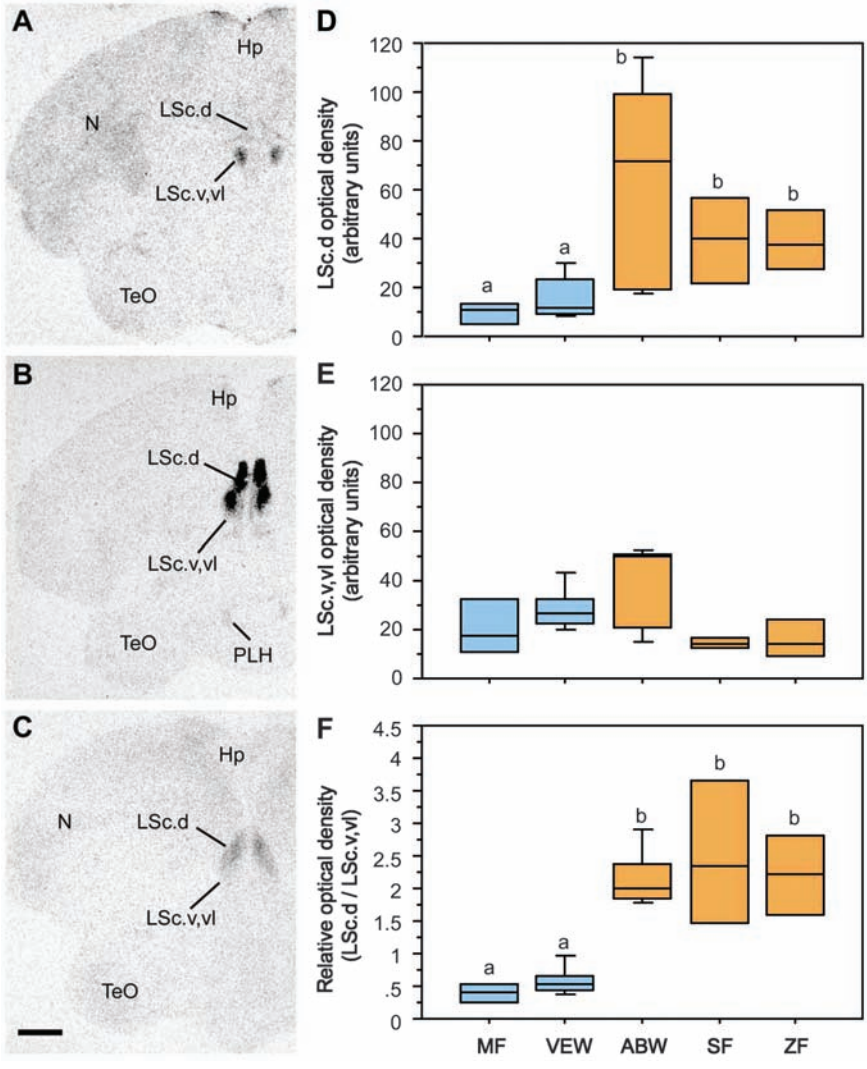

Fig. 4. [I^{125}]-OVTA binding sites in the LS differentiate territorial and flocking finch species. Hp indicates hippocampus; LSc.d, dorsal zone of the caudal lateral septum; LSc.v,vl, ventral and ventrolateral zones of the caudal lateral septum; N, nidopallium; PLH, posterolateral hypothalamus; and TeO, optic tectum. (**A** to **C**) Representative autoradiograms of binding sites in two sympatric congeners, the territorial violet-eared waxbill (A) and the gregarious Angolan blue waxbill (B) plus the highly gregarious zebra finch (C). (**D**) Densities of [I^{125}]-OVTA binding sites in the dorsal (pallial) LSc of two territorial species (melba finch, MF, and violet-eared waxbill, VEW), a moderately gregarious species (Angolan blue waxbill, ABW), and two highly gregarious species (spice finch, SF, and zebra finch, ZF). Territorial and flocking species are shown in blue and orange, respectively. No sex differences are observed and sexes were pooled. Total $n = 23$ (SOM text). Different letters above the boxes denote significant species differences (Mann-Whitney $P < 0.05$) following significant Kruskal-Wallis. Box plots show the median (line), 75th and 25th percentiles (box), and 95% confidence interval (error bars). (**E**) Binding densities tend to reverse in the subpallial LSc (Kruskal-Wallis $P = 0.06$), suggesting that species differences in sociality are most closely associated with the relative densities of binding sites along a dorso-ventral gradient, as confirmed in (**F**).

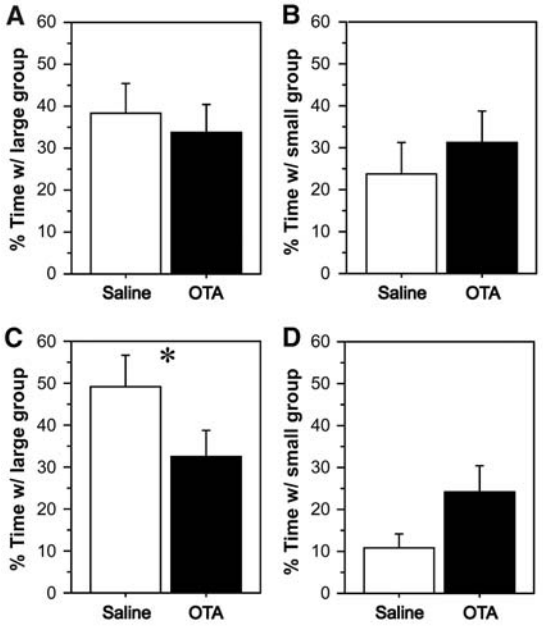

Fig. 5. Female zebra finches were tested in a group size choice paradigm (as in Fig. 1B) after administrations of OTA or saline into the LS or a telencephalic control region adjacent to the lateral ventricle (medial striatum). Data plotted are mean ± SE. (**A** and **B**) OTA infusions into the telencephalic control region produced no significant effect on time spent with the large group (A) or the small group (B). (**C** and **D**) OTA infusions into the LS significantly reduced time with the large group [*P < 0.05 (C)] and tended to increase time with the small group [P = 0.08 (D)].

ANOVA). Central infusions of OTA produced similar results (with novel conspecifics), although only a main effect of treatment was observed (Fig. 3C; $F_{1,1,22} = 6.704$, $P < 0.05$). Lastly, VT and MT were administered intracerebroventricularly, and subjects were exposed to novel same-sex conspecifics. As predicted by the OTA findings, a significant sex by treatment interaction was obtained, resulting from a female-specific increase in time with the large group after infusions of MT (Fig. 3D; $F_{1,2,31} = 3.021$, $P < 0.05$). Across these four experiments, time with the small group tended to be inversely related to time with the large group (Fig. 3, E to H).

These findings demonstrate that nonapeptide receptors influence group-size choices in zebra finches and suggest the hypothesis that evolution in sociality is mediated at least in part by selection on the distribution of these receptors. In order to test this hypothesis, we quantified binding of an [I^{125}]-labeled ornithine vasotocin analog (OVTA) in tissue sections from five species of estrildid finches. Two of these species are highly gregarious and breed colonially (zebra finch and spice finch, *Lonchura punctulata*), two are highly territorial and live as male-female pairs year-round (melba finch, *Pytilia melba*, and violet-eared waxbill, *Uraeginthus granatina*), and one is moderately gregarious (Angolan blue waxbill, *U. angolensis*). Other than sociality, these species are generally similar in other aspects of behavior and ecology (*3*–*5*, *22*).

We observed binding sites for [I^{125}]-OVTA in numerous forebrain areas, including the medial striatum, mediobasal hypothalamus, and lateral septum (LS), consistent with other passerines (*23*). Of these, the LS is of particular interest in relation to sociality (*3*), and this is the only area where binding densities correlated with differences in sociality across species. All three of the gregarious (i.e., flocking) species exhibited significantly higher binding densities in the dorsal (pallial) zone of the caudal LS (LSc) than did the two territorial species (Fig. 4, A to D; Kruskal-Wallis $H = 13.682$, $P < 0.01$), but this direction of species differences tends to reverse within the ventral and ventrolateral (subpallial) zones of the LSc (Fig. 4E; $H = 8.994$, $P = 0.06$), suggesting that the relative concentrations of binding sites along the dorso-ventral axis of the LSc may be important determinants of sociality. Indeed, flocking and territorial species were most clearly differentiated when dorsal and ventral binding densities were expressed as a difference measure [supporting online material (SOM) text] or as a ratio (dorsal/ventral; Fig. 4F; $H = 16.648$, $P < 0.01$). No sex differences were observed (all $P > 0.5$).

In order to directly demonstrate that OT-like binding sites in the LS influence sociality, we fitted female zebra finches with guide cannulae directed at the LS or a telencephalic control area adjacent to the lateral ventricle (medial striatum) and conducted sociality tests after infusions of OTA or saline, as described above. Infusions into the telencephalic control area produced no significant effects (Fig. 5, A and B). However, as predicted, intraseptal administration of OTA significantly reduced the amount of time that subjects spent in close proximity to the large group (Fig. 5C; $P < 0.05$, paired t test) and tended to increase the time spent with the small group (Fig. 5D; $P = 0.08$).

Previous experiments (immediate early gene, lesion, and peptide binding studies) (*3*) likewise suggest that the LS is a focal point of neural processes that titrate sociality, and species differences in sociality are strongly and positively related to the density of binding sites for a VP V_{1A} receptor agonist throughout the LS complex (*4*). VT, VP, and V_{1a}-like receptors play important roles in stress, aggression, and affiliation behaviors across vertebrate taxa (*3*, *10*), and the VT cells of the BSTm (which project heavily to the LS) increase their immediate early gene activity selectively in response to affiliation-related social stimuli (*24*). The lack of VT effects observed here is therefore surprising. However, it is important to note that VT neurons in the BSTm are functionally opposed to those in the paraventricular nucleus of the hypothalamus (PVN) (*3*), and thus VT effects are apt to depend on specific patterns of release across brain areas, which are not readily mimicked by intracerebroventricular infusions.

OT release in mammals reduces anxiety (*25*) and promotes social recognition (*26*, *27*), monogamous partner preferences (*12*, *28*), trust (*29*), and maternal behaviors (*7*–*11*), including maternal aggression (*30*). Limited behavioral functions of OT homologs have also been established for teleost fish (*15*, *16*), but comparable data have been wholly absent for MT. The present findings suggest that affiliative functions are conserved for all of the OT-like nonapeptides, although total affiliation time (time at both ends of the test cage combined) was not significantly affected by treatments in any of our seven behavioral experiments (all $P > 0.10$). Rather, treatments selectively influenced social preferences, and overall our findings suggest that the activation of nonapeptide receptors by endogenous MT promotes sociality (preferences for larger groups) and the preference for familiar social partners. Thus, the combined findings that isotocin modulates social communication and approach in fishes (*15*, *16*) and that MT promotes sociality in birds (present data) strongly suggest that OT-like peptides have influenced social groupings for most of vertebrate history.

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Materials and Methods
SOM Text
Fig. S1
References

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The Transcriptional Repressor DEC2 Regulates Sleep Length in Mammals

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Sleep deprivation can impair human health and performance. Habitual total sleep time and homeostatic sleep response to sleep deprivation are quantitative traits in humans. Genetic loci for these traits have been identified in model organisms, but none of these potential animal models have a corresponding human genotype and phenotype. We have identified a mutation in a transcriptional repressor (hDEC2-P385R) that is associated with a human short sleep phenotype. Activity profiles and sleep recordings of transgenic mice carrying this mutation showed increased vigilance time and less sleep time than control mice in a zeitgeber time– and sleep deprivation–dependent manner. These mice represent a model of human sleep homeostasis that provides an opportunity to probe the effect of sleep on human physical and mental health.

Although sleep is an essential process for life, the brain circuits regulating sleep and the cellular and/or molecular mechanisms involved in this complex process are still enigmatic (*1*–*3*). Sleep or a "sleeplike" behavior is present in virtually every animal species where it has been studied. Total sleep deprivation can be fatal, and partial deprivation of sleep has serious consequences on cognition, mood, and health (*4*–*6*). It is obvious that situational increases in behavioral drive can transiently delay sleep, but very little is known about chronic partial sleep curtailment as a possible consequence of a persistent elevation in waking behavioral drive. The latter trait, sometimes referred to as a "hyperthymic" temperament (*7*), is a theoretical third influence on sleep habits.

Murine Dec2 (mDec2) is a negative component of the circadian clock (*8*–*10*). It belongs to a basic helix-loop-helix (bHLH) protein family in which members can dimerize with each other and can affect gene transcription by binding to specific DNA sequences (*11*). While performing candidate gene resequencing in DNAs from human families, segregating alleles for extremely early wake up times, we identified an h*DEC2* point mutation in a small family with two affected individuals (Fig. 1A) (*12*). Subjects carrying this mutation had lifelong shorter daily sleep times than normal individuals (Table 1). The self-reported nonworkday habitual sleep-offset times of the mutation carriers were much earlier than those of the noncarriers (including noncarrier family members and general controls). However, these two individuals have sleep-onset times that are similar to that of conventional sleepers. The habitual self-reported total sleep time per 24-hour day was much shorter in mutation carriers (average 6.25 hours) compared with the noncarriers (average 8.06 hours) in this family. Thus, they represent "natural short sleepers" who routinely sleep less than individuals with familial advanced sleep-phase syndrome (FASPS) or general controls (Table 1). The average total sleep time for American adults on nonworkdays is ~7.4 hours (www.sleepfoundation.org). The mutation changes a C to G in the DNA sequence of *DEC2*, which is predicted to cause a proline-to-arginine alteration at amino acid position 385 of DEC2 (Fig. 1B). This change was not found in over 250 control DNA samples. The proline at position 385 of DEC2 (P385) is conserved in mammals but not invertebrates. P385 is located in a highly conserved region within a proline-rich domain of unknown function and is close to the C-terminal histone deacetylase (HDAC)–interacting region of DEC2 (Fig. 1B). Activity-rest recording in one mutation carrier using 10-day sleep logs with coincident wrist actigraphy demonstrated an extended active period each day (Fig. 1C).

To examine the effect of the P385R mutation on Dec2 repressor activity, a wild-type (WT) or a P385R mDec2 construct was used in a luciferase assay, and the results showed that P385R attenuated Dec2 repressive activity of Clk/Bmal1-mediated transactivation (fig. S1A). The reduction in Dec2 repressive activity was moderate compared with that of the R57A/K mutations (in which arginine 57 was replaced by alanine or lysine) reported before (*13*). Dec2 was previously shown to preferentially bind to class B E-box elements (CACGTG) as a homodimer and to repress the transcription of target genes in an HDAC-dependent manner (*13*). The effect of HDAC on the mutant Dec2 repression was then analyzed by monitoring m*Per2* promoter-driven luciferase activity with or without a general HDAC inhibitor trichostatin A (TSA) (fig. S1B). HDAC inhibition resulted in similar increases in luciferase activity for both WT and mutant Dec2. Coimmunoprecipitation was then performed for mDec2 (WT or P385R) and human sirtuin-1 (hSIRT1). HEK293 cells were transiently cotransfected with green fluorescent protein (GFP)–tagged (WT or mutated) m*Dec2* and FLAG-tagged h*SIRT1*, followed by FLAG-peptide pull-down and detection of GFP with antibodies on Western blots. The results showed similar physical interactions between WT or P385R mDec2 and hSIRT1 (fig. S1C). Taken together, these results suggest that the P385R mutation affects Dec2 transcriptional repression activity independently from its interaction with HDAC/SIRT.

Because there are only two human mutation carriers in this study, the question remained whether the natural short sleep phenotype was caused by the *DEC2* mutation. Thus, we generated WT and P385R *DEC2* transgenic (Tg) mice using a human bacterial artificial chromosome (BAC) clone (RP11-288E19) carrying the entire h*DEC2* gene to test this hypothesis. As *DEC2* has been established as a component of circadian clock (*9*, *14*), we first set out to determine the circadian period (τ) of *DEC2-P385R* mice. Mice with *Dec2* deleted [knockout (KO) mice] (*10*) and WT littermates were tested in parallel as controls. No significant differences in τ were detected among mice of different genotypes (table S1).

Because the mutation was identified in human short sleepers who, presumably, have cor-

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respondingly longer total daily activity periods, we next determined the duration of the activity period (α) for these mice. *DEC2-P385R* mice retained the WT pattern of rest and activity (running primarily during the dark phase). However, α was ~1.2 hours longer for *DEC2*-mutant transgenic mice (Fig. 2A) than for wild-type mice, *DEC2-WT* Tg mice, and *Dec2* KO mice, which suggests that the expression of the *DEC2-P385R* allele leads to a dominant increase in the quantity of wakefulness in mice. In agreement with this notion, the α was lengthened further (~2.5 hours) when the endogenous *Dec2* alleles were removed by crossing *DEC2-P385R* mice onto the *Dec2* KO background.

To study sleep directly (versus activity rhythms) and to investigate a possible role for *DEC2* in sleep-quantity regulation, electroencephalography (EEG) and electromyography (EMG) were performed. Because we did not observe a change in α for *DEC2-WT* Tg mice (Fig. 2A) and because human mutation carriers have one normal allele with one mutant allele, we chose to perform EEG and EMG on *DEC2-P385R* mice and their WT littermates. Mice of both genders (female:male/1:1) were included in all EEG studies to exclude the possibility of sex differences noticed in other reports (*15*). *DEC2-P385R* mice were awake (as defined by EEG) ~8% longer than WT mice in the light phase (Fig. 2B, table S2). The short-sleep phenotype of these mice was reflected in the significant shortening of both non–rapid eye movement (NREM) and rapid eye movement (REM) during sleep in the light phase for *DEC2-P385R* when compared with control mice (Fig. 2C and table S2). NREM sleep was ~6% less and REM sleep was ~2% less in *DEC2-P385R* versus WT mice during the light phase. Sleep architecture was further characterized by counting sleep and wakefulness episodes. Over a 12-hour period, *DEC2-P385R* mice showed more episodes of wakefulness than WT mice (193 ± 12 versus 133 ± 10, $P < 0.05$), but the mean duration of each episode was slightly shorter during the light phase (97 ± 10 s versus 116 ± 12, $P < 0.05$) (Fig. 2D and table S3). Consistent with this, *DEC2-P385R* mice also showed more NREM episodes during light periods (190 ± 10 versus 139 ± 9, $P < 0.05$) but each episode was shorter (118 ± 3 s versus 184 ± 5, $P < 0.05$) (Fig. 2E and table S3). REM episodes were similar in abundance (41 ± 5 versus 53 ± 6) and duration (63 ± 3 s versus 64 ± 3) for *DEC2-P385R*

Table 1. Sleep schedule comparison for human subjects. Age refers to when data were collected. Status: C, mutation carrier; NC, nonmutation carrier. Sleep offset is local standard clock time of "average" final morning awakening, and sleep onset is evening time of first falling asleep as stated by individuals recalling extended vacations based on structured interviews. Values are ± SD.

Subjects	Age	Status	Sleep offset	Sleep onset	Sleep length (hour)
44990	69	C	4:00	22:00	6.0
47690	44	C	4:30	22:00	6.5
101174	51	NC	6:00	22:35	7.4
100785	51	NC	7:00	22:45	8.3
100656	44	NC	5:00	21:30	7.5
100654	16	NC	7:45	24:00	7.7
100655	10	NC	7:00	21:35	9.4
FASPS			4:30 ± 1.33	19:45 ± 1.33	8.66 ± 0.80 (n = 16)
Control			6:12 ± 2.45	21:50 ± 1.76	8.37 ± 1.67 (n = 15)

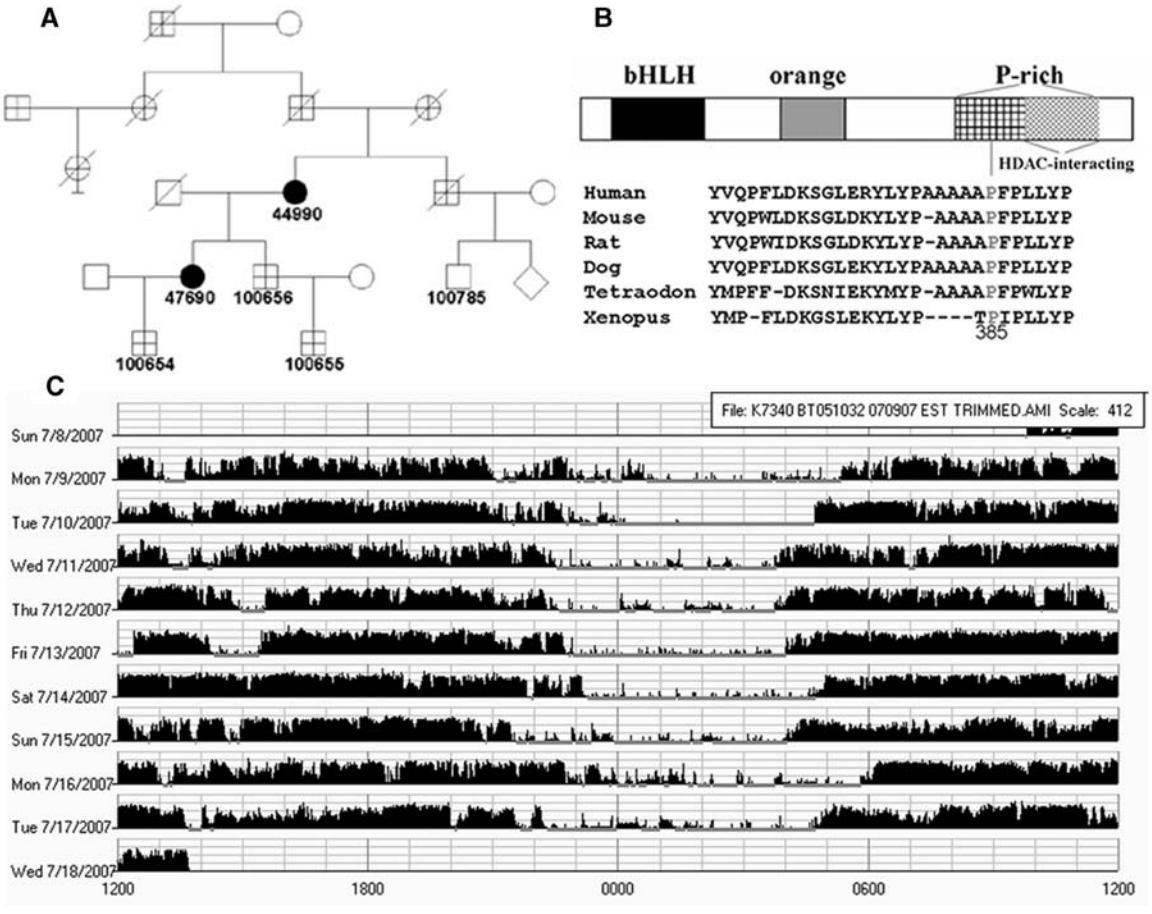

Fig. 1. A *DEC2* point mutation was identified in a short sleep family. (**A**) Pedigree of K7430 family carrying *DEC2* mutation (P385R). (**B**) P385 is localized in the C-terminal proline-rich domain and its flanking sequences are highly conserved among mammalian DEC2 orthologs. (**C**) Activity recording by wrist actigraphy for one mutation carrier demonstrates the extended active period each day.

and WT mice (Fig. 2F and table S3). These results suggest that the sleep structure of *DEC2-P385R* mice is more fragmented than that of WT mice (particularly NREM sleep). Although a slightly increased NREM delta power in the EEG is visible in *DEC2-P385R* mice, we did not see a statistically significant difference in NREM delta or REM theta power during daytime or nighttime sleep, which suggested that, despite decreased sleep duration and continuity, sleep depth under baseline conditions is not significantly affected in mutant transgenic mice (Fig. 2G). These results indicate that the amount and consolidation of wakefulness, NREM, and REM in *DEC2-P385R* mice are affected mainly during the light phase. The only significant difference observed in the dark phase was the mean duration of NREM. Together, these results strongly suggest that the changes in sleep measurements for *DEC2-P385R* mice are caused by the P385R point mutation.

We next examined the response of *DEC2-P385R* mice to sleep deprivation to further probe the role of *DEC2* in sleep regulation. *DEC2-P385R* and WT littermate mice were subjected to 6 hours of continuous sleep deprivation. Both genotypes showed a rebound in NREM and REM sleep in the remaining 6 hours of the light period and the subsequent 12 hours of darkness, although the degree of NREM and REM elevation was much smaller in the *DEC2-P385R* mice during the 12 hours of darkness (Fig. 3, A and B). During the dark phase (D1 and D2), NREM sleep was increased less in *DEC2-P385R* (ΔD1, 17.1% and ΔD2, 2.0%) versus WT mice (ΔD1, 71.8% and ΔD2, 27.2%) (Fig. 3B and table S2). Similarly, REM sleep was increased less for *DEC2-P385R* (ΔD1, 74.4%, ΔD2, 82.3%) than for WT mice (ΔD1, 175.2%, ΔD2, 122.4%). As seen in Fig. 3C, cumulative sleep loss and recovery data relative to the baseline hours of sleep across the deprivation and recovery periods show that WT mice lost more sleep during the L1 sleep deprivation period, probably because they had a baseline of more sleep during L1 periods. The slower recovery of acutely lost sleep in the *Dec2-P385R* mice is therefore even more remarkable because they started the experimental sleep deprivation with chronically less sleep. Immediately after sleep deprivation, mutant *DEC2-P385R* mice also had less compensatory gain in NREM compared with WT mice (Fig. 3C), consistent with a role for *Dec2* in sleep homeostasis. A significantly lower NREM delta power density change after sleep deprivation

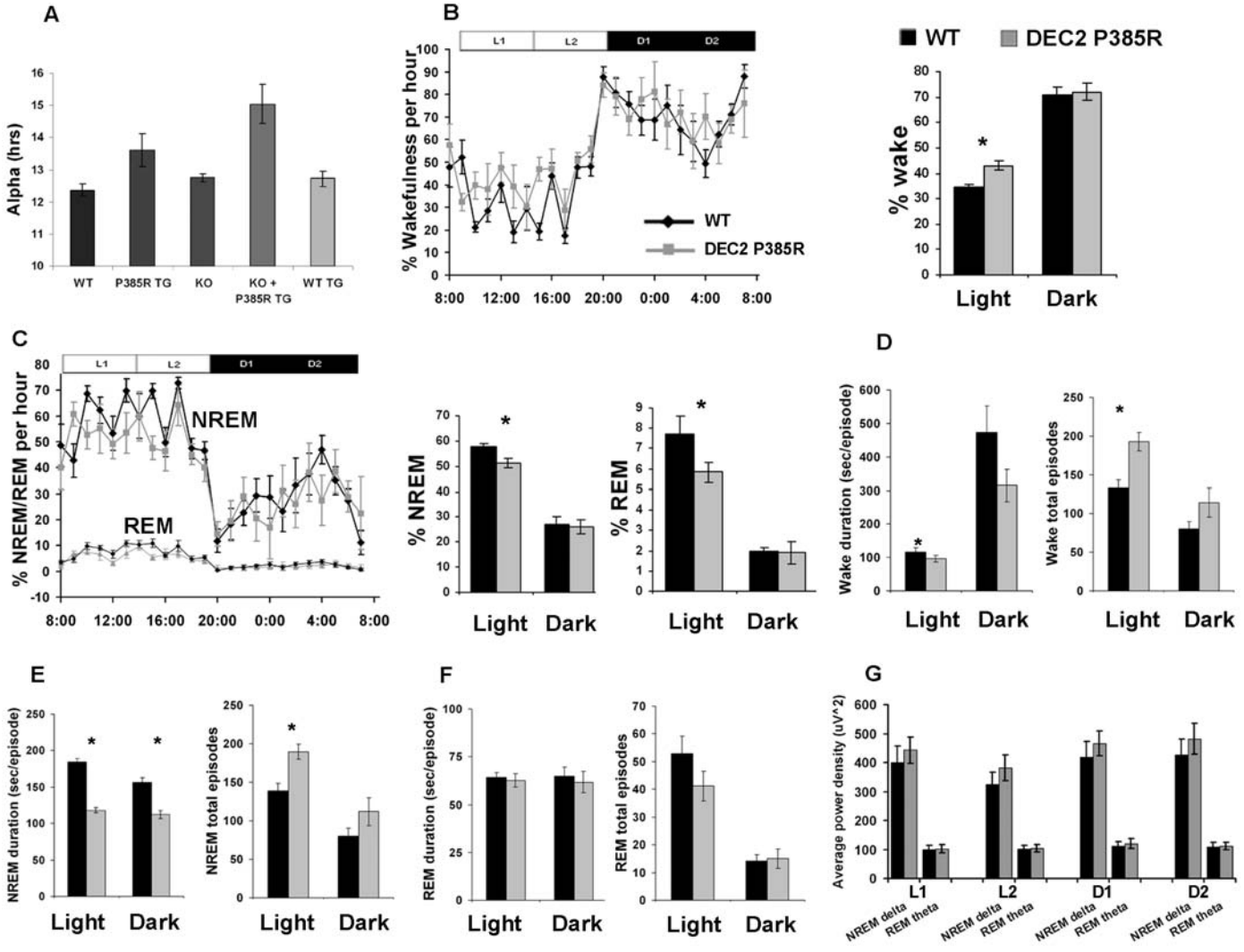

Fig. 2. Baseline sleep-wake characterizations of *DEC2-P385R* and WT littermate mice. (**A**) Duration of the active phase (α) for mice of the indicated genotype. (**B**) Percentage of time in a wakeful state was significantly increased in *DEC2-P385R* ($n = 5$) compared with their WT littermates ($n = 8$), especially in light phase (ZT 0–12, ZT 0 = 8 A.M.). Horizontal bar indicates light and dark phases. L1, ZT 0–6; L2, ZT 6–12; D1, ZT 12–18; D2, ZT 18–24. (**C**) Percentage of time spent in NREM and REM during the light phase was shortened in *DEC2-P385R* mice. Comparisons of mean duration time and episode number of wakefulness (**D**), NREM (**E**), and REM (**F**) for *DEC2-P385R* (gray bar) and WT littermates (dark bar). (**G**) Average spectral power was calculated for each of the 6-hour periods (from left to right: L1, L2, D1, and D2) as indicated by the horizontal bar in (B). NREM delta and REM theta power were compared for the two genotypes. Significant differences were marked with asterisks. $P < 0.05$, by one-tailed and two-tailed Student's *t* test. All data are expressed as means ± SEM. Error bars represent SEM.

was found in *DEC2-P385R* compared with WT mice, which further confirmed the altered NREM homeostasis in mutant mice (Fig. 3D). The differences of REM qualities between WT and *DEC2-P385R* mice were much less than those of the NREM and did not reach statistical significance. These results demonstrate that the P385R mutation leads to alterations in sleep rebound and NREM intensity after sleep deprivation in mice.

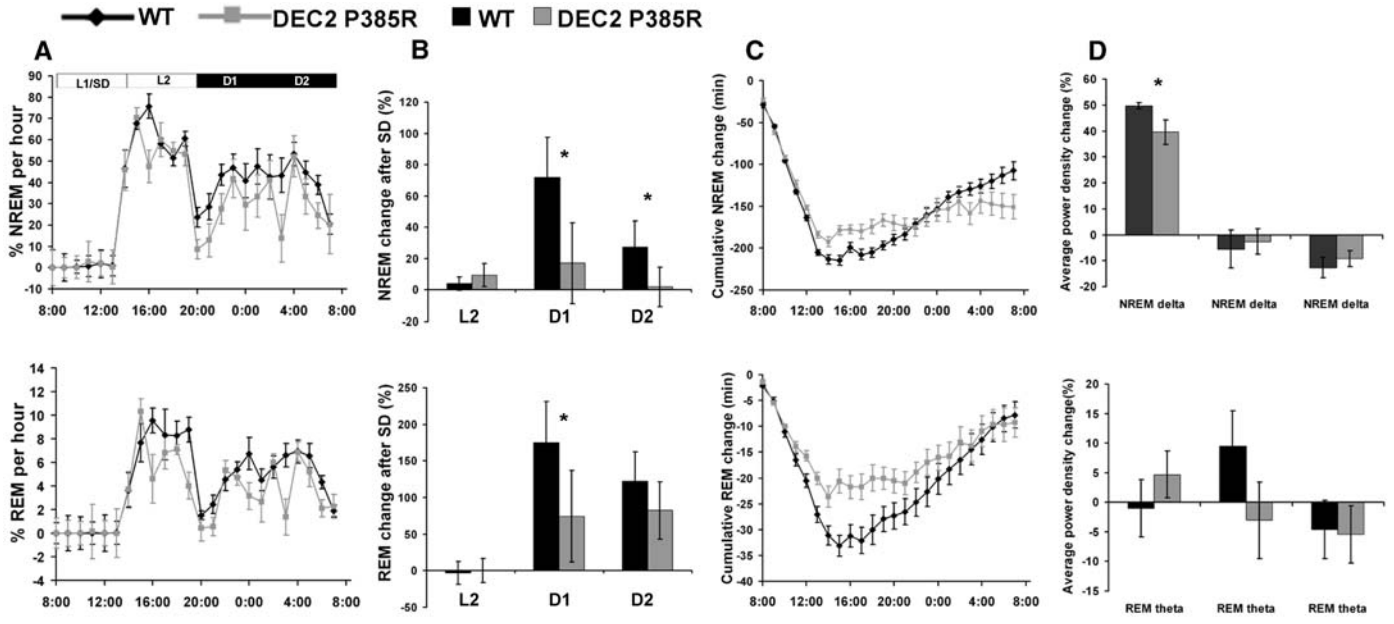

Fig. 3. Altered sleep regulation in *DEC2-P385R* mice. Data for NREM sleep are shown in top panels and for REM sleep are shown in bottom panels. (**A**) Time course of NREM and REM sleep as percentage of time spent every hour during and after sleep deprivation for one day. (**B**) Percentage changes of time after sleep deprivation in comparison with the baseline condition for NREM and REM in L2, D1, and D2 for *DEC2-P385R* and WT mice. (**C**) Cumulative NREM and REM sleep loss and gain compared with baseline conditions for the sleep deprivation experiment. (**D**) Analysis of spectral sleep power changes compared with baseline conditions for L2, D1, and D2. Significant differences are marked with asterisks. $P < 0.05$, by one-tailed and two-tailed Student's *t* test. Error bars represent SEM.

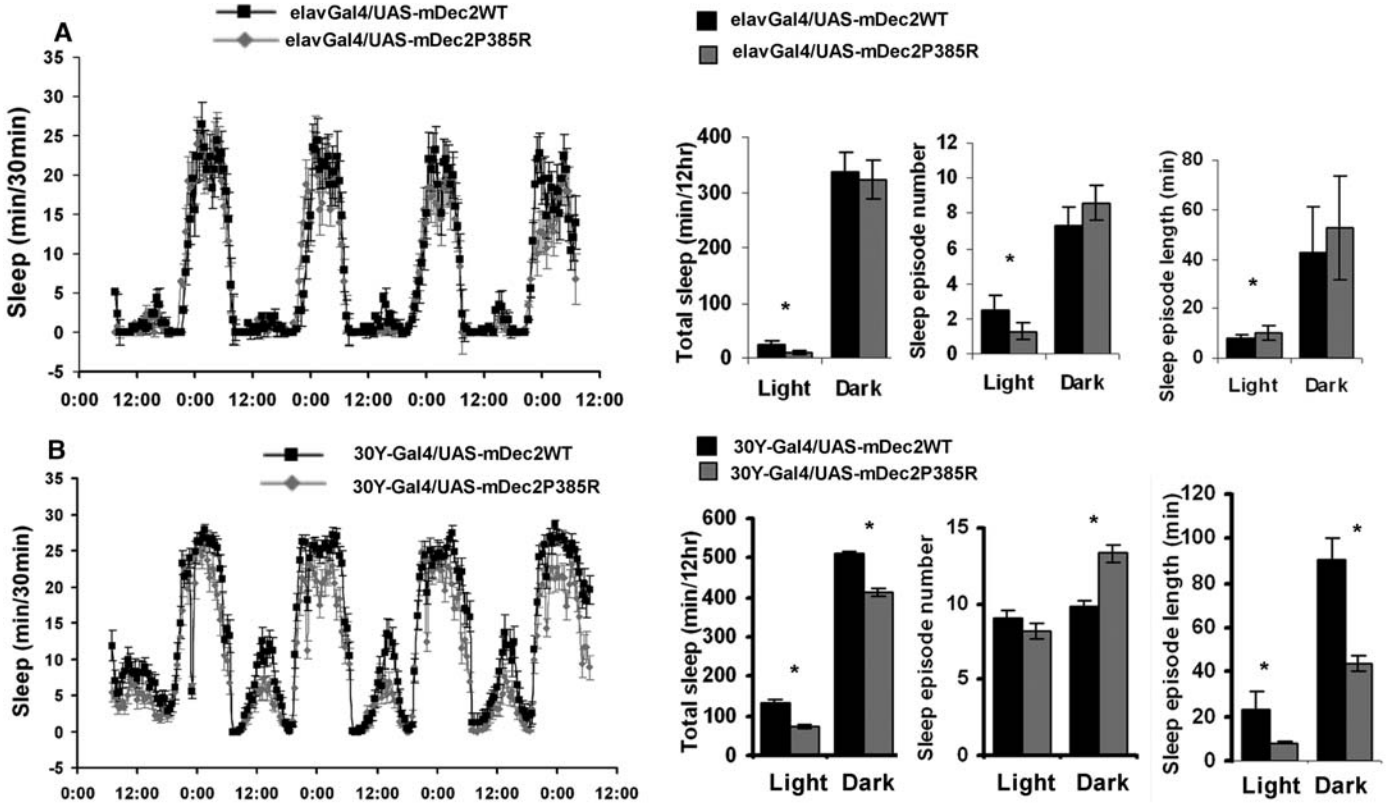

Fig. 4. Characterization of sleeplike behavior in transgenic flies overexpressing WT (black) or P385 (gray) *mDec2* with *elav*-GAL4 (**A**) or *30Y*-GAL4 (**B**) drivers. Profile of daily sleep-like behavior (amount of sleep during each 30-min period), total sleep time, sleep episode number, and mean sleep episode length in the light and dark periods of the day were plotted. Significant differences by one-tailed Student's *t* test are marked with asterisks ($P < 0.05$). Error bars represent SEM.

These changes in sleep homeostasis in the mutant mice provide a testable hypothesis for future work examining why human subjects with the mutation lead such active lives despite their persistently shorter sleep.

We performed EEG and EMG on *Dec2* KO mice and their WT littermates. Baseline wakefulness, NREM, and REM percentages showed that *Dec2* KO mice sleep slightly more than the WT mice and that the difference is mostly in the dark period (table S4 and fig. S2). During the light period, only the NREM sleep of *Dec2* KO mice was more abundant than that of WT mice and only slightly so. NREM rebound after sleep deprivation for *Dec2* KO mice was much slower, which implied that Dec2 is an important factor regulating sleep recovery.

We next set out to test whether m*Dec2P385R* can cause a similar rest-sleep phenotype in *Drosophila*. The closest homologous protein to DEC2 in *Drosophila* [CG17100, clockwork orange (*16*, *17*)] shares <18% amino acid sequence similarity, <11% identity, and P385 is not conserved. We therefore generated transgenic flies with expression-inducible UAS-*mDec2WT* and UAS-*mDec2P385R* on the *w1118* background. When these flies were crossed with *elav*-GAL4, driving pan-neuronal overexpression (*18*), *mDec2P385R* flies showed significantly lower daytime sleep-like behavior with reduced rest bout number and lengthened rest bout durations compared with WT flies (Fig. 4A). Because mushroom bodies were shown to be the likely sleep-rest behavior center in *Drosophila* (*19*), we also overexpressed P385R and WT m*Dec2* under the control of a mushroom body *30Y*-GAL4 driver (*20*). It was noteworthy that *mDec2P385R* transgenic flies driven by the *30Y*-GAL4 showed significantly less sleeplike behavior with significantly shorter sleep bout duration in both light and dark phases than *mDec2WT* flies (Fig. 4B). However, rest or sleep bout number was significantly higher only in the dark phase for *mDec2P385R* transgenic flies. These results indicate that the behavior of flies with mushroom body driver expression of *mDec2P385R* echoed those of the *DEC2-P385R* transgenic mice (Fig. 2, B, C, and E, and Fig. 4B).

The power of human genetics in studying human behavioral traits was demonstrated in the identification of mutations and the subsequent molecular characterization of FASPS (*21*–*23*). As currently understood, FASPS is primarily a circadian rhythm variant leading to altered phase; total daily sleep time is normal (*21*, *23*, *24*). We have applied a similar approach and identified a gene involved in regulation of sleep quantity. This provides a unique opportunity for exploring human sleep quantity regulation. *DEC2-P385R* mutation gave a short sleep phenotype, which was recapitulated in transgenic mouse and fly models but was not found in *Dec2* KO mice. In addition, this phenotype was enhanced by the absence of endogenous *Dec2* alleles, which suggested that P385R leads to a dominant-negative mutation. Our results demonstrate that *DEC2* plays an important role in regulating daily total sleep time in mammals and that the control of sleep-like behavior may be conserved and regulated in a similar manner as far back in evolution as invertebrates.

We did not see statistically significant differences in the NREM delta or REM theta power in the *DEC2-P385R* mice during day- or night-time sleep, although there is a trend toward increased NREM delta in these mice. It is possible that a small deficit of sleep in the short term does not significantly affect sleep power, whereas a long-term accumulation will. Alternatively, the attenuated NREM delta power enhancement, together with slow and incomplete NREM sleep recovery after sleep deprivation in the *DEC2-P385R* mice, suggests that these mice are able to cope with shorter sleep because of altered sleep homeostasis. It is noteworthy that recent data on human sleep emphasizes the importance of cumulative sleep debt even if it is only due to partial sleep deprivation. Understanding the regulatory mechanisms of sleep quality and quantity will facilitate the development of interventions to alleviate pathologies associated with sleep disturbance.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5942/866/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S4
References

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Protein Friction Limits Diffusive and Directed Movements of Kinesin Motors on Microtubules

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Friction limits the operation of macroscopic engines and is critical to the performance of micromechanical devices. We report measurements of friction in a biological nanomachine. Using optical tweezers, we characterized the frictional drag force of individual kinesin-8 motor proteins interacting with their microtubule tracks. At low speeds and with no energy source, the frictional drag was related to the diffusion coefficient by the Einstein relation. At higher speeds, the frictional drag force increased nonlinearly, consistent with the motor jumping 8 nanometers between adjacent tubulin dimers along the microtubule, and was asymmetric, reflecting the structural polarity of the microtubule. We argue that these frictional forces arise from breaking bonds between the motor domains and the microtubule, and they limit the speed and efficiency of kinesin.

Friction is the force that resists the relative motion of two bodies in contact. Contact is mediated by adhesive bonds between individual molecules, and friction arises from the forces necessary to deform and break these bonds (*1*). When a bond breaks, the energy stored in its deformation is dissipated. Adhesive interactions occur between proteins (*2*, *3*), and how they might give rise to protein friction has been discussed theoretically (*4*). Protein friction is expected to be

especially important for motor proteins because these nanomachines bind stereospecifically to discrete sites on a filamentous track and because, in order to move, they must break the bond at one site before rebinding at a new site in the direction of motion. In theoretical models of force generation by molecular motors, protein friction is predicted to limit both speed and efficiency (*5*–*9*). However, the frictional forces acting on motors have not been measured, and how they depend on the speed and direction of motion is not known.

Protein friction is related to diffusion according to the Einstein relation, $D = k_B T/\gamma$ (D diffusion coefficient, k_B Boltzmann constant, T absolute temperature, γ frictional drag coefficient). The higher the protein friction, the slower the diffusion. Diffusion of proteins along polymers—i.e., randomly directed stepping, as opposed to the directed adenosine triphosphate (ATP)–driven stepping of motor proteins—is functionally important. In the case of microtubules, diffusion along the microtubule lattice rapidly targets proteins to the microtubule ends [MCAK and XMAP215 (*10*, *11*)]; maintains dynamic contact between chromosomes and the growing or shrinking ends of microtubules [Dam1 and Ndc80 (*12*, *13*)]; and allows sliding of bundled microtubules [Ase1p and Ncd (*14*, *15*)]. The diffusion coefficients vary by over three orders of magnitude, which implies that the frictional interactions must also vary considerably in strength. It has been hypothesized that the diffusive interaction between these proteins and microtubules is mediated by electrostatic forces between the negatively charged, glutamic acid–rich C termini of α and β tubulins (the E-hooks) and positively charged amino acids in the diffusing proteins (*16*). Because the E-hooks are spaced every 4 nm, this electrostatic model predicts 4-nm diffusive steps, different from the 8-nm directed steps between adjacent tubulin dimers that motor proteins such as kinesin take. However, the binding sites associated with diffusion, the size of the diffusive steps, and the relation between diffusion and protein friction has not been experimentally studied.

The interaction of budding yeast kinesin-8, Kip3p, with microtubules is an ideal model system in which to study protein friction and its relation to diffusion. Kinesin-8 motors accelerate depolymerization of microtubules (*17*, *18*) and thereby regulate the lengths of mitotic spindles in yeast and other cells (*19*). In the presence of ATP, Kip3p is a highly processive motor that moves toward the plus, or rapidly growing, end of the microtubule (*17*); however, in the absence of ATP, we found that it diffuses on the microtubule lattice for several seconds (Fig. 1, A and B). Using total internal reflection fluorescence (TIRF) microscopy (*20*), we measured the diffusion coefficient in adenosine diphosphate (ADP, 1 mM) from the mean-squared displacement to be D_{TIRF} = 0.0043 ± 0.0005 μm^2/s (Fig. 1C). (All errors are SEM unless otherwise noted.) The diffusion coefficient in the absence of nucleotides was much smaller, 0.00020 ± 0.00003 μm^2/s (fig. S1). The value of the diffusion coefficient in ADP corresponds to a frictional drag coefficient of γ_{TIRF} = 0.95 ± 0.11 μNs/m. This implies that imposed velocities on the order of $\upsilon \approx 1$ μm/s will lead to frictional forces of $\gamma\upsilon \approx 1$ pN, which should be measurable with optical tweezers.

We used optical tweezers (*21*, *22*) to directly measure the friction force between Kip3p and microtubules in the presence of ADP (*20*). To quantify the motor-filament interaction forces as a function of velocity, we dragged Kip3p-coated microspheres over immobilized microtubules (Fig. 2A). The concentration of Kip3p molecules on the microspheres was low enough to ensure that only single molecules interacted with the microtubule (*20*). We moved the microtubules relative to the laser trap back and forth with a constant speed, using a piezo-electric translation stage, and recorded the position of the Kip3p-coated microsphere. To correlate the friction forces with the polarity of the microtubule, we exchanged the buffer at the end of each experiment with one containing ATP and, for the same microtubule, identified the polarity by the plus end–directed motility of the Kip3p-coated microspheres.

A representative experiment in 1 mM ADP is shown (Fig. 2B) in which both the centroid of a microsphere and the center of the laser trap are plotted against time. Upon Kip3p binding (arrows), the microsphere transiently slowed down and then accelerated to follow the trap, lagging on average a constant displacement Δx behind the trap center. Using the calibrated trap stiffness κ_{trap} [~0.037 pN/nm (*21*, *23*)], we then calculated the friction force $F = \kappa_{trap}\Delta x$ during such drag events (Fig. 2C). After the transient response, the friction force reached a steady state (Fig. 2C, dashed lines) and its magnitude increased with increasing speed.

The motor-microtubule friction force depended on speed and, in the case of large speeds, additionally on direction (Fig. 3). For small stage speeds, υ, the friction force increased linearly according to $F = \gamma\upsilon$. In the experiment shown in Fig. 3, the frictional drag coefficient was 1.11 ± 0.07 μNs/m, in good agreement with γ_{TIRF} (no significant difference by a t test, t = 1.23, $P < 0.95$). This frictional drag coefficient was much larger than the hydrodynamic (viscous) drag coefficient of the molecule (fig. S3A) and microsphere (fig. S4). The frictional drag coefficients inferred from the initial slopes of nine force-velocity curves in 1 mM and 2 mM ADP (table S1) were in good agreement with the ones calculated from measurements of the diffusion coefficient at the same ADP concentrations (*20*). This agreement with the Einstein relation was expected because, in the presence of ADP, which does not serve as an energy source for kinesin motors, the system is close to thermal equilibrium. Thus, both the drag and the diffusion arise from bond rupture dynamics.

At higher velocities, the frictional forces depended nonlinearly on speed, leveled off, and were significantly lower when the motor was dragged toward the microtubule's plus end compared with the minus end (Fig. 3). The nonlinearity can be well accounted for by a model for protein friction in which an energy barrier has to be overcome to break an adhesive bond (*4*). The external force F_{trap} alters the diffusive stepping rate according to the Arrhenius theory (*9*). If there is a single energy barrier between adjacent binding sites, then the mean motor velocity υ_m is equal to the product of the step distance, δ, and the difference between the mean forward, k_+, and backward, k_-, stepping rates, which depend exponentially on the force:

$$\upsilon_m = \delta(k_+ - k_-) \text{with } k_\pm = k_0 e^{\pm F_{trap}(\frac{1}{2}\delta \pm \Delta)/(k_B T)} \quad (1)$$

where k_0 is the stepping rate without a force. The asymmetry parameter Δ corresponds to the distance between the midpoint of the binding

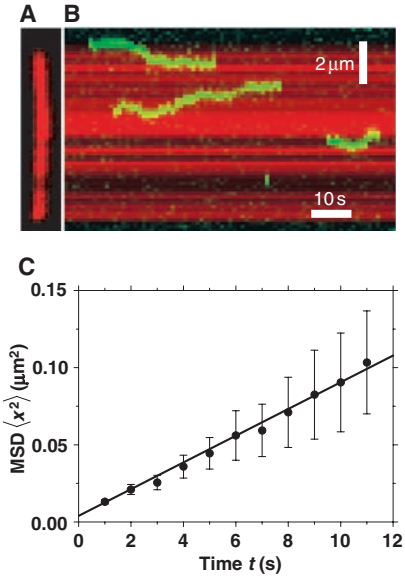

Fig. 1. Diffusion of Kip3p (kinesin-8) along microtubules in the presence of 1 mM ADP. (**A**) Image of a dimly rhodamine-labeled microtubule (red). (**B**) Kymograph of sequential frames of single Kip3p-GFP (green fluorescent protein) molecules (green) overlaid on the image of the microtubule (red) depicted in (A). (**C**) Mean-squared displacement MSD $\langle x^2 \rangle$ plotted against time t. A linear fit yielded the diffusion coefficient quoted in the text via $\langle x^2 \rangle = 2Dt + 2\varepsilon^2$ with tracking precision ε = 44 nm.

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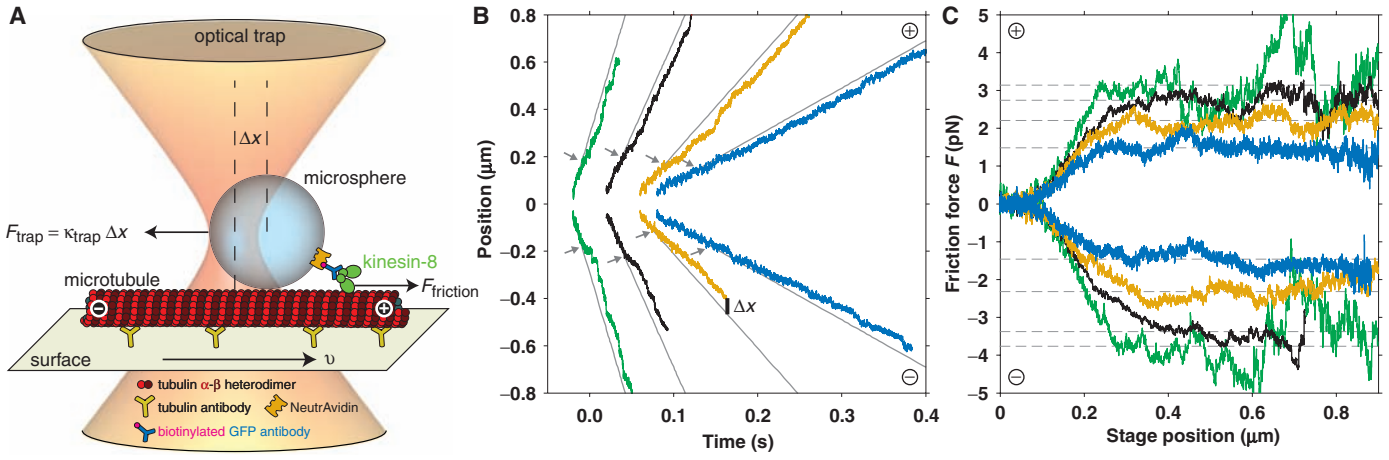

Fig. 2. Kip3p dragged over a microtubule in the presence of 1 mM ADP. (**A**) Schematic of the experiment: Using a focused laser, a Kip3p-coated microsphere is trapped close to an immobilized microtubule (not drawn to scale). Moving the stage with constant velocity υ past the stationary laser drags the microtubule lattice underneath the kinesin-8 molecule, creating a friction force $F = -F_{trap}$ (neglecting the very small hydrodynamic drag arising from the viscosity of the aqueous solution). Positive stage velocity is defined as the microtubule moving with its plus end leading; in this case, the laser and the motor are moving toward the minus end. (**B**) Time traces of the positions of the trap center (gray lines) and the microsphere relative to the microtubule plus end ($|\upsilon|$ = 2, 4, 8, and 12 μm/s for blue, yellow, black, and green, respectively). The arrows indicate the start of Kip3p binding events. (**C**) Trap force as a function of stage position. After a transient phase, a plateau value (- - -) is reached. Data are from the same experiment as in (B); each trace is an average of several drag events. Similar results were found for eight other microtubules.

sites and the position of the energy barrier, which corresponds to a transition state (Fig. 3, schematic inset). Provided that the fluctuations in the force are small, as is the case (Fig. 2C and fig. S5), Eq. 1 relates the friction force $F = -F_{trap}$ to the stage velocity $\upsilon = -\upsilon_m$. In the limit of low forces, a Taylor expansion of Eq. 1 results in $\upsilon = [\delta^2 k_0/(k_B T)]F = F/\gamma$, corresponding to the linear force-velocity relation observed at low speeds.

A global fit of Eq. 1 to nine force-velocity curves obtained on nine different microtubules in different experiments encompassing a total of 2015 drag events (Fig. 3, graph inset, and table S1) gave a step distance $\delta = 7.8 \pm 0.3$ nm, an asymmetry parameter $\Delta = 0.33 \pm 0.01$ nm, and a stepping rate $k_0 = 90 \pm 15\ s^{-1}$. The spatial asymmetry, though small, can lead to large differences in translocation velocities, depending on the magnitude and direction of the force (Fig. 3).

To obtain direct evidence for stepwise movement along the lattice, we examined displacement traces recorded at intermediate velocities. We observed steps of roughly 8 nm in size (Fig. 4A). To objectively estimate step-sizes over the whole range of velocities, including the ones with poor signal-to-noise ratios, we performed a fluctuation analysis [Fig. 4B and fig. S6] (*24*). For $|\upsilon| \geq 4$ μm/s, we obtained an average step size $\delta = 7.9 \pm 0.3$ nm. At lower speeds, we expected to overestimate the step size because of the presence of backward steps (*9*) (Fig. 4B, inset, dashed line). However, we observed a decrease in step size, which suggested that several rate-limiting processes at low forces exist, as has been observed for kinesin-1 (*25*). Thus, the fluctuation analysis supports the direct observation of 8-nm steps during frictional slipping of the motor along the microtubule.

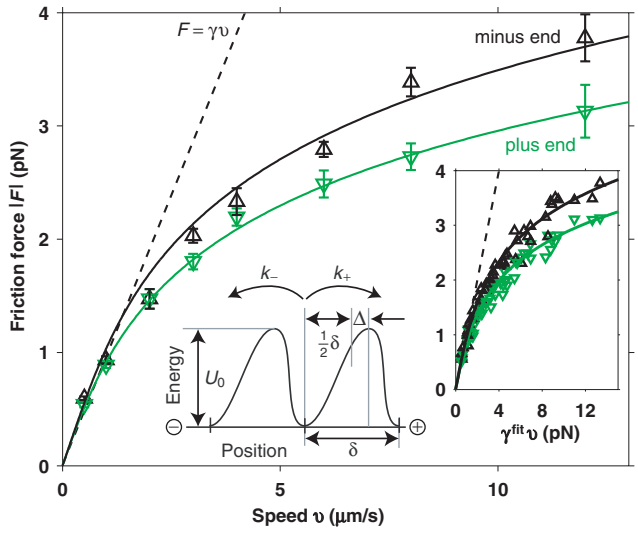

Fig. 3. Force-velocity relation. Absolute value of the friction force as a function of drag speed for the same Kip3p molecule and microtubule shown in Fig. 2, B and C. Drag direction toward the microtubule's plus end (▽) and minus end (△) are indicated. Theoretical force-velocity relations are based on (i) viscouslike friction $F = \gamma_{TIRF}\upsilon$ (- - -) and (ii) protein friction (fit with Eq. 1: directed toward the plus or minus end). (**Graph inset**) Master curve of nine sets of measurements from nine microtubules with a rescaled abscissa: The stage speed is multiplied by the individual linear frictional drag coefficients for each microtubule-motor set. In this way, the linear force-velocity relation (- - -) has a slope of one. (**Schematic inset**) Asymmetric potential landscape defining the periodicity δ, the asymmetry parameter Δ, the potential well depth U_0, and the forward k_+ and backward k_- rates. The asymmetry in the schematic is exaggerated to illustrate the asymmetry parameter.

Considering both the velocity-dependence of the frictional force and the fluctuation analysis, we conclude that the motor's binding sites on the microtubule are spaced 8 nm apart and separated by a single activation barrier. By comparing the hydrodynamic drag coefficient of Kip3p in solution measured by fluorescence correlation spectroscopy, $20 \pm 2\ \mu m^2/s$ (fig. S3A), with the frictional drag coefficient on the microtubule and using a first-passage time analysis, we calculated the height of the energy barrier to be $U_0 = 13 \pm 2\ k_B T$ (*20*). Therefore, our measurements of protein friction allow us to estimate the asymmetric periodic interaction energy landscape between a molecular motor and its track, in a specific nucleotide state.

What is the molecular basis for the 8-nm steps? Our measured step size of ~8 nm suggests that during diffusion and the frictional interaction, Kip3p is stepping from one tubulin dimer [8.2 nm in length (*5*)] to the adjacent one and not following the periodicity imposed by the E-hooks. In support of this conclusion, we found that the diffusion coefficient and the interaction time were not

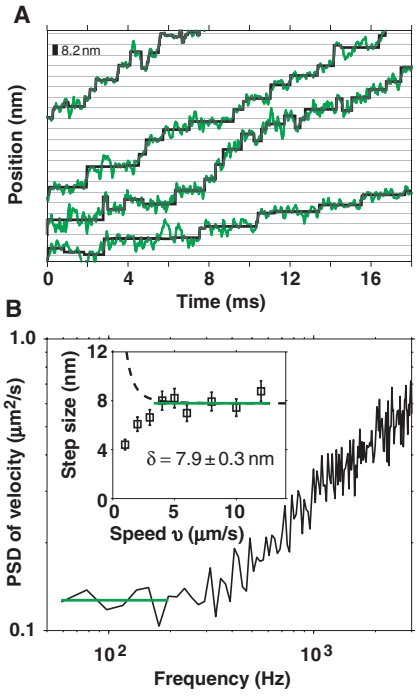

Fig. 4. Step determination methods. (**A**) Stepwise movement in exemplary regions (υ = 8 μm/s). The underlying grid has an 8.2-nm periodicity equal to the length of a tubulin dimer. Step detectors find steps consistent with ~8 nm. (**B**) Fluctuation analysis: Velocity power spectral density (PSD, drag velocity υ = 8 μm/s). The plateau $p = 2\upsilon\delta$ at low frequencies indicates that the microsphere moved in a stepwise manner (green horizontal line: average plateau value). (Inset) Step size based on the fluctuation analysis as a function of drag speed. The dashed line (- - -) shows the expected behavior for a Poisson stepper including backward steps. The green solid line indicates the mean step size value for $|\upsilon| \geq 4$ μm/s ($N = 6$).

greatly changed when the E-hook was removed by proteolysis (fig. S1). We therefore propose that Kip3p diffusion in the presence of ADP predominantly involves interactions with the canonical kinesin-tubulin–binding site. The long association time in ADP suggests that diffusion of Kip3p occurs via a hand-over-hand mechanism whereby the two motor domains bind to adjacent tubulin dimers along a protofilament, and that when one unbinds it can rebind either in front or behind the other motor domain.

In addition to providing information about the molecular mechanism of protein diffusion along microtubules, our measurement of protein friction provides mechanical insight into the speed and efficiency of Kip3p and other motors. In the conventional chemical view of molecular motors (*5*), mechanical motion is tightly coupled to transitions between different chemical states in the ATP hydrolysis cycle. The rates of the transitions, in turn, depend on the load. This picture can be turned around to produce a mechanical view of a molecular motor interacting with its track in which a force-generating element is opposed by a frictional element that dissipates energy and limits the speed (*26*). The friction arises because the breaking of bonds associated with chemical states slows the transitions between the mechanical states even in the presence of an external force. Kip3p is a very slow motor, with a maximum speed of 0.05 μm/s. Given that Kip3p can generate a force F_m larger than 1 pN (fig. S7), our measurement of the frictional drag coefficient in ADP of 0.75 μNs/m indicates that drag in the ADP state is not limiting the speed ($F_m/\gamma >> \upsilon_m$). Instead, the maximum speed of Kip3p is likely to be limited by the drag in strongly bound states, such as the nucleotide-free state with a frictional drag coefficient of ~20 μNs/m, as estimated from the diffusion coefficient. In contrast, measurements on kinesin-1 (*27*, *28*) indicate a frictional drag coefficient toward the microtubule plus end of ~10 μNs/m in ADP. Given that the maximum force is 7 pN (*29*) and that detachment in the ADP state is thought to be the rate-limiting step for kinesin-1 (*28*), we predict a maximum speed of 0.7 μm/s, similar to the maximum speed of kinesin-1.

Protein friction also gives insight into the efficiency of kinesin. The slope of the force-velocity curve can be interpreted as the friction coefficient of an active motor. For kinesin-1, this is ~8 μNs/m (the maximum force divided by the maximum speed). At maximum speed, such a friction element dissipates $\gamma\upsilon_m\delta \approx 50 \times 10^{-21}$ J per 8-nm step. Thus, about 50% of the energy from ATP hydrolysis (*5*) is dissipated as friction between the motor and its substrate. Internal conformational changes in the motor head associated with the ATP hydrolysis cycle lead to additional energy dissipation.

Protein friction is important in muscle (*30*) and should apply to other molecular motors, such as myosin V (*31*), DNA enzymes (*32*, *33*), and rotary engines (*34*, *35*). Diffusion of proteins along polymers and microtubules plays a central role in several biological processes, as discussed in the introduction. In the case of diffusion, no net energy is dissipated. In the case of actively driven proteins, energy should predominantly be dissipated through protein friction. Our single-molecule studies indicate that bond rupture dynamics underlie both diffusion and protein friction.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5942/870/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Table S1
References

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Mechanistic Analysis of a Dynamin Effector

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Dynamin-related proteins (DRPs) can generate forces to remodel membranes. In cells, DRPs require additional proteins [DRP-associated proteins (DAPs)] to conduct their functions. To dissect the mechanistic role of a DAP, we used the yeast mitochondrial division machine as a model, which requires the DRP Dnm1, and two other proteins, Mdv1 and Fis1. Mdv1 played a postmitochondrial targeting role in division by specifically interacting and coassembling with the guanosine triphosphate–bound form of Dnm1. This regulated interaction nucleated and promoted the self-assembly of Dnm1 into helical structures, which drive membrane scission. The nucleation of DRP assembly probably represents a general regulatory strategy for this family of filament-forming proteins, similar to F-actin regulation.

Dynamin-related proteins (DRPs) comprise a family of large guanosine triphosphatases (GTPases) that self-assemble into structures that associate with and remodel intracellular membranes (*1*–*3*). In vitro, DRPs are sufficient to mediate membrane constriction and scission (*4*–*8*). In vivo, however, DRP-associated proteins (DAPs) are required for DRP-dependent membrane remodeling (*1*, *9*–*15*). Although many DAPs have been identified, their mechanistic roles are unknown. We examined the role of Mdv1, a DAP required for mitochondrial division, which is driven by the action of the DRP Dnm1 (*3*). Guanosine triphosphate (GTP) drives the self-assembly of Dnm1 into helical structures, which can constrict lipid membranes (*5*). Self-assembly stimulates Dnm1 GTP hydrolysis, which is required to complete mitochondrial scission (*5*, *16*). Dnm1 self-assembly proceeds via a rate-limiting, Dnm1 concentration–dependent nucleation event, which may be exploited as a means to regulate the assembly and thus the function of Dnm1 in vivo (*5*).

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Dnm1-driven mitochondrial division also requires Fis1 and Mdv1 (*10*, *17*–*19*). Mdv1 functions as a molecular bridge between mitochondrial-anchored Fis1 and soluble Dnm1, and together Fis1 and Mdv1 function to target Dnm1 to the mitochondrial surface (*10*, *20*–*22*). Mdv1 also functions after targeting to facilitate division (*16*, *20*) [supporting online material (SOM) text and fig. S1]. To examine the post-targeting roles of Mdv1, we purified it from yeast cells. Co-overexpression of Fis1 lacking the transmembrane domain Fis1ΔTM was required to produce a soluble form of full-length Mdv1, and Fis1ΔTM co-purified with Mdv1 at stoichiometric levels (fig. S2A). However, after purification, the Mdv1/Fis1ΔTM complex dissociated (fig. S2B).

We asked whether the interaction of Mdv1 and/or Fis1 with Dnm1 was regulated and whether they affected Dnm1's kinetic and structural properties. Using a nonphysiological liposome composition, we targeted Dnm1 directly to liposomes. Dnm1 bound efficiently to liposomes in a manner that was independent of guanine nucleotides (Fig. 1A). Consistently, the Dnm1 mutants Dnm1S42N and Dnm1K41A, which are deficient in nucleotide binding or nucleotide hydrolysis, respectively, also efficiently bound to liposomes (*5*, *16*). Only the assembly-defective mutant Dnm1G385D, which exists as a dimer under assay conditions, exhibited a decreased association with liposomes (*5*, *23*). Thus, unassembled Dnm1 has a relatively weak affinity for liposomes, and the interaction between oligomeric Dnm1 and liposomes is strengthened via avidity.

In contrast to Dnm1, the recruitment of Mdv1 to liposomes required Dnm1 and either GTP or the nonhydrolyzable GTP analog GMPPCP (Fig. 1B). In contrast to Mdv1, only a small fraction (10 to 20%) of Fis1ΔTM was recruited to Dnm1-liposome complexes, and the addition of a 10-fold molar excess of Fis1ΔTM did not alter the amount of Dnm1 associated with liposomes nor the amount of Mdv1 recruited to Dnm1-liposome complexes (fig. S2C). Thus, Fis1 had no measurable effect on the Dnm1-lipid or Dnm1-Mdv1 interaction.

Consistent with a GTP-regulated Dnm1-Mdv1 interaction, Dnm1K41A also was able to efficiently recruit Mdv1 to liposomes in the presence of both GTP and GMPPCP, whereas Dnm1S42N was not able to recruit Mdv1 to liposomes (fig. S3A and B). The increased recruitment of Mdv1 to liposomes by Dnm1K41A with GTP as compared to wild-type Dnm1 suggests that upon nucleotide hydrolysis, the Dnm1-Mdv1 interaction was destabilized (Fig. 1B and fig. S3A). Assembly-defective Dnm1G385D was not able to efficiently recruit Mdv1 to liposomes in the presence of GTP or GMPPCP, a finding that is consistent with previous studies (fig. S3C) (*16*).

Thus, the Dnm1-Mdv1 interaction is dependent on a combination of avidity and the GTP-specific conformational state of Dnm1. Liposomes facilitated the assembly of Dnm1 helical structures in a nucleotide-independent manner, in contrast to the strict GTP requirement for the assembly of Dnm1 helices in the absence of liposomes (fig. S4A) (*5*). Thus, although avidity is probably important, the GTP-specific conformational state of Dnm1 is the critical determinant for the Dnm1-Mdv1 interaction. Indeed, qualitative differences in the helical structures formed in the presence of GMPPCP were observed (fig. S4A). Thus, Mdv1

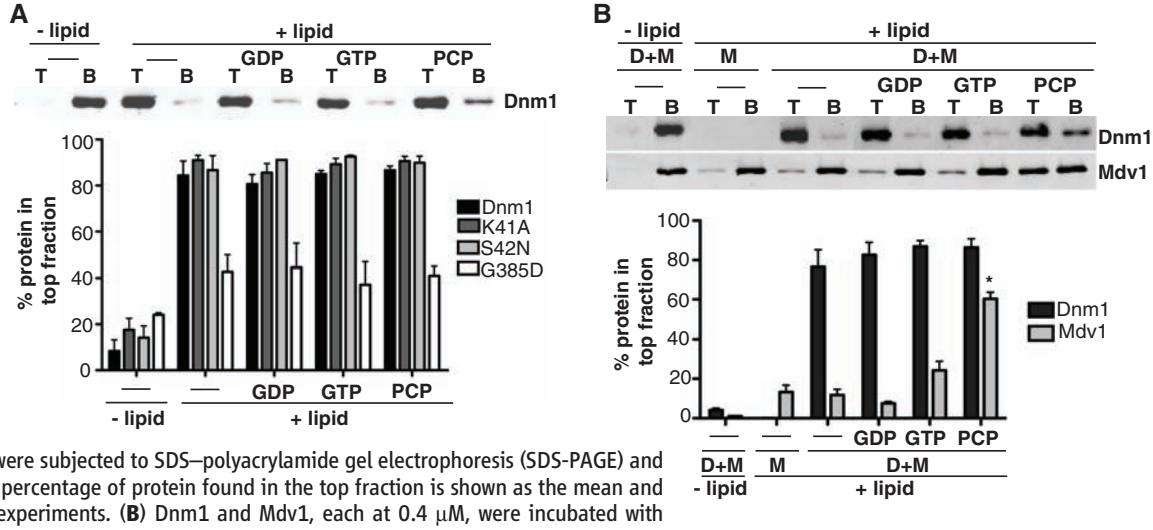


Fig. 1. Mdv1 preferentially interacts with the GTP-bound form of Dnm1. (**A**) Dnm1 and the indicated Dnm1 mutants at 0.4 μM were incubated with liposomes in the absence and presence of various nucleotides. The association of the protein with liposomes was assessed by its ability to float with liposomes after equilibrium sucrose gradient centrifugation. Equivalent amounts of the top (T) and bottom (B) fractions of the gradients were subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. The percentage of protein found in the top fraction is shown as the mean and SEM; $n = 3$ independent experiments. (**B**) Dnm1 and Mdv1, each at 0.4 μM, were incubated with liposomes in the absence and presence of various nucleotides and subjected to liposome floatation and analysis as described in (A). Data are shown as the mean and SEM; $n = 3$ independent experiments. *$P \leq 0.05$.

preferentially interacts with the GTP-bound, assembled form of Dnm1, and this interaction is regulated by GTP hydrolysis, which probably reflects the importance of a dynamic Dnm1-Mdv1 interaction in division in vivo (*16*).

A GTP-regulated Dnm1-Mdv1 interaction is consistent with a role for Mdv1 in regulating Dnm1 self-assembly and activity. To test this idea, we examined whether purified Mdv1 altered the kinetic properties of Dnm1 (*5*). As previously observed, Dnm1 exhibited a concentration-dependent kinetic lag in assembly-driven GTP hydrolysis before reaching a steady state, indicating that a nucleation event is required for self-assembly (*5*) (Fig. 2, A and B). The addition of Mdv1 dramatically decreased this kinetic lag in a concentration-dependent manner (Fig. 2, A and B). Thus, Mdv1 acts as a nucleator for Dnm1 self-assembly. The nucleation of Dnm1 self-assembly was also stimulated by liposomes with a mitochondrial outer membrane composition (OMC) (Fig. 2A) (*24*). Under the conditions tested, OMC liposomes were less effective than Mdv1 in decreasing the kinetic lag and, in combination with Mdv1, did not further reduce the kinetic lag.

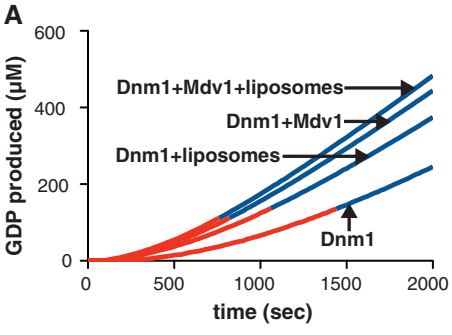
Mdv1 also increased the steady-state level of GTP hydrolysis by Dnm1, which also indicates that Mdv1 stimulated Dnm1 self-assembly (Fig. 2, C and D). The addition of Mdv1 also reduced the apparent Hill coefficient for GTP binding (Fig. 2D), which suggests that Mdv1 is inducing and/or stabilizing a GTP-dependent conformation of Dnm1 to promote self-assembly. The addition of Mdv1 resulted in a 1.4 ± 0.1–fold increase (n = 3 independent experiments) in the amount of GTP that could be cross-linked to Dnm1 by ultraviolet light (fig. S5), consistent with Mdv1 increasing the affinity of Dnm1 for GTP and preferentially binding to the GTP-bound form of Dnm1 (Fig. 1B). No significant effect on Dnm1 activity with or without Mdv1 was observed with a 15-fold molar excess of Fis1ΔTM, suggesting that Fis1 does not act directly on Dnm1 during mitochondrial division (fig. S2, D and E).

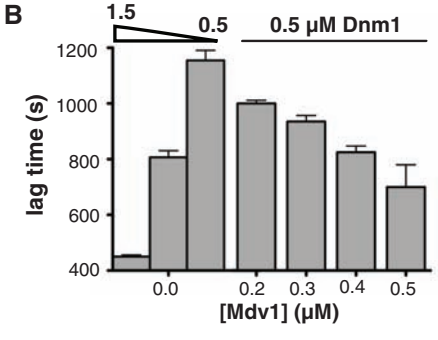
Mdv1 modulated Dnm1 activity only within the range of Dnm1 concentrations observed in vivo (Fig. 2E and table S1). OMC liposomes also had the greatest stimulatory effect on Dnm1 activity within this range, albeit to a lesser extent than Mdv1 (Fig. 2E). When added in combination with Mdv1, OMC liposomes did not further stimulate the activity of Dnm1. At relatively high nonphysiological concentrations, Dnm1 was able to effectively nucleate its own self-assembly, and the addition of Mdv1 and OMC liposomes had no further effect on the rate of GTP hydrolysis. Thus, because maximal GTP hydrolysis by Dnm1 is unaltered by Mdv1, Mdv1 does not stimulate the intrinsic ability of Dnm1 to hydrolyze GTP and thus acts as a positive effector to nucleate and drive Dnm1 assembly at the mitochondrial outer membrane, and consequently the GTP hydrolysis activity of Dnm1 is stimulated.

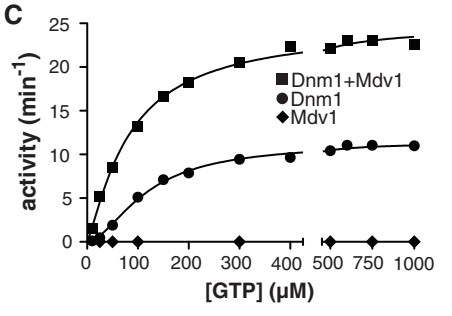
Kinetic data indicate that Mdv1 acts as a nucleator to drive Dnm1 self-assembly. Velocity sedimentation analysis consistently revealed that a greater fraction of Dnm1 was recovered in the assembled pellet fraction in the presence of Mdv1 and either GTP or GMPPCP, as compared to that recovered under identical assay conditions with Dnm1 alone (Fig. 3A). Moreover, a greater fraction of Mdv1 was recovered in the pellet in the presence of Dnm1 and either GTP or GMPPCP. Thus, Mdv1 acts to facilitate Dnm1 assembly by preferentially interacting with the GTP-bound form of Dnm1 and nucleating its assembly. In agreement with this idea, Mdv1 counteracts the effects of the Dnm1 small-molecule inhibitor mdivi-1, which attenuates the GTP-dependent assembly of Dnm1 (*25*) (SOM text and fig. S6).

To determine the structural basis for Mdv1-dependent Dnm1 nucleation, we analyzed GMPPCP Dnm1 structures by negative-stain electron microscopy (EM) (Fig. 3, B and C). At physiological Dnm1 concentrations, in the absence of Mdv1, we observed both simple rings and helices (Fig. 3B). In contrast, in the presence of Mdv1, we exclusively observed helices (Fig. 3B), which were uniformly decorated with additional electron density (Fig. 3, B and C). Consistently, Dnm1 structures assembled in the presence of liposomes and Mdv1 displayed an increase in electron density, quantified as an increase in the distance from the lipid membrane to the outer edge of the assembled protein, as well as a qualitative difference in structural features (Fig. 3, D and E). Thus, Mdv1 coassembled with Dnm1 helical structures, probably at a stoichiometric ratio as suggested by our binding data (Fig. 1B and fig. S3).

Based on our analyses, we propose two models for Mdv1-dependent Dnm1 nucleation, which are not mutually exclusive (fig. S7). Our finding that Mdv1 binds preferentially to Dnm1-GTP supports a model in which it acts as a conformational nucleator to induce or stabilize a GTP-like conformation of Dnm1 that is more favorable for self-assembly. Alternatively, Mdv1 may function as a structural nucleator by acting as a physical scaffold to facilitate the formation or stabilization of oligomeric Dnm1 assemblies. To test this possibility, we examined whether Mdv1 itself could oligomerize. In yeast two-hybrid studies, the central coiled-coil domain of Mdv1 can mediate self-interaction (*20*). Examina-







D

	- Mdv1	+ Mdv1
k_{cat} (min^{-1})	9.2 ± 1.2	24 ± 2.7
$K_{0.5}$ (μM)	87 ± 15	90 ± 5.8
Hill Coefficient	1.6 ± 0.1	1.2 ± 0.0

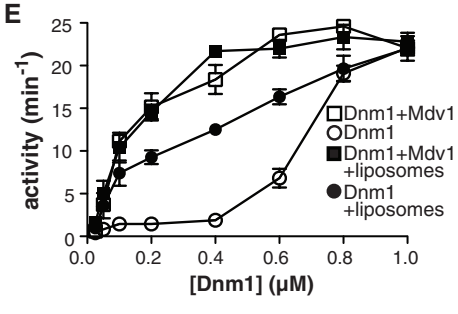
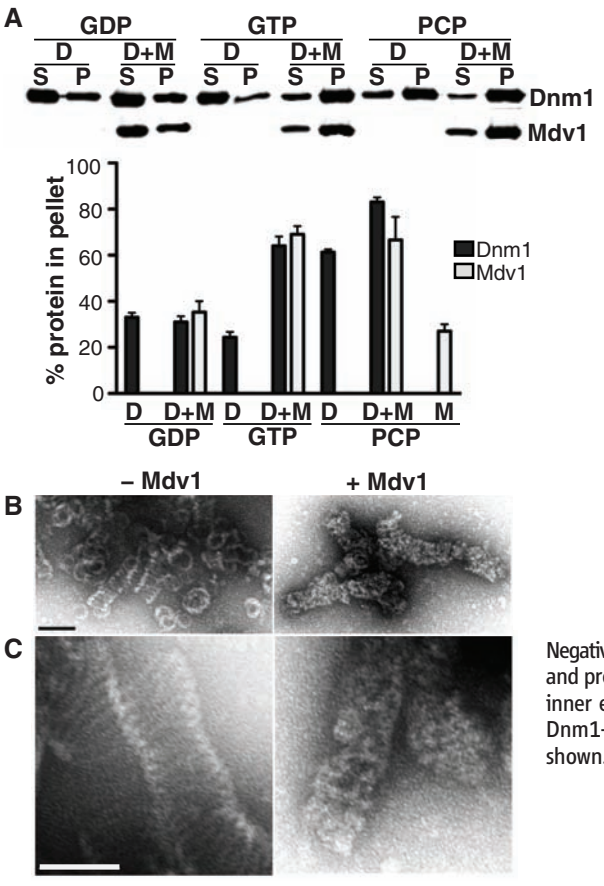


Fig. 2. Mdv1 acts to nucleate Dnm1 self-assembly. (**A**) A plot of Dnm1 GTPase activity in the absence and presence of Mdv1 and OMC liposomes versus time after the initiation of self-assembly. The lag time as determined by linear regression analysis is shown in red and the steady-state region of the curve is shown in blue. Dnm1 and Mdv1 were present at 0.5 and 0.4 μM, respectively. OMC liposomes were present at 0.05 mg/ml, and GTP was present at 500 μM. (**B**) Quantification of lag time in the presence of various concentrations of Mdv1. Dnm1 was present at 1.5, 0.8, or 0.5 μM, as indicated, and GTP was present at 500 μM. Data are shown as the mean and SEM; n = 3 independent experiments. (**C** and **D**) Steady-state kinetics of Dnm1 in the absence and presence of Mdv1. Dnm1 and Mdv1 were present at 0.5 and 0.4 μM, respectively. A representative kinetic experiment is shown in (C). The table in (D) shows kinetic parameters determined as described in methods (*32*). k_{cat}, turnover number; $K_{0.5}$, substrate concentration where velocity is one-half maximal. Data are shown as the mean and SEM; n = 3 independent experiments. (**E**) Steady-state GTP hydrolysis activity of Dnm1 at various Dnm1 concentrations in the absence and presence of 0.4 μM Mdv1 and 0.05 mg/ml OMC liposomes, as indicated. GTP was present at 500 μM. Data are shown as the mean and SEM; n = 3 independent experiments.



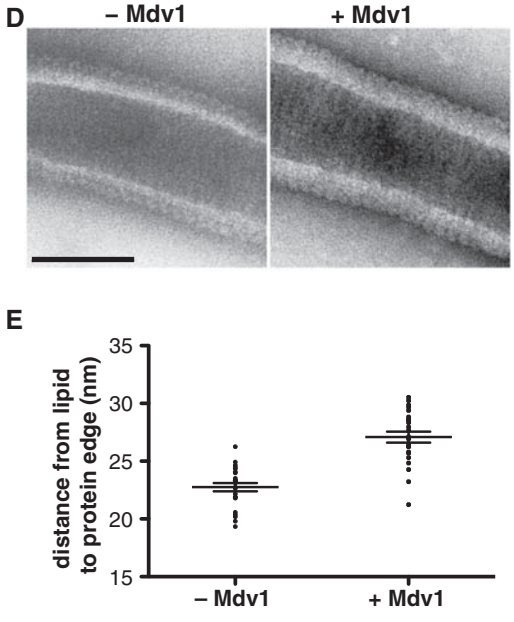


Fig. 3. Mdv1 stimulates Dnm1 self-assembly. (**A**) Velocity sedimentation of Dnm1 in the absence and presence of Mdv1. Dnm1 and Mdv1 were present at 0.2 μM. A representative Western blot is shown, and quantification data are shown as the mean and SEM; $n = 3$ independent experiments. (**B** and **C**) Analysis of GMPPCP-dependent Dnm1 self-assembly by negative-stain EM in the absence (left panels) and presence (right panels) of Mdv1. Different fields are shown in (B) and (C); the images in (C) were taken at a magnification 15,000 times higher than those in (B). Scale bars, 100 nm. (**D**) Negative-stain EM images of Dnm1-GMPPCP assembled on liposomes in the absence (left panel) and presence (right panel) of Mdv1. Scale bar, 100 nm. (**E**) Quantification of the distance from the inner edge of the lipid membrane to the outer edge of the assembled protein on Dnm1- and Dnm1-Mdv1 coated liposomes; $n = 24$ protein-coated liposomes. The mean and SEM are also shown.

tion of the subunit composition of purified Mdv1 by hydrodynamic analysis indicated that it exists as a dimer (Fig. 4A). At higher concentrations, we also detected Mdv1 tetramers and hexamers after treatment with a chemical cross-linker (Fig. 4B). Thus, like Dnm1, dimeric Mdv1 is probably the building block for larger Mdv1 oligomers, which is consistent with Dnm1 and Mdv1 acting in a stoichiometric manner (*5*). In addition, these results support the idea that through self-assembly, Mdv1 can also act as a structural nucleator for Dnm1 self-assembly.

It is likely that the nucleation of DRP assembly will emerge as a general strategy for the regulation of DRP function in vivo by DAPs. Indeed, the assembly of dynamin can be promoted by DAPs, such as amphiphysin and Snx9 (*9*, *13*–*15*). Mdv1 is tethered to the mitochondrial surface by Fis1 (*10*). Thus, Mdv1-dependent nucleation serves to spatially activate Dnm1's membrane remodeling function within the cell. The temporal and contextual regulation of DRPs by DAPs is probably also important. This is apparent in mammalian cells, where the mammalian Dnm1 ortholog, Drp1, is less assembled than Dnm1 at a steady state, and mitochondrial division is stimulated by a variety of signaling pathways, which serve to integrate mitochondrial division with cellular physiology or to perform other functions, such as the regulation of mitochondrial outer membrane permeabilization during apoptosis (*25*–*28*). Although no Mdv1 ortholog has been identified in mammalian cells, multiple DAP candidates have been identified for Drp1, raising the possibility that each may function as an effector that responds to a different cellular input (*29*–*31*). Finally, it is also possible that mechanistically distinct DAPs exist that serve as partial, negative, or neutral effectors. Thus, DAPs might also function in cells to expand the repertoire of DRP functions.

A

Hydrodynamic parameters	
Stokes' radius (A)	49.5
Sedimentation coefficient (S)	7.4
Molecular mass (kD)	149
Estimated number of subunits	2

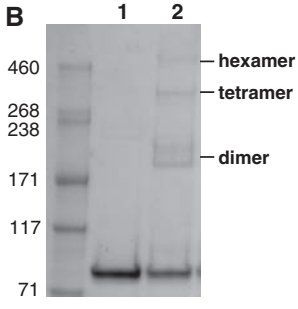


Fig. 4. Mdv1 oligomerizes. (**A**) Hydrodynamic parameters of Mdv1 were determined by sucrose gradient and gel filtration analyses and calculated as described in methods (*32*). (**B**) Analysis of Mdv1 oligomerization by chemical cross-linking. SDS-PAGE Coomassie-stained gel is shown of Mdv1 samples without (lane 1) and with (lane 2) a 5-M excess of cross-link reagent.

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ER Stress Controls Iron Metabolism Through Induction of Hepcidin

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Hepcidin is a peptide hormone that is secreted by the liver and controls body iron homeostasis. Hepcidin overproduction causes anemia of inflammation, whereas its deficiency leads to hemochromatosis. Inflammation and iron are known extracellular stimuli for hepcidin expression. We found that endoplasmic reticulum (ER) stress also induces hepcidin expression and causes hypoferremia and spleen iron sequestration in mice. CREBH (cyclic AMP response element–binding protein H), an ER stress–activated transcription factor, binds to and transactivates the hepcidin promoter. Hepcidin induction in response to exogenously administered toxins or accumulation of unfolded protein in the ER is defective in CREBH knockout mice, indicating a role for CREBH in ER stress–regulated hepcidin expression. The regulation of hepcidin by ER stress links the intracellular response involved in protein quality control to innate immunity and iron homeostasis.

The iron-regulatory hormone hepcidin, a defensin-like peptide produced by the hepatocytes in response to iron and inflammation, is a central humoral mediator of innate immunity and host defense (*1*). The primary antimicrobial strategy of this circulating peptide may be that of limiting vital iron that is needed by invading microorganisms, thus contributing to host defense. Hepcidin accomplishes this task by triggering degradation of the iron exporter ferroportin, thereby limiting iron egress from enterocytes and macrophages (*2*). If perpetuated, hepcidin stimulation may lead to the anemia of chronic diseases that is seen during infection, malignancy, and chronic inflammation (*3*). Thus, hepcidin qualifies as an acute-phase protein and an important component of the innate immune response.

Recently, the acute inflammatory response has been linked to endoplasmic reticulum (ER) stress, a state that is associated with disruption of ER homeostasis and accumulation of unfolded or misfolded proteins in the ER. CREBH, an ER stress–associated liver-specific transcription factor, responds to the immune response–inducing factors lipopolysaccharide (LPS) and interleukin-6 (IL-6) and directly activates the transcription of acute-phase response genes, such as serum amyloid P component (SAP) and C-reactive protein (CRP) in the liver (*4*). Both LPS and IL-6 are also potent inducers of hepcidin in the liver (*5*, *6*). Based on these premises, we wondered whether hepcidin, the iron hormone, is controlled by ER stress and investigated the contribution of CREBH to this activity.

We first tested three known ER stressors in the HepG2 hepatoma cell line: brefeldin A, the A23187 ionophore, and tunicamycin (Tm) (*7*). As expected, all tested compounds induced ER stress, as shown by the appearance of the spliced form of XBP1, an indicator of ER stress (*8*) (Fig. 1). Under such circumstances, the expression of hepcidin mRNA was markedly induced, particularly by Tm and A23187 (Fig. 1, A to C). Actinomycin D completely prevented Tm-induced hepcidin mRNA stimulation, indicating that hepcidin response to ER stress is due to transcriptional activation (Fig. 1D).

To confirm the in vitro data, we performed further experiments in mice treated with Tm and found that ER stress readily induces hepatic hepcidin gene expression in vivo (Fig. 2A). In agreement with the hepcidin model of iron regulation, Tm-treated mice

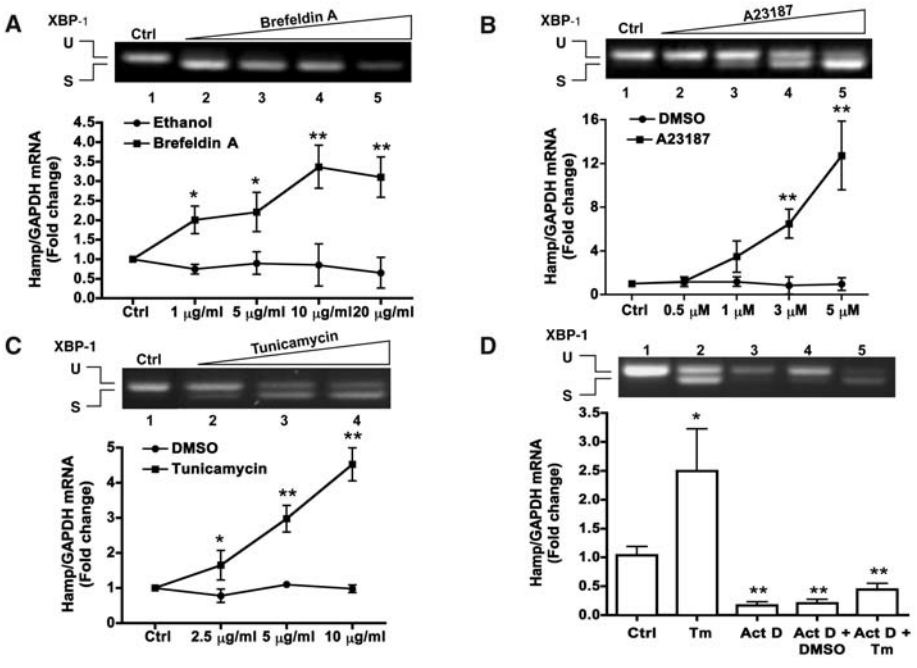

Fig. 1. ER stress induces hepcidin gene expression in vitro. (**A** to **C**) HepG2 cells were treated with different ER stressors, as indicated. RNA was extracted and subjected to semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) for unspliced (U, 442 bp) or spliced (S, 416 bp) XBP1 mRNA forms (shown in the upper panel by ethidium bromide stain) or to quantitative RT-PCR (qRT-PCR) for hepcidin mRNA (HAMP) (shown in the lower graph). Data are the mean ± SD of three independent triplicate experiments with mean control values set to 1.0 (*$P < 0.01$; **$P < 0.001$). (**D**) HepG2 cells were cultured in the absence or presence of actinomycin D (Act D, 1 μg/ml) and tunicamycin (Tm, 10 μg/ml). RNA was extracted and processed as above. Data are presented and analyzed as above (*$P < 0.03$; **$P < 0.005$).

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presented with hypoferremia (Fig. 2B) and iron sequestration in macrophages, mostly evident in the spleen (Fig. 2C). This was accompanied with lower hepatic and spleen ferroportin expression (fig. S1).

HFE, a protein required for hepcidin regulation by iron (*9*) and involved in human hemochromatosis (*10*), is not required for hepcidin induction by ER stress, because Hfe knockout mice readily up-regulated hepcidin expression in response to Tm challenge (fig. S2).

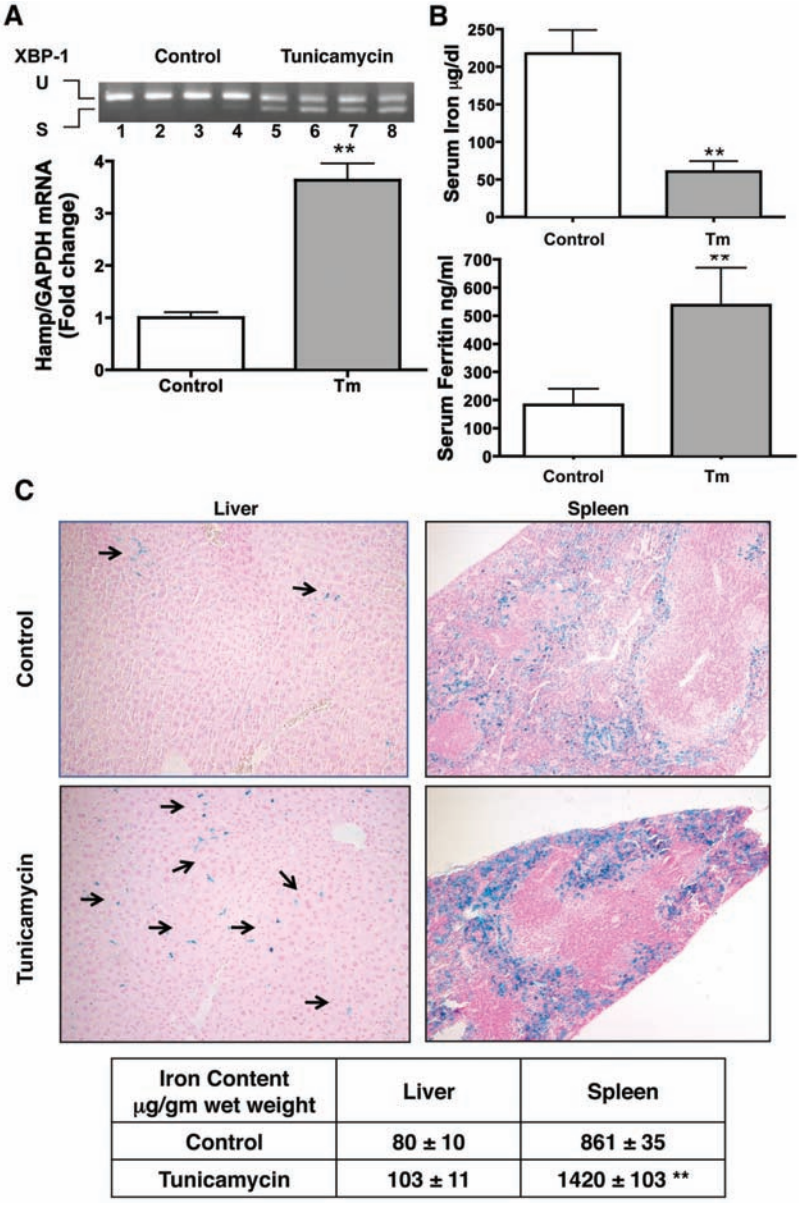
We then investigated the mechanism(s) for transcriptional activation of hepcidin and explored the role of CREBH. CREBH small interfering RNA (siRNA) specifically and markedly down-regulated hepcidin mRNA in HepG2 cells, without affecting expression of liver-specific albumin mRNA (Fig. 3A). Similarly, CREBH siRNA also prevented Tm-stimulated hepcidin mRNA expression (Fig. 3A). The 5′ promoter region of the hepcidin gene (*HAMP*) has been extensively investigated in other experimental settings (*11*–*17*).

The activity of a 789–base pair (bp) *HAMP* promoter fragment was comparably decreased by cotransfecting HepG2 cells with CREBH siRNA or an expression vector encoding CREBH protein lacking its transactivation domain (Fig. 3B), whereas a vector expressing the CREBH N-terminal active fragment greatly enhanced HAMP promoter activity (Fig. 3B). In 3T3 fibroblastic cells, which lack CREBH, exogenously expressed active CREBH increased HAMP promoter activity by 20-fold, as shown in HepG2 cells (fig. S3). These data indicate that CREBH is key for constitutive and inducible expression of the HAMP promoter in vitro.

The investigated *HAMP* genomic region displays two conserved putative binding sites for CREBH (fig. S4A). The −175/+14 promoter segment, where site A lies, accounted for most basal luciferase activity in transfected HepG2 cells (Fig. 3C). To further clarify the role of sites A and B in driving *HAMP* promoter activity, we performed transfection experiments with promoter variants carrying point mutations at the CREBH binding sites A and/or B. *HAMP* promoter activity decreased by ~60% after mutation of site A and remained unchanged when site B was mutated, whereas simultaneous mutation of both sites did not further decrease promoter activity (Fig. 3D). Accordingly, cotransfection of a CREBH dominant negative expression plasmid impaired luciferase activity of wild-type but not mutant AB promoter fragment (fig. S5), whereas combined site AB mutation blunted Tm-stimulated *HAMP* promoter activity (Fig. 3E). HepG2 nuclear proteins can specifically bind site A and B (fig. S6, A and B). CREBH binding to these elements (particularly site A) in the endogenous promoter was confirmed by chromatin immunoprecipitation (ChIP) assay with a specific anti-CREBH antibody (Fig. 3F). The data indicate the importance of the identified *HAMP* promoter elements and, particularly, the key role of site A occupancy by CREBH in driving *HAMP* promoter activity in vitro. However, we consistently observed a greater stimulatory effect of Tm on endogenous HAMP mRNA, suggesting that other regions and factors may be involved in the activation of HAMP promoter reporter in vitro. At least one such factor is X-box binding protein 1 (XBP1).

Basic region leucine zipper (B-ZIP) transcription factors, such as CREBH and XBP1, form either homo- or heterodimers to bind specific DNA sequences and regulate gene transcription (*18*, *19*). In vitro, the increase in hepcidin mRNA in response to different ER stressors was accompanied with the simultaneous appearance of spliced XBP1 mRNA (Fig. 1). Coexpression of mHAMP-775/+14-luc with plasmids expressing the spliced form of XBP1 and the active form of CREBH led to a greater stimulation of basal *HAMP* promoter activity than CREBH alone (fig. S7).

To clarify the role of CREBH in hepcidin expression during ER stress in vivo, we used *Crebh*$^{-/-}$ mice. Tm treatment led to marked hepcidin activation in wild-type mice, but failed to appreciably induce hepcidin expression or cause iron perturbation, including spleen iron accumulation, in *Crebh*$^{-/-}$ mice (Fig. 4, A and B). In vivo expression of mutant human FVIII, a protein that inherently misfolds in the ER (*20*) and elicits a milder form of ER stress than Tm treatment (fig. S8A), also increased hepcidin mRNA in wild-type mice (although to a lesser extent than Tm) but not in *Crebh*$^{-/-}$ mice (fig. S8B).



Iron Content µg/gm wet weight	Liver	Spleen
Control	80 ± 10	861 ± 35
Tunicamycin	103 ± 11	1420 ± 103 **

Fig. 2. ER stress induces hepcidin gene expression and perturbs iron homeostasis in vivo. 129S6/SvEvTac wild-type mice were treated with Tm or dextrose (Control) (*7*). Two independent experiments were performed with 10 mice per group. Reported data are from one representative experiment. (**A**) Hepatic XBP1 and HAMP mRNA were analyzed and the data are presented as in Fig. 1 (**$P < 0.0002$). (**B**) Serum iron and serum ferritin were measured in control and treated (Tm) animals (*7*) (**$P < 0.0001$). (**C**) Liver and spleen histopathologic pictures: Perls' Prussian blue stain for iron showing iron accumulation in Kupffer cells (arrows) and splenic macrophages. Lower table: chemical iron assessment (**$P < 0.0001$).

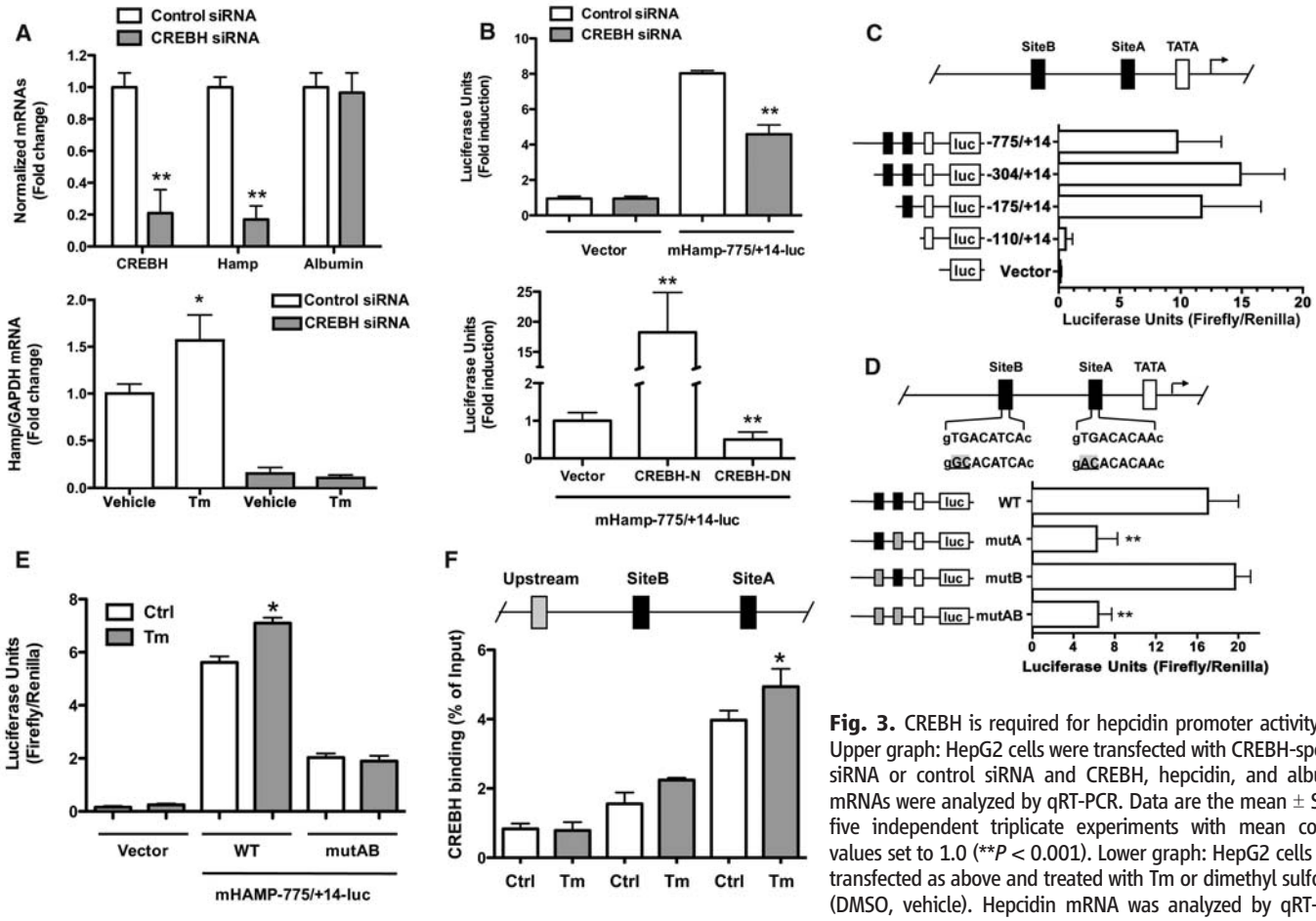


Fig. 3. CREBH is required for hepcidin promoter activity. (**A**) Upper graph: HepG2 cells were transfected with CREBH-specific siRNA or control siRNA and CREBH, hepcidin, and albumin mRNAs were analyzed by qRT-PCR. Data are the mean ± SD of five independent triplicate experiments with mean control values set to 1.0 (**$P < 0.001$). Lower graph: HepG2 cells were transfected as above and treated with Tm or dimethyl sulfoxide (DMSO, vehicle). Hepcidin mRNA was analyzed by qRT-PCR. Data are presented as above (*$P < 0.03$). (**B**) Upper graph: HepG2 cells were cotransfected with the pGL3-Basic plasmid (Vector) or the mHAMP-775/+14-luc plasmid and CREBH specific or control siRNA (**$P < 0.005$). Lower graph: the mHAMP-775/+14-luc plasmid was cotransfected with active (CREBH-N) or dominant negative (CREBH-DN) CREBH plasmids into HepG2 cells. Data are presented and analyzed are as in Fig. 1 (**$P < 0.0005$). (**C**) Different hepcidin promoter segments were cloned in the pGL3-Basic plasmid (Vector) and transfected in HepG2 cells. (**D**) HepG2 cells were transfected with wild-type mHAMP-775/+14-luc (WT) or mutant promoter constructs and luciferase activity assessed (**$P < 0.005$). (**E**) HepG2 cells transfected with the pGL3-Basic (Vector) or WT or site A and B–mutated mHAMP-775/+14-luc plasmids were treated with either DMSO (Ctrl) or Tm; data are the mean ± SD of five independent experiments (*$P < 0.04$). (**F**) Immunoprecipitation of HepG2 chromatin from cells exposed to either DMSO (Ctrl) or Tm was performed with anti-CREBH antibody. Promoter regions (100 to 150 bp) spanning site A, site B, or an upstream control region were amplified, as depicted. Percentage of DNA immunoprecipitated with anti-CREBH antibody relative to input chromatin is shown; data are the mean ± SD of three independent experiments (*$P < 0.05$).

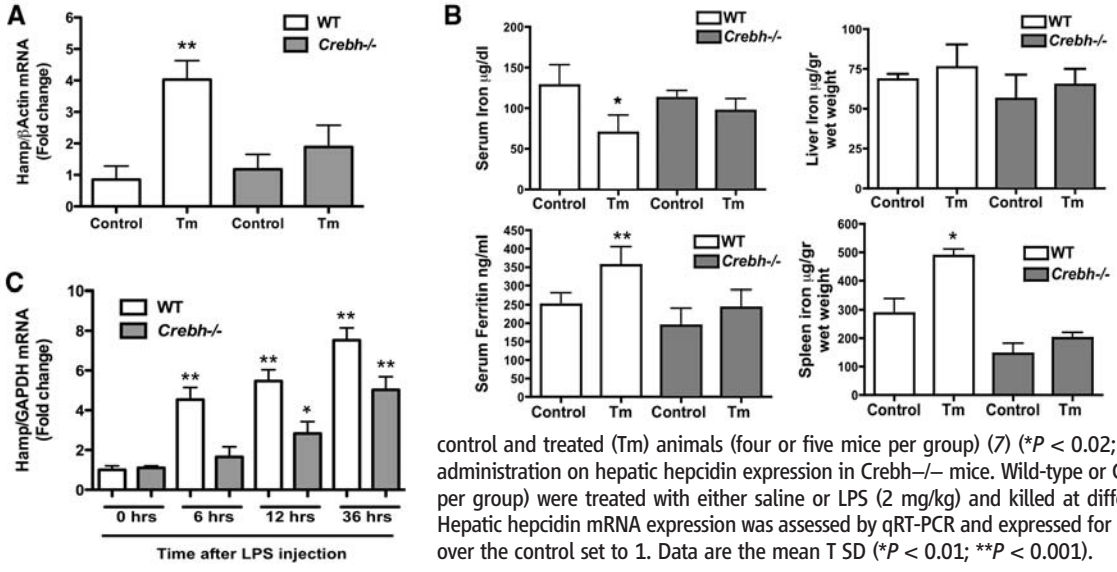


Fig. 4. ER stress induction of hepcidin expression is defective in *Crebh*$^{-/-}$ mice. (**A**) WT or *Crebh*$^{-/-}$ mice were treated with either DMSO (Control) or Tm. Hepatic hepcidin mRNA expression was analyzed by qRT-PCR. Data are the mean ± SD from one representative experiment (four or five mice per group) of four experiments (**$P < 0.0001$). Mean control values were set to 1.0. (**B**) Serum iron, serum ferritin, and tissue iron were measured in control and treated (Tm) animals (four or five mice per group) (7) (*$P < 0.02$; **$P < 0.004$). (**C**) Effect of LPS administration on hepatic hepcidin expression in Crebh–/– mice. Wild-type or Crebh–/–mice (four or five mice per group) were treated with either saline or LPS (2 mg/kg) and killed at different time points, as indicated. Hepatic hepcidin mRNA expression was assessed by qRT-PCR and expressed for each time point as fold increase over the control set to 1. Data are the mean T SD (*$P < 0.01$; **$P < 0.001$).

LPS and IL-6 are known stimulators of both CREBH and hepcidin (*5*, *6*). We tested whether CREBH is required for hepcidin response to LPS in vivo by analysis in wild-type and *Crebh*$^{-/-}$ mice. CREBH deficiency did not impair induction of the hepcidin gene in response to inflammatory challenge, although the increased expression of hepatic hepcidin mRNA was lower in *Crebh*$^{-/-}$ animals compared to wild-type mice (Fig. 4C). The data suggest that the CREBH-hepcidin axis may cooperate with other well-characterized signaling pathways (such as IL-6/STAT3) to stimulate hepcidin expression during inflammation (fig. S9).

The liver regulates drug detoxification, lipid metabolism, glucose homeostasis, and, as emerged after the discovery of hepcidin, iron homeostasis. As professional secretory cells, the hepatocytes likely face a subtle but persistent condition of ER stress due to the extremely high requirement for protein folding within the ER lumen (*21*). Homeostasis in the ER is tightly monitored through a series of adaptive programs, called the unfolded protein response (UPR). The UPR not only regulates protein folding capacity within the ER, but also modulates fundamental physiological processes, such as differentiation of specialized cell types and cell metabolism (*22*). Here, we show that this adaptive program also influences iron metabolism, through activation of hepcidin, the iron hormone. CREBH stable occupancy of the hepcidin promoter may serve as a "stress sensor" for intracellular or extracellular signals perturbing homeostasis (fig. S9). CREBH may act alone or recruit other stress-related transcription factors, such as XBP1, as shown here. Under conditions of severe ER stress, hepcidin activation and iron withdrawn from the bloodstream may facilitate a general defense mechanism and an innate immune response, in a manner similar to that which occurs during hepcidin activation in response to systemic inflammation (*1*). Overall, it seems that, at variance with other ER stress–induced factors, CREBH activates the expression of classic acute-phase response genes, such as SAP and CRP (*4*) and hepcidin (this report), a peptide that also qualifies as a main acute-phase response gene.

The regulation of hepcidin by ER stress links the cellular response involved in protein quality control to innate immunity and iron homeostasis. Apparently, hepcidin "senses" not only extracellular stimuli, such as iron fluctuations, erythroid factors, and cytokines (*1*), but also stress signals arising intracellularly (fig. S9).

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Supporting Online Material

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Capuchin Monkeys Display Affiliation Toward Humans Who Imitate Them

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During social interactions, humans often unconsciously and unintentionally imitate the behaviors of others, which increases rapport, liking, and empathy between interaction partners. This effect is thought to be an evolutionary adaptation that facilitates group living and may be shared with other primate species. Here, we show that capuchin monkeys, a highly social primate species, prefer human imitators over non-imitators in a variety of ways: The monkeys look longer at imitators, spend more time in proximity to imitators, and choose to interact more frequently with imitators in a token exchange task. These results demonstrate that imitation can promote affiliation in nonhuman primates. Behavior matching that leads to prosocial behaviors toward others may have been one of the mechanisms at the basis of altruistic behavioral tendencies in capuchins and in other primates, including humans.

In everyday life, we often unintentionally imitate the body postures, gestures, and mannerisms of our social interaction partners (*1*), a phenomenon that has been termed the "chameleon effect" (*2*). This form of imitation, which occurs completely unconsciously, can have profound effects on subsequent social interactions: The imitated person reports increases in shared rapport, liking, and feelings of empathy with the interaction partner (*2*) and is also more likely to display prosocial behaviors such as helping others, leaving more generous tips, or donating money to charity (*3*, *4*). Imitation therefore increases affiliation, empathy, and rapport between individuals, and it undoubtedly plays an important role in maintaining harmonious relationships with others (*5*).

Being able to cultivate successful social relationships is likely to carry substantial adaptive value. Individuals with strong social bonds who receive support from others are thought to have an evolutionary advantage over those who are ostracized from a group (*3*, *5*). Nonconscious imitation may therefore be an evolutionary adaptation that facilitates group living and may occur among other social primate species. Great apes and macaques share with us the capacity to recognize imitation (*6*–*8*), but it is presently unclear whether imitation may also facilitate positive social interactions in nonhuman primates. That is, do other primates show increased levels of affiliation toward an individual who displays behaviors similar to their own? We addressed this question by studying the effects of imitation on the behavior of capuchin monkeys, a highly social and socially tolerant New World primate species. Observational and experimental evidence suggests that capuchins are easily influenced by others' behavior (*9*–*11*) and are thus likely to recognize when others display behaviors matching their own actions. Moreover, because capuchins are strongly bonded into social groups, they may share with humans this mechanism to facilitate social group living; namely, increasing affiliation toward those who display matching behaviors. We therefore tested whether capuchins recognize imitation and whether imitation positively affects capuchins' social interactions.

In experiment 1, we investigated whether capuchins differentiate between an experimenter imitating them and an experimenter performing

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contingent actions but not imitating them. Following the established research designs with infants (*12*, *13*), two experimenters, each holding a small plastic ball, faced each monkey (Fig. 1). During the baseline phase, both experimenters performed actions that are commonly performed by capuchins (poking the ball with their fingers, mouthing the ball, and pounding the ball on a surface) (*14*). Analysis of visual preferences showed that monkeys did not discriminate between experimenters [mean, 36.23 s and 40.05 s; $t(10) = -0.81$, $P = 0.44$]. During the manipulation phase, monkeys were given an identical ball. One experimenter imitated the monkeys' ball-directed actions, whereas the other experimenter performed contingent but nonmatching actions (*14*). Monkeys looked longer at the imitator while they were manipulating the ball and hence while they were being imitated by the imitator [$t(10) = 2.23$, $P = 0.050$] (Fig. 2). Thus, capuchins are sensitive to the actions of others that match their own actions, and they prefer to look at imitating individuals.

Proximity to others is a reliable indicator of underlying affiliative relationships in capuchins and other primates (*15*). In experiment 2, we tested whether capuchins show increased proximity to an imitator rather than a non-imitator. At the start of the experiment, two experimenters faced each monkey for a 1-min period, one standing on the left side and one standing on the right side of a test cage. Monkeys could freely move within the three chambers of the test cage and choose whether to spend time in front of one of the experimenters or in a neutral middle position equidistant between experimenters (Fig. 1). During the first proximity measurement, monkeys spent similar amounts of time in front of both experimenters [41.2 and 36.0% of the trial; $t(9) = 0.65$, $P = 0.53$] (Fig. 3). As in experiment 1, monkeys were then given a small plastic ball, and one experimenter imitated the monkey's ball-directed actions while the other performed temporally contingent but structurally nonmatching actions. Monkeys looked longer at the imitator while manipulating the ball and hence while being imitated [$t(9) = 2.95$, $P = 0.016$] (Fig. 2). After the manipulation phase, imitator and non-imitator switched positions in front of the test cage, and for 1 min, monkeys could again choose whether to spend time in front of an experimenter or in the middle of the test cage equidistant between experimenters. Monkeys now spent significantly more time in front of the imitator than the non-imitator [mean imitator, 44.0%; mean non-imitator, 27.1% of the trial; $t(9) = 2.29$, $P = 0.048$] (Fig. 3).

One possible explanation for this effect might be that it is not imitation but rather the difference in looking time during the manipulation phase that led to the monkeys having greater familiarity with the imitator and thereby causing the shift in the monkeys' preference for the imitating experimenter. Alternatively, the imitator may have been perceived as being more attentive to the monkeys and therefore may have been preferred in the subsequent proximity test. To test whether attention alone may have caused the observed effect on social preference, in experiment 3 we first conducted a baseline proximity preference as in experiment 2. Two experimenters stood in front of the monkeys, one to the left and one to the right of the test cage, and for 1 min, monkeys could choose to spend time in front of an experimenter or in the middle of the test cage equidistant between experimenters. Monkeys spent similar amounts of time in front of both experimenters [36.9 and 32.0% of the trial; $t(10) = 0.74$, $P = 0.48$] (Fig. 3). Monkeys were then given a small plastic ball, and one experimenter faced and looked at the monkeys, whereas the other experimenter turned around and faced away from the monkeys. Both experimenters remained passive and did not move while the monkeys manipulated the ball. Being sensitive to gaze (*16*), monkeys looked significantly longer at the experimenter facing them during the manipulation phase [$t(10) = 6.41$, $P < 0.001$] (Fig. 2). After the manipulation phase, the two experimenters switched places in front of the test cage, and monkeys could again choose for 1 min whether to spend time in front of one of the experimenters or in the middle of the test cage equidistant between experimenters. Unlike in experiment 2, monkeys now spent similar amounts of time in front of experimenters [mean looking at monkey, 37.8%; mean looking away from monkey, 27.4% of the trial; $t(10) = 1.56$, $P = 0.15$] (Fig. 3). Thus, it appears that it is the process of being imitated rather than simple familiarity that caused monkeys to increase proximity to the imitator in experiment 2.

To investigate whether imitation also affects social interactions, we tested the effects of imitation on monkeys' interactions with an imitator in a token exchange task. A token exchange by its very nature is an interaction between two partners, one providing a token and the other providing a food item (*17*). Several factors may influence a monkey's willingness to exchange tokens with an experimenter; for example, a monkey might refuse to exchange tokens with a person whom they fear [supporting online material (SOM) text]. Therefore, a monkey's emotional reaction toward an experimenter may

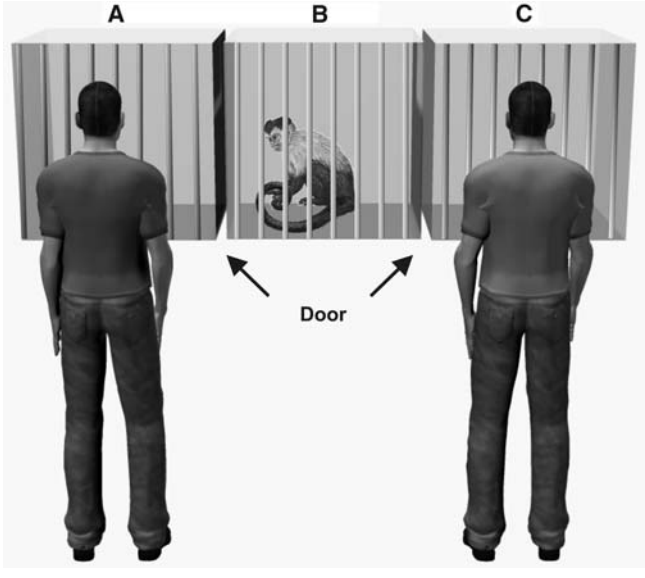

Fig. 1. Schematic of experimental setup in experiments 1 through 5. For proximity measures and token exchanges, monkeys had access to cages **A** through **C**. Monkeys were considered to be in proximity to an experimenter when they entered cage A or C. During the manipulation phases (imitation or gaze control), monkeys were restricted to cage B.

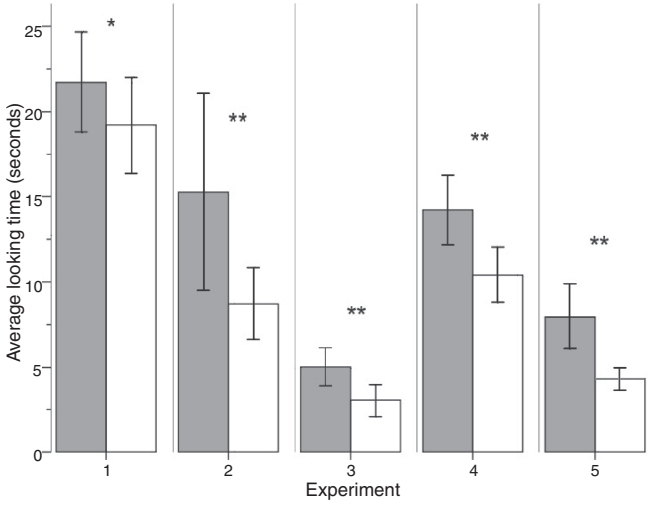

Fig. 2. Average looking time at experimenters while monkeys were manipulating the ball during manipulation phases (imitation or gaze control) in experiments 1 through 5. Gray bars represent the imitator/experimenter facing the monkey; white bars represent the non-imitator/experimenter facing away from the monkey. *$P = 0.05$, **$P < 0.05$, two-tailed t tests [experiments 1 and 3, $n = 11$ monkeys; experiments 2, 4, and 5, $n = 10$ monkeys; see (*14*)]. Error bars represent SEM.

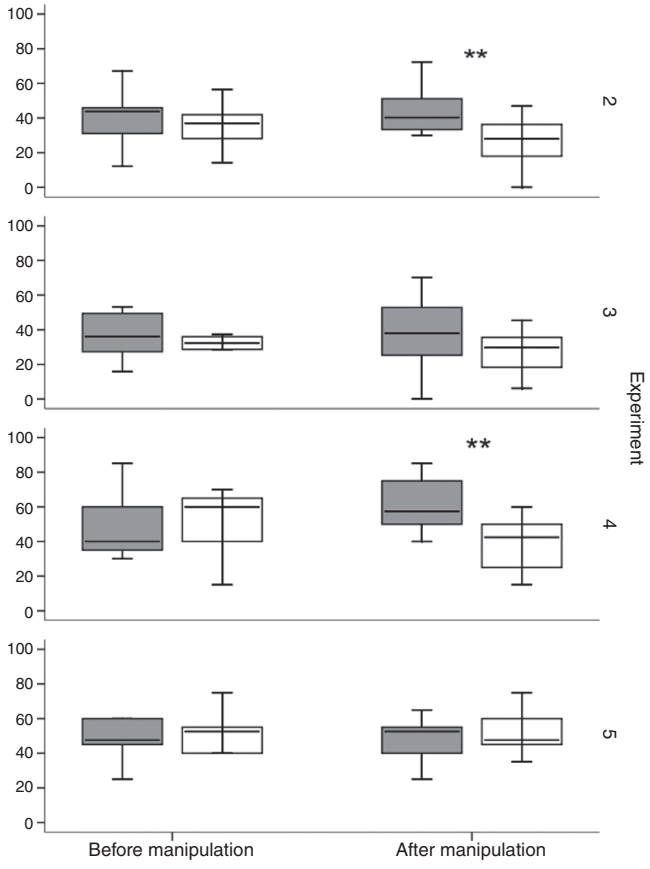

Fig. 3. Average behavioral preferences (proximity and token exchanges) for experimenters in experiments 2 through 5 before and after the manipulation phases (imitation or gaze control). Proximity measures in experiments 2 and 3 were converted into a percentage of the total phase duration. Token exchanges in experiments 4 and 5 were converted into a percentage of total exchanges (10 trials per phase = 100%). The top of the boxes represents the 75th percentile, the bottom of the boxes represents the 25th percentile, and the line in the middle represents the median. Gray boxes represent the imitator/experimenter facing the monkey; white boxes represent the non-imitator/experimenter facing away from the monkey. $^{**}P < 0.05$, two-tailed t tests.

significantly affect a monkey's willingness to exchange tokens. All of our monkeys had previously been trained to exchange small metal or plastic tokens with human experimenters for food rewards. In experiment 4, monkeys could choose to exchange a token with one of two experimenters, both offering the same food reward (a small piece of marshmallow). During the first token exchange session, monkeys did not prefer either experimenter for their token exchanges [mean, 4.55 and 5.15 exchanges; $t(9) = -0.25$, $P = 0.81$] (Fig. 3). Immediately after this first token exchange session, we replicated experiment 1. During the baseline imitation phase, monkeys did not visually discriminate between the experimenters [mean, 37.25 and 40.19 s; $t(9) = -0.93$, $P = 0.37$]. During the experimental imitation manipulation, monkeys showed a visual preference for the imitator during imitated actions [$t(9) = 3.12$, $P = 0.012$] (Fig. 2). Finally, we conducted a second token exchange session, in which monkeys could choose to exchange a token with either the imitator or the non-imitator, again receiving the same food reward from both experimenters. Monkeys now exchanged significantly more frequently with the imitator [mean imitator, 6.05; mean non-imitator, 4.95 exchanges; $t(9) = 2.30$, $P = 0.047$] (Fig. 3), indicating that being imitated increased the frequency of the monkeys' interactions with the imitator.

To confirm that, like the proximity measurements, the token exchanges were not merely facilitated by increased familiarity or perceived attentiveness of the imitator, we ran experiment 5 as a control study. At the start of experiment 5, monkeys could exchange tokens with one of two experimenters, who offered identical food rewards. As in experiment 4, monkeys initially did not prefer either experimenter [mean, 4.55 and 5.45 exchanges; $t(9) = -0.95$, $P = 0.37$] (Fig. 3). We then replicated the gaze control manipulation from experiment 3; that is, one experimenter faced and looked directly at the monkeys, whereas the other experimenter turned around and faced away from the monkeys, which led to monkeys looking significantly longer at the experimenter facing them [$t(9) = 3.51$, $P = 0.007$] (Fig. 2). A second token exchange session, which immediately followed the manipulation phase, revealed, however, that monkeys did not prefer to exchange tokens with the experimenter who had faced them during the manipulation phase [mean looking at monkey, 4.8; mean looking away from monkey, 5.2 exchanges; $t(9) = -0.49$, $P = 0.64$] (Fig. 3). Experiment 5 therefore confirms that it is the process of being imitated that led to increased interactions with the imitator.

These experimental results demonstrate that imitation significantly affects the behavior of capuchin monkeys: They look longer at imitators, spend more time in proximity to imitators, and prefer to interact with imitators in a token exchange task. As control experiments 3 and 5 show, these behavioral preferences cannot be solely explained by familiarity or the perceived attentiveness of the imitator. Thus, imitation positively affects subsequent social interactions not only in humans but also in capuchin monkeys.

Increased affiliation in human studies is observed after the matching of subtle gestures (*2*, *5*) or synchronized movement between individuals (*18*), not the conspicuous imitation shown in the present study. Moreover, it is generally accepted that capuchins do not explicitly match actions in the precise and timed manner of the human imitator in the present study (*9*, *15*, *19*). How well do these effects, which were observed under controlled laboratory conditions, transfer to capuchins' natural group environment? Precise data on this phenomenon in group settings are clearly needed, but it is known that wild capuchin groups routinely synchronize their behavior; for example, for travel, feeding, and predator defense (*15*). It is possible that such group synchronization may provide a sufficient degree of behavioral matching to produce positive effects on subsequent social interactions. Moreover, monkeys are unlikely to understand others' intentions to imitate them (SOM text) (*8*), so that explicit and implicit matching of behaviors are likely to affect them in similar ways. Matching or synchronization of behaviors may therefore carry substantial adaptive value not only as a mechanism of social learning (*20*) but also through its effects on subsequent social interactions.

It has been argued that the link between behavior matching and increases in affiliation might have played an important role in human evolution by helping to maintain harmonious relationships between individuals (*21*). We propose that the same principle also holds for other group-living primates. Matching or coordination of behaviors may lead to higher levels of tolerance and affiliation as well as decreases in aggressive behaviors, thereby increasing group cohesion. Behavior matching can therefore be regarded as a type of "social glue," helping to bind individuals together (*21*). The effects of behavior matching are not necessarily restricted to only two interaction partners, but may also lead to prosocial behaviors toward other individuals who were not directly involved in the social imitative exchange (*3*). An empathic connection resulting from behavior matching (*2*) may therefore extend to others in the social environment and promote altruistic behavioral tendencies in capuchins (*22*) and humans (*3*, *4*).

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Mindblind Eyes: An Absence of Spontaneous Theory of Mind in Asperger Syndrome

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Adults with Asperger syndrome can understand mental states such as desires and beliefs (mentalizing) when explicitly prompted to do so, despite having impairments in social communication. We directly tested the hypothesis that such individuals nevertheless fail to mentalize spontaneously. To this end, we used an eye-tracking task that has revealed the spontaneous ability to mentalize in typically developing infants. We showed that, like infants, neurotypical adults' (n = 17 participants) eye movements anticipated an actor's behavior on the basis of her false belief. This was not the case for individuals with Asperger syndrome (n = 19). Thus, these individuals do not attribute mental states spontaneously, but they may be able to do so in explicit tasks through compensatory learning.

Impairment in reciprocal social interaction and communication is a core feature of autism spectrum disorders, regardless of age and ability. This core feature is manifest in a wide range of social impairments, including characteristic deficits in comprehension and use of pretend play, expressive gestures, deception, and irony (*1*). One influential account that can explain these varied and characteristic impairments proposes that they are a consequence of a failure in the neurologically based capacity to "mentalize," that is, the automatic ability to attribute mental states to the self and others. The first evidence for this hypothesis, which is also known as a deficit in theory of mind (ToM) or "mindblindness" (*2*, *3*), comes from the finding that children with autism fail the verbally instructed Sally-Anne false-belief task (FBT), whereas 4-year-old neurotypical children pass, as do children with Down syndrome of similar verbal mental age (*4*).

In this task, which is considered a stringent test of ToM (*5*), one character (Sally) places a marble in a basket and leaves the room. In her absence, another character (Anne) moves the marble to a box. When Sally returns, children are asked where she will look for her marble. If children understand that Sally's actions will be based on what she believes to be true, rather than the actual state of affairs, they should answer that she will look in the basket, rather than the box. This correct answer requires the child to predict Sally's behavior based on her now false belief.

Despite still exhibiting atypical social features characteristic of autism, individuals of higher verbal ability, in particular those with Asperger syndrome, can pass such false-belief attribution tasks (*6*–*9*). This competence presents a puzzle for the mindblindness hypothesis (*10*) and has prompted the proposal that these high-ability individuals have acquired the ability to reason explicitly about false beliefs by compensatory learning, whereas difficulties in spontaneous mental-state attribution may nevertheless persist (*11*). To date, there is only indirect evidence in support of this hypothesis (*12*–*16*). In this study, we seek to provide direct evidence by contrasting the ability to pass the standard FBT with spontaneous looking behavior during a nonverbal form of this task.

In a groundbreaking study, Onishi and Baillargeon (*17*) used an FBT scenario to exploit infants' tendency to look longer at events that they do not expect. The authors showed that 15-month-old infants looked substantially longer when an actor searched in a location where an object was hidden that she could not know about (that is, when her behavior was incongruent with her belief). Southgate *et al.* (*18*) extended this paradigm so that, rather than measuring whether young children look longer at unexpected outcomes, they measured whether children actually anticipate the outcomes before they happen. They designed a task that made it possible to assess directly whether children had an understanding of the content of an actors' belief. Briefly, 25-month-old children were familiarized to an event in which a puppet hid a ball in one of two boxes (Fig. 1A), and then an actor reached through one of two windows to retrieve the ball from the box (Fig. 1C). Before she reached, a light and simultaneous chime signaled that the actor was about to open a window to retrieve the hidden object (Fig. 1B). In the test trial, the puppet transferred the ball from one box to another and then removed it altogether while the actor was looking away (Fig. 1D). An eye tracker was used to assess whether children expected (by making anticipatory eye movements) the actor to open the door, which would be consistent with her having a false belief about the location of the ball. Southgate *et al.* found that these typically developing children made eye movements toward the window above the box, which was consistent with the actor's belief about the location of the ball, despite the fact that it no longer contained the ball. These children, who would not be able to perform the traditional verbally instructed FBT, thus correctly anticipated the actor's behavior in line with her false belief.

It is this task (detailed above) that we used for the present study (see also movies S1 and S2). We asked whether adults with Asperger syndrome would, through their anticipatory looking, reveal a similar spontaneous capacity for false-belief attribution. At the same time, we had to establish that neurotypical adults would show the same anticipatory looking as young

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children. Before the main analyses, we confirmed that all the participants showed anticipatory looking toward the correct location during familiarization trials (*19*). Written informed consent was obtained from each participant before the study began. The study was approved by the University College London Research Ethics Committee.

As shown in Table 1, the Asperger and neurotypical groups in our study were very similar in age and intelligence quotient (IQ). As no sex differences were shown in any of our measures, we pooled results over gender in each group. We found that the participants with Asperger syndrome performed at the same level as neurotypical adults on verbally instructed versions of a variety of standard ToM tests, including the previously described Sally-Anne task. There were no group differences in the composite ToM score or in the Strange Stories test (both $ts < 1.61$, both $ps > 0.1$, t test). All participants with Asperger syndrome passed the two standard FBTs.

However, the eye-tracking version of the FBT revealed a very different picture. We used differential looking score (DLS) (*20*), which is considered a highly reliable measure of looking bias (*21*, *22*), compiled over a 6-s period (*19*). The Asperger group showed significantly less looking bias toward the correct window than did the neurotypical group [Fig. 2A, main effect of group: $F_{1,32} = 4.93$, $P = 0.003$, $\eta_p^2 = 0.13$, analysis of variance]. Follow-up t tests revealed that the neurotypical group scored significantly above zero [mean = 0.42, $t(16) = 2.76$, $P = 0.014$, Cohen's $d = 0.67$, t test], meaning that this group showed a significant bias toward the correct target, in line with the actor's false belief. This was not the case for the Asperger group, whose bias did not differ from zero [mean: –0.001, $t(18) = –0.010$, $P = 0.99$, Cohen's $d = 0.002$, t test].

We also coded the direction of first saccade (Fig. 2B). Here, 13 out of 17 neurotypical participants made their first eye movement toward the correct location, which was significantly above chance ($P = 0.049$, binominal test). In contrast, only 8 out of 19 individuals in the Asperger group made a correct saccade first, but this did not significantly differ from chance ($P = 0.647$, binominal test). These results are consistent with the DLS reported above. However, the first saccade, which represents a broad categorical response, did not differentiate significantly between the groups ($P = 0.30$, Fisher's exact test, two-tailed).

Could our results be due to gaze abnormalities in the Asperger group? It is important to note that they could not be attributable to an avoidance of eye gaze because the actor's eyes were hidden beneath a visor. However, the duration of fixations to the actor's face was significantly shorter in the Asperger group (mean = 1.9 s) than in the neurotypical group (mean = 3.1 s) [$t(34) = –2.48$, $P = 0.018$, Cohen's $d = 0.827$, t test]. This was not due to overall shorter fixations in the Asperger group, as the duration of fixations toward the windows (correct and incorrect combined) did not differ between groups [mean = 2.5 s in Asperger group and 1.9 s in neurotypical group; $t(34) = 1.53$, $P = 0.14$, Cohen's $d = 0.506$, t test]. The correlation between DLS and the duration of face fixation was not significant in either the Asperger or neurotypical group. Furthermore, total time spent looking at the five regions of interest (face, two windows, and two boxes) did not differ between groups (4.8 s in Asperger group and 5.1 s in neurotypical group, see supporting online material text for further details). Thus, the lack of bias toward the "correct" target in the Asperger group is not explained by gaze abnormalities.

Our study demonstrates that adults with Asperger syndrome do not spontaneously anticipate others' actions in a nonverbal task, closely modeled on the standard FBT that they pass with ease. In particular, the contrast with neurotypical 2-year-olds who show spontaneous looking to the correct location on the same task (*18*) is quite notable. It is unlikely that differences in motivation are to blame, because neurotypical adults showed the same bias as typically developing children, and the Asperger group exhibited correct anticipatory looking on familiarization trials

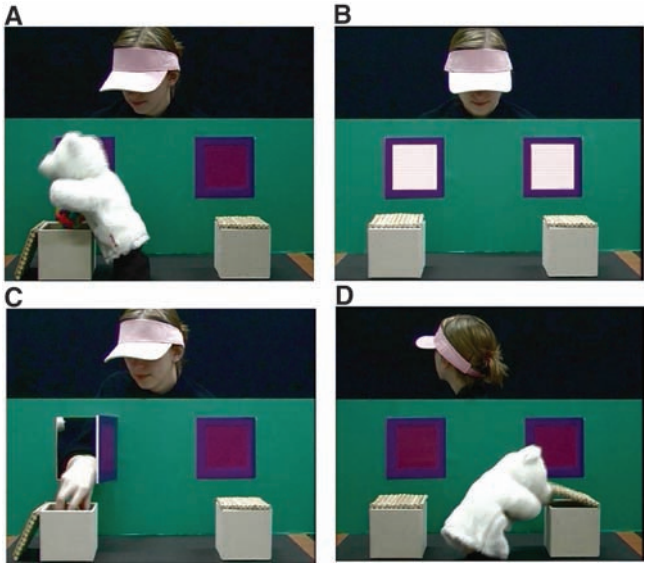

Fig. 1. Selected scenes from stimulus movies (see also movies S1 and S2). In familiarization trials, participants were familiarized to an event in which (**A**) the puppet placed a ball in one of two boxes, (**B**) both windows were illuminated and a chime sounded, and (**C**) an actor reached through the window above the box in which the ball was placed and retrieved the ball. The participants were familiarized to the contingency between (B) and (C). In (**D**), the puppet moves the ball while the actor is looking away. This operation induces a false belief in the actor about the location of the ball.

Table 1. Mean chronological age (CA), verbal IQ (VIQ), performance IQ (PIQ), full-scale IQ (FIQ) (WAIS-III UK), composite ToM score (ToM), Strange Stories test score (SS), scores of autism quotient (AQ), and autism diagnostic observation schedule–generic (ADOS-G).

Group	Asperger syndrome			Neurotypical		
	Mean	SD	Range	Mean	SD	Range
CA	36.8	14.3	21–67	39.6	11.7	26–63
VIQ	116.8	14.4	85–144	116.1	13.2	91–138
PIQ	109.6	13.0	80–132	111.5	10.6	97–132
FIQ	115.6	14.9	89–144	115.3	11.0	95–129
ToM*	9.7	2.0	4–13.5	10.6	1.3	8.5–12.5
SS†	13.2	1.8	10–16	13.6	1.3	12–16
AQ‡	34.9	7.6	17–48	16.5	7.6	6–37
ADOS-G	7.9	4.7	0–17	–	–	–

*The ToM tests consisted of five first-order FBTs [Sally-Anne (*4*), Smarties (*23*), interpretational false belief (*24*), belief-emotion and real-apparent emotion (*25*)] and two second-order FBTs [ice cream van (*26*) and coat story (*6*)]. †The Strange Stories test was taken from (*27*) and required the participant to either interpret another's behavior or understand another's emotion. ‡AS and NT groups differed significantly on the autism-spectrum quotient confirming their diagnostic status [AQ: (*28*), $t(34) = 7.23$, $P < 0.001$, Cohen's $d = 2.41$, t test]. No other variables were significantly different between the two groups.

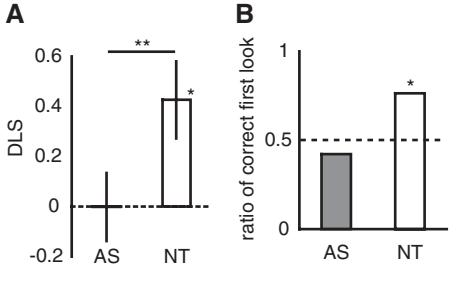

Fig. 2. (**A**) Mean (± SEM) DLS (*19*) and (**B**) the ratio of the number of participants who made correct first saccades in each group. AS, participants with Asperger syndrome ($n = 19$); NT, neurotypical participants ($n = 17$). *$P < 0.05$; **$P < 0.01$. Dotted lines indicate chance level. Statistical test used: (A), t test; (B), binominal test.

when no belief reasoning was required. The current results confirm indirect indications (*12*–*15*) that individuals with Asperger syndrome have a persistent impairment in spontaneous mentalizing and are also consistent with a previous finding (*16*) that children with autism are more likely to give a correct verbal answer than a correct anticipatory look when asked to infer someone's preference.

Although it is plausible that the documented early emerging spontaneous capacity to mentalize (*17*, *18*) is a prerequisite for the later ability to justify behavior in terms of mental states in verbal tasks, our results suggest that this need not necessarily be the case. Instead, our data raise the surprising possibility that an early developing form of the cognitive ability to mentalize, evident in spontaneous looking behavior, is not a necessary precursor of the later developing form of mental-state attribution, which supports explicit reasoning. The former would require spontaneous encoding of socially relevant information and automatic online computation of others' mental states, whereas the latter could also be achieved by verbally mediated reasoning prompted by explicit task structure and instructions.

We suggest that compensatory learning can circumvent neurophysiological limitations, even without removing the original cause of the limitation. Such compensatory learning might explain the apparent paradox between success on explicit FBTs and continued difficulty in everyday social interaction for individuals with Asperger syndrome.

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References
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POSITIONS OPEN

UNIVERSITY of ROCHESTER MEDICAL CENTER

FACULTY POSITION
SYSTEMS MICROBIOLOGIST

The University of Rochester School of Medicine and Dentistry and the Department of Microbiology and Immunology are expanding their existing scientific and research program in microbiology. The Department wishes to recruit a tenure-track Systems Microbiologist at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level. Preference will be given to candidates working in the field of bacteriology. Commensurate with proposed rank, candidates will be expected to develop, or to already have, an independent research program that will complement existing programs. The successful candidate will be joining a highly interactive faculty (**website: http://www.urmc.rochester.edu/smd/mbi/**) housed in a new research building with state-of-the-art core facilities (**website: http://www.urmc.rochester.edu/research/core-facilities.cfm**), and will participate in the University's new program in Genomics and Systems Biology (**website: http://www.urmc.rochester.edu/strategic-plan-in-action/isp/genomics.cfm**). The University is ranked among the nation's top 10 nonprofit workplaces for life scientists, according to *The Scientist* magazine's annual assessment of "Best Places to Work" (**website: http://www.urmc.rochester.edu/strategic-plan-in-action/strategic-plan-news/10-best.cfm**). An attractive salary, competitive startup package, and new laboratory space adjacent to the other microbiologists in the Department are available. Interested individuals should submit a letter of application, curriculum vitae, a statement of research interests and goals, and the names, addresses, and telephone numbers of three references. These materials should be sent to:

Steve Dewhurst, Ph.D.
Professor and Chair, Department of Microbiology and Immunology
c/o Brenda Knorr
University of Rochester Medical Center
601 Elmwood Avenue, Box 672
Rochester, NY 14642-0672 USA
Telephone: 585-275-3402; fax: 585-473-9573
E-mail: bknr@mail.rochester.edu

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

RESEARCH FACULTY POSITION
The University of Rochester
School of Medicine and Dentistry
Division of Endocrinology and Metabolism

The Division of Endocrinology and Metabolism at the University of Rochester School of Medicine is seeking new Research Faculty members of all ranks. Candidates must have an M.D. or Ph.D. and should be performing endocrine-related basic or translational research. The Division is under new leadership and is seeking to rapidly expand its academic programs. Thus, the University of Rochester offers an exciting opportunity for research scientists interested in endocrinology. Please electronically transmit curriculum vitae and cover letter to: **Stephen Hammes, Chief, Division of Endocrinology and Metabolism, University of Rochester School of Medicine, e-mail: stephen_hammes@urmc.rochester.edu.**

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POSTDOCTORAL POSITION. Open immediately in the Microbiology and Cell Science Department, University of Florida, for a qualified *U.S. citizen or permanent resident* with a demonstrated interest in prokaryotic molecular genetics and regulation. The position is funded by an NIH Recovery Act Supplement for "Mechanism of CsrA-Mediated Global Control," to investigate a global regulatory system involving an RNA binding protein (CsrA) and noncoding regulatory RNAs. Applicants should electronically submit their curriculum vitae and three references to: **Tony Romeo, Ph.D., e-mail: tromeo@ufl.edu.**

POSITIONS OPEN

COLLEGE of CHARLESTON
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

CHEMICAL BIOLOGY

The College of Charleston Department of Chemistry and Biochemistry, located in historic downtown Charleston, South Carolina, invites applications for a tenure-track position as an **ASSISTANT PROFESSOR** in chemical biology to begin August 2010. Applicants must possess a terminal degree. Postdoctoral experience is highly desired. Teaching responsibilities will inlude both lecture and laboratory. The successful candidate must initiate and sustain a research program that includes undergraduates. Review of applications will begin October 1, 2009, and continue until the position is filled. For more information visit **website: http://www.cofc.edu/~chem.** Questions may be directed to **e-mail: heldrichr@cofc.edu; telephone: 843-953-5515.**

ENDOWED PROFESSORSHIP
IN NEUROPHYSICS OR BIOPHYSICS
Trinity University, San Antonio, Texas

The Department of Physics and Astronomy at Trinity University invites applications for the Williams Endowed Professorship in Neurophysics or Biophysics, to begin in August 2010. This position, along with three recently filled positions (two senior and one junior), will enrich our rapidly growing undergraduate major in neuroscience at Trinity, which was established with support from the Howard Hughes Medical Institute (**website: http://www.trinity.edu/org/hhmi/Curriculum_Development/neuroscience_major.html**). The Neuroscience Program is interdepartmental with six faculty from biology, psychology, and chemistry, and with this position will now include physics. It is a vibrant Program with research interests ranging from behavioral psychology to reproductive behavior to the neurochemistry of neurodegenerative disease. Requirements include a Ph.D. in physics or neuroscience with a strong physics component, a current tenured position at the rank of Associate Professor or higher, and a distinguished record of teaching and research. Preference will be given to candidates who can enhance connections with the Psychology and/or Biology Departments and can contribute to a degree program in neuroscience, and who will establish a research program that includes participation by students. Candidates in experimental, theoretical, or computational research are all encouraged to apply. Please submit curriculum vitae and detailed statements of experience and interests in teaching and research to: **Chair of the Search Committee, Endowed Professorship in Neurophysics or Biophysics, Department of Physics and Astronomy, Trinity University, One Trinity Place, San Antonio, TX 78212**. Applicants must also arrange for three letters of reference to be sent to the Search Committee. Review of complete application files, with letters, will begin on December 1, 2009. The Department of Physics and Astronomy (**website: http://www.trinity.edu/physics**) has six faculty and attracts outstanding students. Active research areas include astrophysics/cosmology, atmospheric physics, dynamics and pattern formation in soft matter and biological systems, nanomaterials/plasmonics, and gravitational wave detection. *Trinity University is an Equal Opportunity Employer. We strongly encourage applications from qualified women and minority candidates.*

The Department of Pathology and Cell Biology at Columbia University seeks highly qualified individuals for faculty positions in surgical pathology, anatomic pathology, and research. Appointments can be at the **ASSISTANT, ASSOCIATE, PROFESSOR,** or **ASSOCIATE RESEARCH SCIENTIST** level depending on experience and qualifications. Clinical positions require Board certification and a license in New York State prior to the start of service. Research positions require record of publication in leading journals and a statement of research directions. To apply online go to **website: https://academicjobs.columbia.edu/applicants/Central?quickFind=51975.**

Columbia University takes Affirmative Action toward Equal Employment Opportunity. Women and minorities are encouraged to apply.

Finding a Partner for Your Ph.D.

The selection of a doctoral program: it's not quite marriage, but it's a long-term commitment that could turn into a lifetime relationship, perhaps impacting—positively or negatively—the rest of your research career. **By Emma Hitt**

Most people graduating with their Ph.D. in science will say that graduate school represents a very different scenario than simply attending classes and passing exams, as during their undergraduate studies. A doctoral degree can sometimes take six or more years—and there's no guarantee that the joyous day ending with a diploma in hand will even materialize. Unlike the undergraduate experience, earning a doctoral degree is largely dependent on one's efforts to conduct research, write journal articles, and most important, complete a dissertation. Success in these areas arises at least in part from picking a graduate school and a program that represents a suitable match, selecting the right adviser, and perhaps experiencing a little luck with your research projects. On what basis, therefore, should a graduate school be selected? And how, exactly, can one sift through the myriad of potential matches to make this momentous decision?

Patricia Burchat

The Right Match

According to data from the Ph.D. Completion Project conducted by the Council of Graduate Schools (CGS)—the main organization representing graduate students in the United States—fewer than 60 percent of students entering graduate school in the sciences will complete their doctoral degree within a 10-year time frame. About one in five people in the life sciences drop out entirely during the program. And by about year six, according to the project data, only about 42 percent of doctoral students in the life sciences will have completed their degree, 34 percent will still be slaving away at it, and 24 percent will have thrown in the towel. For the math and physical sciences, only about 39 percent complete their degree by year six, 27 percent are still going, and 34 percent have dropped out. These data underscore the need to pick a graduate school wisely.

"Completing a doctoral degree has much more to do with the right match of a graduate program to a given student than it does with personality characteristics or even the academic preparation of an individual student," says **Debra W. Stewart,** president of the CGS. An excellent, motivated student may fail to succeed in a program that is not an appropriate match; whereas a less academically prepared student may blossom in the right environment.

One important aspect of the match has to do with academic climate, Stewart says. "Some students might do well in a kind of dog-eat-dog, highly competitive environment; other students would be crushed by that, and would do much better in a much more laid back, laissez faire–type environment," she says. "You have to know yourself, and you have to really know the kind of climate you need to ensure that you grow and develop in your intellectual and professional life as a result of this program."

> "A new lab rotation each quarter allows students to learn more about the research pursued by the group and gives a student the opportunity to test the 'culture' in the group before making a decision."

Quality Counts

"We increasingly encourage students to gain information, including about placement of graduates of the program, how long it typically takes a student to get a degree, and what percentage of the students who begin programs actually complete them," says Stewart. "All of those are dimensions of quality," she says.

Above all, "the most important criteria for selecting a graduate school should be the quality and commitment of the faculty," says **G. Steven Martin,** department chair of Molecular and Cell Biology, at the University of California, Berkeley. Factors to consider when evaluating the quality of the faculty include the number, impact, and significance of scientific publications, in addition to any honors and recognition received, he says. If a PubMed search on a potential academic adviser pulls up only a few publications in recent years, or if the publications are all in specialized or archival journals, then this may indicate that the faculty member has a limited interest in research, and perhaps spends continued »

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BS/MS: FINDING THE RIGHT GRAD PROGRAM

"Increasingly, many programs recruit students to visit the school maybe after acceptance, but before the student makes a decision, to try ensure that it is the right match."
—Debra W. Stewart

more time as an administrator or in teaching activities. Likewise, if the faculty member's institutional or lab website does not clearly describe the lab's achievements or convey a sense of excitement about the research or future directions, then this, too, may indicate that selecting a more active lab might provide a more productive and satisfying graduate school experience.

An Interest in Interests

A key question with respect to match, also, is whether the faculty available at an institution meshes with a student's particular research interests, Stewart says. "You can have a fine program across the board, but it might not have a sufficient number of faculty members in a specialty area desired by the student." Even within a specific scientific discipline, for example biology, the range of study—genetics, cell biology, botany, entomology, marine biology, zoology to name a few— can be very diverse. The same holds true for the physical sciences. Making sure a doctoral program can accommodate a student's specific interest is an important first step in the selection process.

For students with a clearly defined idea of what they want to study, perhaps the best resource will be a professor at an undergraduate institution who currently serves as an adviser or mentor. A student who has worked with a professor in a lab during undergraduate studies and wants more of the same may ask for a recommended professor or program of related research at another institution.

However, not all students have a clearly defined path when they go to graduate school, so one strategy for a successful match is to pick a larger school, with many different types of programs and qualified faculty. "The interests of students often develop and change between undergraduate and graduate studies because the student is exposed to many more research options as a first-year graduate student than they likely were even aware of as an undergraduate," says **Patricia Burchat,** department chair, Physics, Stanford University. "Therefore, I think students should look for schools with a variety of programs of interest, rather than focusing on one particular research area, laboratory, or adviser," she says. The more opportunities that are available, the better the chance will be of selecting a lab that will match your interests and for completing the venerable degree program.

Some graduate science programs offer the opportunity to perform research rotations in various laboratories and with different professors. Rotations are a way to make sure that a lab is a good match, both personally and professionally, before settling in. This is especially important since it is often difficult to switch labs later on, and doing so may add months or years to a degree program. The Ph.D. programs in physics and applied physics at Stanford give first-year graduate students the opportunity to rotate through different research groups during their first year. "A new lab rotation each quarter allows students to learn more about the research pursued by the group and gives a student the opportunity to test the 'culture' in the group before making a decision," Burchat says. "The rotation system also allows the adviser to interact with the student before making the very significant commitment to mentor and support the student for her/his graduate career, and helps promote better matches between student advisees and faculty advisers and research areas," she adds.

Financially and Geographically Speaking

Other aspects to consider include more practical issues, such as financial support, that may or may not be available to the student. One question to ask is, what is the average number of years it takes students to complete the program? since each year spent in graduate school represents tens of thousands of dollars of foregone income. In the sciences, many Ph.D. programs offer a tuition waiver and a stipend in return for committing to an intensive schedule working in a laboratory and passing up other employment. Graduate programs will typically state this information on their website. Some package deals are better than others. The cost of living of a specific area must also be considered, as should personal preferences: a student attending a college that is nestled in the countryside is likely to miss the big city after five or six years, even though it's "just college" and they haven't "settled down" yet.

Other questions about the design of the program include the coursework required—does it closely match a student's interests? Does the school draw important seminar speakers within a field of research, and how often do these events take place? Does a student have the ability to participate in the academic life of the department and campus, such as by serving on faculty search committees, inviting seminar speakers, organizing student-led courses and seminars, participating in graduate student organizations? "These are all important aspects of graduate school life," says Martin.

"One trend we have written about is that an increasing number of research universities are building large, open 'megalabs,' where investigators share space and supplies," says **Jennifer Ruark,** a deputy managing editor at the *Chronicle of Higher Education*. "The goal is to get scientists interacting with each other and to foster interdisciplinary research—particularly in the life sciences, such as among developmental biologists, structural continued »

Featured Participants

Chronicle of Higher Education
www.chronicle.com

Council of Graduate Schools
www.cgsnet.org

Google
www.google.com

PhDs.org
www.phds.org

Quintessential Careers
www.quintcareers.com

Stanford University
www.stanford.edu

University of California, Berkeley
berkeley.edu

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BS/MS: FINDING THE RIGHT GRAD PROGRAM

"The problem with most rankings is that someone else is determining what is important."
—Geoff Davis

10 Questions To Ask ...

... A Current Student	... The Faculty
1. What do you see as the strengths of the program?	1. What are the major strengths of this program?
2. What do you see as the weaknesses of the program?	2. What are potential weaknesses of the program?
3. How accessible are the program faculty?	3. How would you describe the faculty?
4. What are the research facilities like—does there seem to be adequate funding, supplies, and equipment?	4. Are the classes team taught or taught by one individual?
5. If you had it to do over, would you select this graduate school again? Why/why not?	5. How would you describe the academic environment here compared with other schools?
6. What is the surrounding location of the school campus like? Are there leisure activities, good housing, and other amenities?	6. What attributes does a graduate student need to be successful at this school/in this program?
7. What is campus life like—do you have an opportunity to become involved and/or take on leadership roles?	7. Do you see any common characteristics in students who have quit their doctoral degree programs here—if so, what were they?
8. Do you feel that there is an adequate level of financial and academic support to complete your studies?	8. What research facilities are available and how adequate is intramural and extramural funding for the labs?
9. How competitive and difficult are the academic standards here?	9. What have recent graduates of the same program done with their degrees?
10. Is there anyone else I might talk to who could help provide an accurate picture of the program?	10. What unique features are offered by this school compared to other similar schools?

biologists, and chemists," she says. "Such a learning environment may represent an attractive feature for some students."

Strategies for Sleuthing a School

One obvious step for evaluating a potential graduate program is to visit the school. This is often part of the graduate school admissions process, during which prospective applicants have a chance to meet with students and faculty. "Increasingly, many programs recruit students to visit the school maybe after acceptance, but before the student makes a decision, trying to ensure that it is the right match," Stewart says.

According to **Randall Hansen,** education and career coach and founder of Quintessential Careers, four steps can be taken to evaluate a graduate program. The first step is to find a list of schools that offer the degree (and specialization) sought. "There are any number of books, websites, and even some professional organizations that offer this information." The second step is to spend time researching each graduate program, starting with each program's website. "Review what they say about themselves, their faculty, their students, and their graduates. Look at costs, location, accreditation, culture, financial assistance, and anything else the program says about itself." The third step is to review any rankings or outside reviews and solicit the opinions of professors regarding the programs. The fourth step is to visit the top two or three programs and talk with current students about their likes and dislikes about the program to get a sense of the university and its surroundings.

One tempting approach is to look at the online rankings and apply to the top schools in your intended area of study. Online rankings, such as those from *Newsweek* and *US News and World Report,* "can be useful but not totally reliable," Berkeley's Martin says. "Rankings are a popular way to evaluate graduate programs," says **Geoff Davis,** a mathematician who developed the PhDs.org Graduate School Guide, and now is a researcher with Google. "The problem with most rankings is that someone else is determining what is important," he says. "One-size-fits-all definitely does not apply for graduate schools—there is no 'best' program—what's great for one person might be terrible for another," he says.

With so many options to choose from, making the right decision about graduate school can be bewildering. As was inscribed by the ancient Greeks at the entrance to the Temple of Apollo at Delphi, perhaps the most important piece of advice is to "know thyself." Doing so will go a long way toward choosing the right path. Another piece of advice, perhaps not from the ancient Greeks, although you never know, is to "do your homework." Thoroughly researching all the options is the best way to ensure selecting wisely. Finally, if all else fails, one can take solace from the fact that "nothing is irreversible," even a decision about graduate school, although let's hope it doesn't come to that.

Resources

Council of Graduate Schools. Resources for students. www.cgsnet.org/Default.aspx?tabid=160

Gradschools.com. A comprehensive online graduate school guide to finding the best graduate schools and graduate degree programs. www.gradschools.com

Petersons. A searchable database of graduate programs based on subject area, location, and other parameters. www.petersons.com

PhDs.org. A website with extensive information on graduate school selection that lets students generate their own rankings. http://graduate-school.phds.org

***Science*/AAAS Graduate Program Finder.** http://sciencecareers.sciencemag.org/career_development/tools_resources/graduate_programs

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National Center for Complementary and Alternative Medicine

The National Center for Complementary and Alternative Medicine (NCCAM), a component of the National Institutes of Health (NIH), Department of Health and Human Services, seeks an accomplished, innovative neuroscientist to serve as Scientific Director of its Intramural Research Program (IRP) and as senior investigator responsible for developing a new research program in mind-body medicine. This individual reports to the Director, NCCAM, and will serve as a member of the NCCAM leadership team.

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Applicants must possess an M.D., Ph.D., or equivalent degree in a biomedical field related to the mission of NCCAM and have professional experience reflecting a broad scientific background and experience in basic and/or clinical investigation. Applicants must be able to interact with full authority; have the demonstrated capability to plan and direct research programs of national and international importance; and have the ability to communicate with and obtain the cooperation of national and international organizations and individuals who represent wide-ranging views and competing priorities. Salary is commensurate with qualifications and experience. Full Federal benefits including leave, health and life insurance, long-term care insurance, retirement, and a savings plan (401k equivalent). Qualified individuals are encouraged to email their CV, bibliography, list of three references, and cover letter outlining their relevant experience and vision for leading the NCCAM IRP to: **nccamsrrecruits@mail.nih.gov, Subject Line: Scientific Director Search. Email receipt of applications and inquiries is preferred; however, candidates needing reasonable accommodation may fax application materials to 301-402-4741**. Application deadline is **October 1, 2009**.

Department of Health and Human Services
National Institutes of Health / National Eye Institute
Deputy Director

POSITION: The National Eye Institute (NEI), a major component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is seeking exceptional candidates for the challenging position of Deputy Director, NEI. The NEI is responsible for a national and international program to conduct and support research, training, health information dissemination, and other programs with respect to blinding eye diseases, visual disorders, mechanisms of visual function, preservation of sight, and the special health problems and requirements of individuals who are visually impaired. These activities are conducted in hundreds of extramural laboratories and clinics throughout the United States and in NEI's own facilities in Bethesda, Maryland.

The Deputy Director, NEI serves as second in command and principal advisor to the Director, NEI, in evaluating, planning, directing, and coordinating all activities related to the mission and function of the Institute, in the execution of policies, and in the allocation of resources to carry out these policies. On behalf of the Director, NEI, the Deputy Director represents the NEI in all matters and in this sense is the champion and guarantor for the intellectual and administrative environment at the Institute. The Deputy Director also serves as an ambassador and spokesperson for the Institute.

QUALIFICATIONS REQUIRED: Applicants must possess a Ph.D., M.D., or equivalent doctorate degree in the biomedical sciences, with broad senior-level research experience and experience in direct administration of a research program. Applicants should be known and respected within their profession, both nationally and internationally, as distinguished individuals of outstanding scientific competence and administrative capability. Candidates should have demonstrated leadership; serving as a spokesperson; planning, program assessment, and analysis of program objectives; resolution of operational problems and issues; and the ability to manage financial and human resources including building, motivating, and maintaining a culturally diverse staff. Experience with NIH administrative policies, procedures and operations are highly desirable but not essential.

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HOW TO APPLY: Interested candidates should send a letter of interest, including a brief description of research and administrative experience, curriculum vitae and bibliography, and the names of at least three references to: **Chair, NEI Deputy Director Search Committee at NEISearch@nei.nih.gov or 31 Center Drive, Room 6A03, MSC 2510, Bethesda, MD 20892**. Applications should be received by **September 15, 2009** and review of applications will begin soon thereafter, but applications will continue to be accepted and considered until the position is filled. For questions, contact **Dr. Paul A Sieving, Director, NEI at paul.sieving@nihgov**. Applicants are encouraged to browse the NEI Home Page at **http://www.nei.nih.gov/**

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Lucknow-226 001 (India)

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Central Drug Research Institute, Lucknow is a premier R&D Institute under the aegis of the Council of Scientific and Industrial Research (CSIR) which is an autonomous body under Department of Scientific & Industrial Research, Government of India. Applications on the prescribed forms are invited from the eligible persons of Indian Nationality for the following scientific and technical posts :-

1. Scientist Gr.IV(1): 06 Posts (SC:01, ST:02, OBC:02 & PWD:01) in Pay Scale /Pay Band of Rs.15600-39100 (PB-3) with Grade Pay Rs.5400/- [T.E. = Rs. 33724 pm]. Maximum Age limit 35 years.

Post Code 001: (01 post): **Area:** Information Technology Unit (Reserved for SC); **Post Code 002:** (01 post): **Area:** Microbiology (Reserved for ST); **Post Code 003:** (01 post): **Area:** Tissue & Cell Culture (Reserved for ST); **Post Code 004 :** (01 post): **Area:** Lab. Animals (Reserved for OBC); **Post Code 005:** (01 post): **Area:** S&T Management (PME) for Technical Information, Industrial Liaison & Planning (TIILP) Division (Reserved for OBC); **Post Code 006 :** (01 post) : **Area :** Lab. Animals [Reserved for PWD (for Hearing Impaired Persons having 40% or more disability)]

2. Scientist Gr.IV(2): 14 Posts (Unreserved) in Pay Scale/Pay Band of Rs.15600-39100(PB-3) with Grade Pay Rs.6600/- [T.E. = Rs. 39901 pm]. Maximum Age limit 35 years.

Post Code 007: (01 post): **Area:** Pharmaceutics; **Post Code 008:** (01 post): **Area:** Medicinal and Process Chemistry; **Post Code 009:** (01 post): **Area:** Medicinal and Process Chemistry; **Post Code 010:** (01 post): **Area:** Biometry & Statistics; **Post Code 011:** (01 post): **Area:** Botany; **Post Code 012:** (01 post): **Area:** Clininical & Experimental Medicine ; **Post Code 013:** (01 post): **Area:** Endocrinology; **Post Code 014:** (01 post): **Area:** Lab. Animals; **Post Code 015:** (01 post): **Area:** Molecular & Structural Biology; **Post Code 016:** (01 post): **Area:** Parasitology; **Post Code 017:** (01 post): **Area:** Pharmacokinetics; **Post Code 018:** (01 post): **Area:** Neuropharmacology Unit (Pharmacology); **Post Code 019:** (01 post): **Area:** CVS Unit (Pharmacology); **Post Code 020:** (01 post): **Area:** Pharmacology

3. Scientist Gr.IV(3): 15 Posts (Unreserved) in Pay Scale/Pay Band of Rs.15600-39100 (PB-3) with Grade Pay Rs.7600/- [T.E. = Rs. 45794 pm]. Maximum Age limit 40 years.

Post Code 021: (01 post): **Area:** Medicinal and Process Chemistry; **Post Code 022:** (01 post): **Area:** Medicinal and Process Chemistry; **Post Code 023:** (01 post): **Area:** Sophisticated Analytical Instrument Facility; **Post Code 024:** (01 post): **Area:** Sophisticated Analytical Instrument Facility; **Post Code: 025:** (01 post): **Area:** Biochemistry; **Post Code 026:** (01 post): **Area:** Biochemistry; **Post Code 027:** (01 post): **Area:** Clinical & Experimental Medicine; **Post Code 028:** (01 post): **Area:** Endocrinology ; **Post Code 029:** (01 post) **Area:** Drug Target Discovery & Development; **Post Code 030:** (01 post): **Area:** Molecular & Structural Biology; **Post Code 031:** (01 post): **Area:** Parasitology; **Post Code 032:** (01 post): **Area:** Pharmaceutics; **Post Code 033:** (01 post): **Area:** Pharmacokinetics; **Post Code 034:** (01 post): **Area:** Pharmacology; **Post Code 035:** (01 post): **Area:** Microbiology. For complete advertisement and other details like qualifications, experience and terms & conditions please visit our Website: **http://www.cdriindia.org/situationv.asp.**

Regional Centre for Biotechnology

An institution of research, training and education established within the Health Biotech Science Cluster by the Dept. of Biotechnology, Govt. of India, under the auspices of UNESCO

Career Opportunities in Interdisciplinary Areas of Biotechnology

The Regional Centre for Biotechnology (RCB), a newly created institution in the National Capital Region (NCR) of Delhi, would like to recruit scientists with potential for intellectual leadership and passion for both research and teaching in cutting-edge areas of biotechnology. A demonstrated record of scientific productivity in a wide range of areas including life science, biomedical science, engineering, physical and chemical sciences and material science, in the form of recent peer-reviewed publications in scientific journals of high international repute and/or internationally valid and productive patents is essential, as is some indication of independence in scientific thinking, and a minimum of three years of post-doctoral experience.

The RCB, an institution of excellence established under an agreement with UNESCO for providing opportunities to Indian and other Asian students and scholars, is one of the two major institutions being set up within a Health Biotech Science Cluster (HBSC). The HBSC is being developed by the Department of Biotechnology, Government of India, on a 200-acre site located within the NCR in Faridabad, and is intended to foster innovative conceptual research in a wide range of biotech-related sciences, with an initial focus on health biotechnology. The other sister institution in the cluster is the Translational Health Science and Technology Institute (THSTI). HBSC and the institutions therein will also include mission centres dedicated to vaccines, diagnostics and imaging, molecular medicine, platform technologies, informatics, experimental animal facilities, technology transfer and intellectual property services and clinical trial services. These strongly complementary institutions/centres will have seamless scientific collaborations and are being developed under the mentorship of major national and international research centres.

The primary focus of the RCB will be to provide world-class research, training and education in biotech sciences at the interface of multiple disciplines. The educational programmes of RCB will be designed to create innovative opportunities to engage in research and learn to integrate science, engineering and medicine to provide major breakthroughs of relevance to India and the region. Specialized domain-specific programmes will also be designed by the faculty of RCB and THSTI in new opportunity areas of discovery science and technological innovations such as (but not restricted to) drug discovery and systems biology, cell and tissue engineering, nano science and technology, and synergy of information technology and advanced biomaterials, in order to create highly specialized people who delve across disciplines.

Faculty appointments will be offered from entry to program coordinator levels depending on the quantum of experience and the quality of scientific productivity. Investigators will be provided shared laboratory space and adequate start-up resources. They are expected to generate eventual extramural project-based funding.

The permanent laboratories of the Centre will come up in the HBSC Campus at Faridabad over the next three years. Interim laboratories will function from Gurgaon, neighboring the South Delhi area, which has adequate housing, transportation and schooling facilities.

Interested scientists are invited to send their bio-data containing details of qualification, positions held, professional experience/distinctions, copies of notable publications, and the names, addresses, fax numbers, telephone numbers and electronic mail addresses of three potential referees, to the **Manager, Regional Centre for Biotechnology, c/o National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi –110067, India**. An advance electronic copy of the bio-data may be sent to the e-mail address: **rcb@nii.ac.in**. People working in public sector institutions within India are requested to have their applications forwarded through proper channels. Only short-listed candidates will be contacted for further discussions.

Tier 1 Canada Research Chair in Biorefining Processess or Bioenergy Research

Lakehead University invites applications and nominations of internationally recognized and respected scholars to be considered for a Tier 1 Canada Research Chair (CRC) in Biorefining or Bioenergy Processes. Candidates will focus on the development of novel bioproducts or bioenergy from forest biomass through transformative biological, biochemical, and/or thermo-chemical conversions technologies (such as microbial fermentation, enzymatic transformation, pyrolysis/gasification, and catalytic processing). The Chair should have expertise in one or more following disciplines including biology, applied microbiology, biochemistry, and chemical or bioprocess engineering. The Chair's areas of interest will include identifying biorefining pathways to specialty and commodity bioproducts or bioenergy: lignin, cellulose, hemicellulose or residual streams such as bark, sawdust, and sludge. The Chair's expertise will complement research underway at Lakehead University's Biorefining Research Initiative and support research collaborations with FPInnovations and the forest industry sector.

The successful candidate will collaborate with three Biorefining Research Chairs already established at Lakehead University's Biorefining Research Initiative to build a world-class program to deliver new bioproducts or bioenergy for forestry industry installations. For more information about BRI and the BRI team, visit **http://lubri.lakeheadu.ca**.

The successful candidate will hold a doctorate degree and have excellent research credentials and a demonstrated record of securing external research funding.

Tier 1 candidates must be researchers of international stature who can bring an innovative perspective to the university in carrying out and leading research, training researchers, and contributing to the PhD programs in Biotechnology and/or Forest Sciences. Furthermore, candidates are expected to have extensive research experience and all the qualifications for a tenured appointment at the rank of full professor or associate professor. Associate professors are expected to be promoted to the full professor level within one or two years of the nomination. Appointment is for an initial seven-year term and is renewable every seven years.

Lakehead University offers on campus and community-based programs, continuing education and distributed learning, and graduate programs at the Master's and Doctoral levels. Lakehead is a comprehensive university providing an impressive array of programs in professional, arts, and sciences and the west campus of the Northern Ontario School of Medicine. The University has an enrolment of approximately 7,500 students, with a large Aboriginal contingent at the Thunder Bay campus. The Orillia campus, which has just unveiled its first permanent building, will be the first official Leadership in Energy and Environmental Design (LEED) Platinum university campus in Canada.

For more information on these and all other positions as well as a complete listing of teaching opportunities and detailed course descriptions, please visit our website at

http://hr.lakeheadu.ca/employment.php.

lakeheadu.ca

Applications and nominations including a curriculum vitae, seven-year research plan, and three publications that demonstrate a significant contribution to the field should be sent to:

Dr. Rui Wang, Vice-President Research, Lakehead University, Thunder Bay, Ontario P7B 5E1

In addition, three confidential letters of recommendation should be sent under separate cover to Dr. Wang by the candidate's referees. Review of applications will begin on October 1, 2009.

Please note that all positions are subject to review and final approval by the CRC Secretariat in Ottawa. For additional information on the CRC program, please visit the program website at: **www.chairs.gc.ca**.

Lakehead University is an Equal Opportunity Employer. The CRC program imposes no restrictions with regard to nationality or current country of residence.

Lakehead University is a comprehensive institution with over 7,500 students and over 2,000 faculty and staff. The University has a main campus in Thunder Bay, Ontario and a branch campus in Orillia, Ontario. Lakehead offers a variety of programs at the undergraduate and graduate levels, including the PhD.

Lakehead
UNIVERSITY

香港城市大學
City University of Hong Kong

大隱隱於市 學研研出產
A World-class University
Celebrating 25th Anniversary

Worldwide Search for Talent

City University of Hong Kong is a dynamic, fast developing university distinguished by scholarship in research and professional education. As a publicly funded institution, the University is committed to nurturing and developing students' talent and creating applicable knowledge to support social and economic advancement. It is a forward looking university with over 20,000 students and more than 3,000 full-time faculty and staff from diverse international backgrounds and ethnicities. Currently, the University has six Colleges/Schools. Within the next five years, the University aims to recruit **200 more scholars** in various disciplines from all over the world, including **science**, **engineering**, **business**, **social sciences**, **humanities**, **law, creative media, energy**, **environment**, and **biomedical & veterinary sciences**.

Applications and nominations are invited for:

Dean of College of Science and Engineering

[Ref. A/592/29]

The College of Science and Engineering is the University's largest academic unit and intellectual core. It comprises eight constituent units: Biology and Chemistry; Building and Construction; Computer Science; Electronic Engineering; Mathematics; Manufacturing Engineering and Engineering Management; Physics and Materials Science; and Building Science and Technology. The College vision is to win international recognition as one of the best science and engineering colleges in the Asia-Pacific region. With seven Academicians, eleven IEEE Fellows, and many promising young scholars, the College has a very strong research profile, pioneering in many of the world's most cutting-edge discoveries. The College offers about 60 taught programmes at various levels, including associate degree to master's degree, and research degree programmes at MPhil and PhD levels.

Qualifications for Appointment

Candidates must be a distinguished scholar and researcher of international standing, with strong research and publication records and the capability to provide academic leadership in both undergraduate and postgraduate programmes. Substantial relevant experience in a senior academic position and evidence of strong management skills appropriate to working in an international environment are highly desirable. The successful candidate should also have a strategic vision to lead the College to new heights in academic achievement and an ambitious drive to foster the highest standards of research, scholarship and education.

Salary and Conditions of Service

Remuneration package will be commensurate with the appointee's credentials and experience. Excellent fringe benefits include gratuity, leave, medical and dental schemes, and relocation assistance where applicable. The appointee will be offered the deanship and a chair professorship simultaneously on a fixed-term contract of three years.

Application and Information

Further information on the post and the University is available at http://www.cityu.edu.hk, or from the Human Resources Office, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong [Fax : (852) 2788 1154 or (852) 3442 0311/email : deancse@cityu.edu.hk]. Please send the nomination or application with a current curriculum vitae and ask three academic referees to send their references directly to Ms. Rita FUNG of the Human Resources Office by **30 September 2009**.

Please quote the reference of the post in the application and on the envelope. The University reserves the right to consider late applications and nominations, and not to fill the position. Personal data provided by applicants will be used for recruitment and other employment-related purposes.

City University of Hong Kong was ranked the 18th in Asia according to *The Times Higher Education 2009* survey.
http://www.cityu.edu.hk

UNIVERSITY OF Nebraska Lincoln

JUNIOR AND SENIOR FACULTY POSITIONS DEPARTMENT OF BIOCHEMISTRY and REDOX BIOLOGY CENTER

The Department of Biochemistry is currently expanding its faculty in fundamental biomedical research and invites nominations and applications for three tenure-leading positions. These academic-year, state-funded appointments are part of the strategic plans of **UNL** and the **Institute of Agriculture and Natural Resources** directed to strengthen the molecular life sciences. These positions are housed in the **George W. Beadle Center**, which includes state-of-the-art core facilities in proteomics and metabolomics, genomics, crystallography, bioimaging, flow cytometry, bioinformatics, biophysical spectroscopy, and trace element analysis. It is expected that all three investigators will have or establish nationally recognized and extramurally funded research programs and contribute to the undergraduate and graduate teaching missions of the Department of Biochemistry.

Redox Biochemistry (#090428) – This position, with an **open academic rank**, is in redox biology with preference given to investigators addressing gene regulation, disease pathophysiology, redox-active trace elements, metabolism, proteomics and metabolomics, or microbial pathogenesis. The Department is interested in highly qualified individuals applying for this position at the Assistant Professor level; those applying at the Associate and Full Professor levels are expected to have a nationally recognized and externally supported research program and a minimum of three years experience teaching and/or mentoring students and/or research staff.

Molecular Genetics and Biological Chemistry (#090430) – This position, with preference for individuals at the **Assistant Professor** level, is in the broad areas of molecular genetics, biological chemistry, and signal transduction, with particular consideration given to investigators using systems biology approaches to study complex diseases including cancer, infectious disease or metabolic disease.

Proteomics/Metabolomics Mass Spectrometry (#090431) – This position, at the **Assistant Professor** level in conjunction with **Redox Biology Center**, an NIH-supported Center of Biomedical Research Excellence (CoBRE), is in the area of redox proteomics/metabolomics. Preference will be given to applicants employing advanced mass spectrometry techniques for analyzing modified proteins and metabolites and shotgun proteomics.

A PhD (or equivalent) in biochemistry or related field and two years postdoctoral experience (or equivalent) is required for all three positions. Each position comes with highly competitive start-up packages. Lincoln Nebraska boasts an outstanding quality of life that includes fine culinary and artistic treasures, a budding live music scene and numerous parks, golf courses and bike trails. In 2008, WebMD reported that Lincoln was the healthiest city in the United States.

To learn more about the University of Nebraska, the Biochemistry Department and the Redox Biology Center, visit **http://biochem.unl.edu**. Applicants should go to **http://employment.unl.edu**, search for the requisition number, complete the Faculty Academic/Administrative Information form, attach a letter of application, Curriculum Vitae, a succinct statement of research, and statement of teaching interests. Applicants must arrange for three confidential letters of reference to be sent directly to: **Search Committee, Department of Biochemistry, University of Nebraska, N200 Beadle Center, Lincoln, NE 68588-0664, USA** or by e-mail to **biochemistrysearch@unl.edu**. Review of applications will begin on **September 14, 2009** and continue until the positions are filled or the searches are closed.

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.

INFLUENZA — VIROLOGY/ IMMUNOLOGY

RESEARCH FACULTY POSITION

The Duke Human Vaccine Institute (DHVI) is seeking candidates for a faculty member with expertise in virology, immunology, and immunobiology of influenza virus infections. The faculty member will collaborate with other DHVI investigators and researchers on the isolation and propagation of newly emergent influenza strains. The successful candidate will be skilled in isolation of influenza virus from clinical material or tissue cultures, and will be able to perform antibody neutralization and other relevant assays. Research will be performed in Duke's state-of-the-art BSL-3 NIAID/NIH Regional Biocontainment Laboratory. The candidate should be willing and able to conduct research in BSL-2 and BSL-3 laboratories, including working with animals in biosafety containment environments, and must be able to complete CDC Select Agent Registration. With support from the DHVI team, the faculty member will be expected to develop an independent research program over a three-year period. Qualified candidates must have a MD and/or PhD in a relevant discipline and have completed a minimum of two years post-doctoral training in influenza biology/virology research.

Salary and academic ranking will be commensurate with education, training, and experience level. Candidates should email or send a letter of interest and current CV to: **Search Committee Chair, Duke Human Vaccine Institute, Box 103020, 106 Research Drive, Durham, NC 27710. Email: dhvifacultysearch@notes.duke.edu**

DukeMedicine

Duke is an Equal Opportunity/Affirmative Action Employer. Minorities and women are encouraged to apply.

iavi International AIDS Vaccine Initiative

Senior Director, Vaccine Design

The International AIDS Vaccine Initiative (IAVI) is a global, not-for-profit, public-private partnership organization working to speed the search for a vaccine to prevent HIV infection and AIDS. Founded in 1996 and operational in 24 countries, IAVI, and its network of partners, research and develop vaccine candidates. IAVI also advocates for a vaccine to be a global priority and works to ensure that a future vaccine will be accessible to all who need it.

IAVI is a young, dynamic and mission-driven organization, and the work environment is fast-paced and intellectually stimulating. IAVI's culture, like that of a biotech company, is one that involves working collaboratively with a variety of internal and external peers, using scarce resources effectively, and sharing a common sense of commitment and purpose. In a global search effort, IAVI seeks a Senior Director, Vaccine Design. IAVI's AIDS Vaccine Design and Development Lab, Brooklyn, New York. IAVI has regional offices in Amsterdam, Johannesburg, Nairobi, and New Delhi, with a Human Immunology Lab in London, and a new Neutralizing Antibody Research Center in La Jolla, California.

IAVI is recruiting for a Senior Director, Vaccine Design at its Design and Development Lab, situated in Brooklyn, New York. This individual will be responsible for implementing IAVI's R&D work-plans, milestones and goals. These encompass:

- Identification of promising vaccine concepts in collaboration with IAVI's consortium partners
- Translation of these concepts into vaccine leads
- Transition of leads into and through development
- Generation and testing of immunogens that will elicit broadly neutralizing antibodies
- Generation and testing of potent and safe vector candidates for vaccines
- Rigorous testing of the leads to be sure they can and should move forward
- Identification and implementation of predefined Go/No Go decision points.

IAVI's AIDS Vaccine Design and Development Laboratory (the Design Lab) serves as the hub of IAVI's global vaccine discovery effort, closely linked with IAVI's Live Attenuated Consortium, Neutralizing Antibody Consortium, Vector Consortium, Human Immunology Laboratory (London), and an expanding network of partners.

The Senior Director, Vaccine Design reports directly to the Vice President, Vaccine Design. Its direct reports include the three Senior Directors in charge of Protein and Analytical Chemistry, Immunology, Vector Design, and Lab Facilities. **Other Responsibilities:** Lead the process for the review, evaluation, and investment recommendations of new technologies and innovations for AIDS vaccine discovery, in collaboration with the Vice President for Vaccine Design, other senior members of the R&D staff, and IAVI staff responsible for business development.

Leadership and Management: • Ensure that the highest quality of scientific skill, diligence and rigor is applied to all projects within the Design Lab • Manage short-term and long-term Design Lab resource needs and facility budgets to achieve organizational objectives • Manage Design Lab staff and consultants, staff the Lab effectively to achieve objectives, recruit and evaluate the performance of all personnel, provide coaching and mentoring to staff and develop and maintain an organizational structure with appropriate functional responsibilities to accomplish business goals. Additionally, ensure that scientific staff remains at the cutting edge of the scientific disciplines required for peak performance in their jobs • Establish effective working relationships with other departments at IAVI, and external biotechnology and pharmaceutical partners to advance IAVI's mission.

Qualifications and Experience: We seek an individual who possesses a strong foundation in vaccine science with a solid scientific reputation internationally. The successful candidate will have: • Experience directing a vaccine project from research to clinical development in industry, government and/or academia • Insight into recognizing promising lead candidates early while balancing speed and probability of success • Expertise in generating and interpreting key data demonstrating preclinical vaccine performance • An understanding of priority-setting, resource management, and the bridge-building required to foster scientific cooperation • Ability to formulate scientific based Go/No Go decision points, based on immunological, protection and safety endpoints • A M.D. or Ph.D. degree in a biological science, including immunology, microbiology, virology, biochemistry or bioengineering • Additional specific skill sets and experience in the following areas would be desirable: Animal model testing of vaccines, immunology, antigen design, adjuvants, HIV biology, generation of an IND, and application for peer-review funding

IAVI operates as an intense mission-driven organization trying to solve one of the most difficult and important scientific and policy challenges of our times. As a result, a passion for the mission is a critical attribute.

Leadership and Management Behavioral Competencies: • A builder of strong, productive personal and professional relationships and interconnected networks of people and organizations • Highly developed and effective interpersonal and communication skills, verbal and written; able to communicate sensitively and compellingly with individuals as well as large groups across cultures and languages • Encourages open communication and participation with an emphasis on transparency and the sharing of information • Strong conceptual thinker who can drive quickly to a clear and concise synthesis of even the most complex issues and concepts • A visionary leader who exudes a passion for the mission that is inspiring and contagious • Defines ambitious goals and establishes priorities, designing processes and managing projects and resources that align to achieve those goals • A champion of innovation who responds to complex problems and challenges by applying new perspectives and exploring a variety of relevant possible solutions • A team player and team-builder who is willing to work with and patiently align diverse stakeholders who maintain their individual autonomy and decision-making

Personal Characteristics: Respected for intellect and sound scientific judgment; Creative and flexible in approaching problem-solving and management in a globally diverse and highly matrixed environment; Dynamic, energetic; Strategic; Embraces change; Strong organizational skills; Values-driven with a high degree of integrity and moral authority; Disciplined and methodical; A resilient temperament; Excellent interpersonal skills, influencing skills and cultural competencies, with the ability to effectively manage across a global platform of partners and employees.

To submit your application, please go to **http://www.iavi.org/about-IAVI/careers/Pages/Careers.aspx**. Click on career search and apply to **Job ID 1359**.

THE OHIO STATE UNIVERSITY

The Department of Microbiology at The Ohio State University invites applications for **two tenure-track Assistant Professor** positions.

Preference will be given to applicants performing research in the areas of **Environmental Microbiology/Microbial Ecology** and **Molecular Microbiology**, but candidates of outstanding quality working in any area of Microbiology will also be considered. Applicants must have a Ph.D. or equivalent degree and at least 2 years of postdoctoral research experience. The successful candidates will be expected to establish and maintain an extramurally funded research program in their field of study and to actively support our strong training program including a commitment to excellence in teaching at both the undergraduate and graduate levels.

The Department currently has 15 full-time and 8 adjunct faculty members. For an overview of the Department, visit our website: **http://www.osumicrobiology.org/**.

Applications will be accepted until the positions are filled, but priority will be given to applications received by **October 1, 2009**. For full consideration, applicants should send a letter of intent, *curriculum vitae*, statement of research and teaching interests, and arrange for three letters of recommendation to be sent by e-mail to: **micro.grad@osu.edu**.

Additional questions about this search should be directed to **Dr. Juan D. Alfonzo**, Chair of the Search Committee (**alfonzo.1@osu.edu**)

To build a diverse workforce The Ohio State University encourages applications from minorities, veterans, women and individuals with disabilities. Flexible work options available. Ohio State is an NSF ADVANCE Institution. EEO/AA Employer.

NUS National University of Singapore

Fellows Positions in the Research Centre of Excellence in Mechanobiology

This newly established Research Centre (RCE) at the National University of Singapore (NUS) is based on a collaborative interdisciplinary approach to molecular, cellular and tissue mechanics. Through quantitative biophysical approaches, we will address major problems in biomedical sciences including pathogenesis (from bacterial to cancer), tissue regeneration and function. We are seeking outstanding candidates with strong background in molecular and cell biology, biophysics, biology-related engineering or computer sciences. Candidates should be prepared to advise on interdisciplinary team projects as well as to execute their own research.

We are seeking outstanding postdoctoral candidates at two levels, Junior and Senior Fellows.

Junior Fellows: These positions are for recent Ph.D.s or Ph.D.s who are changing fields. Junior fellows are recruited to work with PIs in the RCE (see faculty at web site).

Senior Fellows: These positions are for more experienced postdocs who will manage a funded team project in the Centre. In addition to funds to complete the project, Senior Fellows will have access to funds to hire personnel and to develop the results for additional funded projects independently.

For more information on the Centre, please see website **www.dbs.nus.edu.sg/mechano/index.html.**

The Centre offers competitive salary package, and housing allowance.

Please submit your application (form downloadable from www.dbs.nus.edu.sg/mechano/index.html), along with curriculum vitae, research interest, names of three external referees and an indication of a PI or PIs you may wish to work with (if any) to:

Chair, Recruitment Committee
RCE in Mechanobiology
National University of Singapore
14 Science Drive 4
Singapore 117543
Fax: (65) 67795671
Email: mechbio@nus.edu.sg

ASSISTANT PROFESSOR IN NATURAL SCIENCES & THE LEARNING SCIENCES

The University of Illinois at Chicago invites applicants for a joint faculty position at the rank of assistant professor in Natural Sciences and the Learning Sciences beginning August 16, 2010.

The position is offered as part of a university-wide interdisciplinary initiative in the Learning Sciences (http://www.lsri.uic.edu), which currently has four split-appointment faculty members in addition to over a dozen active associated faculty. A Ph.D. program for Learning Sciences has recently been approved and has enrolled its first students. The natural science departments (Biological Sciences, Chemistry, Earth and Environmental Sciences, and Physics) situated in the College of Liberal Arts and Sciences have strong programs in teaching and in all areas of basic research, including vibrant Ph.D. programs.

We seek applicants with a record of research and publication focusing at the nexus of an area of Natural Science and the Learning Sciences. Candidates may hold a doctorate in a natural science or a closely related field, or may hold a master's degree in a natural science in addition to a doctorate in the Learning Sciences or a closely related field. They should have a demonstrated record of research focusing on the support of science learners at one or more levels. Position responsibilities include carrying out a program of research and scholarship at the national level, teaching in the natural science department where the person will be appointed and teaching graduate courses in the Learning Sciences Program.

Applicants for the position must submit a vita and statement of research and teaching interests. These should be submitted electronically to lssearch@uic.edu. Three letters of recommendation should be forwarded to the LS/NS Search Committee in care of Deana Donzal (deana@uic.edu). Please contact Deana for surface mail address if you are unable to submit electronically. Review of applications will begin immediately and continue until the position is successfully filled.

UIC is an Affirmative Action/Equal Opportunity Employer seeking applicants who are from diverse backgrounds and/or have disability status. Final authorization of the position depends upon availability of state funding.

UIC University of Illinois at Chicago

NATIONAL CENTRE FOR CELL SCIENCE

(An autonomous Institution of the Department of Biotechnology, Government of India)

Advertisement No. Admn-01-2009

RESEARCH SCIENTISTS

National Centre for Cell Science is a premier institute recognized nationally and internationally for its contribution in different areas of Modern Biology. For its expanding activities, the Centre seeks to recruit Research Scientists in positions of Scientist 'C' and Scientist 'D' to pursue cutting edge research in the areas of Stem Cell Biology / Regenerative Biology, Neurobiology, Developmental Biology, Systems / Computational Biology and translational Medicine. Interested applicants holding Indian Citizenship may send their applications with CV and 3 reference letters to the Director, NCCS. For detailed advertisement, please visit our website http://www.nccs.res.in/admn-01A-2009.html

Director
National Centre for Cell Science
NCCS Complex, Ganeshkhind P.O.,
Pune – 411007, Maharashtra, INDIA
Phone: 91-20-25708121
Fax: 91-20-25692259
E-mail: director@nccs.res.in

The complete application should reach within six weeks from the date of appearance of this advertisement.

Search for Research Leaders in Strategic Areas

Sultan Qaboos University (SQU), the national university of the Sultanate of Oman and is one of the regional leaders in research and postgraduate training, intends to develop focused research programs in areas of

Solar Energy
Enhanced Oil Recovery
Plant Pathology
Marine Biotechnology
Metabolic Syndromes
Cancer
Nanotechnology for Water Desalination

These programs will be led by outstanding researchers who will serve as **Directors of Research Centers** or **Research Chairs** and will be provided with highly competitive salary packages and all resources needed to establish strong research teams. They will be expected to build capacity at SQU, promote collaboration locally, regionally and internationally, and contribute significantly to addressing the strategic research goals of the Sultanate.

SQU calls for expressions of interest by senior academics and researcher having a breadth and depth of experience in these areas. For more details please log in to the University's website: **www.squ.edu.om** at the link: **Job Vacancies.**

SULTAN QABOOS UNIVERSITY
Sultanate of Oman

University of Miyazaki
Assistant Professor Positions in Interdisciplinary Research Areas

The University of Miyazaki, one of the National University Corporations in Japan, has commenced the Model Program for Young Principal Investigators. For this 5-year program, we have 10 assistant professor positions, seeking outstanding young researchers who are able to independently pursue original and innovative research in the four interdisciplinary research areas as follows:

I. Research into pathogenic and industrially important microorganisms based on genome analysis.
II. Searches, basic research, and clinical applications for novel bioactive peptides.
III. Fundamental research and practical applications for biomass/energy resource conversion.
IV. Production of high-quality agricultural, marine, and livestock products and development of functional foods.

Applicants should be able to communicate in English and have a Ph.D. in a related field (awarded within the past 3 to 10 years). Female applicants and/or applicants currently residing outside Japan (both Japanese and foreign nationals) will be preferentially appointed if their research accomplishments are at the same level as those of the other applicants. Appointed assistant professors are expected to effectively and flexibly develop their research, provided with research funds, independent environments and support systems.

For more information, please refer to our official website at:
http://www.miyazaki-u.ac.jp/ir/
Application Deadline:
5:00 pm, September 30, 2009 (Japan Standard Time)

Contact information: Interdisciplinary Research Organization Office
1-1 Gakuenkibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan
TEL: +81-985-58-7859 FAX: +81-985-58-7860
E-mail: iroffice@of.miyazaki-u.ac.jp

WE BELIEVE

THE CURE WILL BE FOUND RIGHT HERE

Faculty Positions: Melanoma Research

The Donald A. Adam Comprehensive Melanoma Research Center at the Moffitt Cancer Center is seeking laboratory-based faculty members with a Ph.D., M.D. or M.D. Ph.D. with an interest in melanoma research. The prospective candidates will be appointed at the Associate or Senior Member level, and it is expected that they would establish an independent funded laboratory research program concentrating on translational melanoma investigation in the fields of tumor microenvironment, apoptosis or the cell cycle.

An outstanding start-up plan is available, as well as a highly competitive salary package with excellent lab space. A specific attraction is the opportunity to interact with ongoing well funded research programs in molecular oncology, drug development, population science and translational immunology/immunotherapy. The Comprehensive Melanoma Research Center brings together clinicians, basic and translational scientists at Moffitt to aggressively pursue new ideas in the etiology, treatment and prevention of melanoma. At the Moffitt Cancer Center, significant growth in basic and translational research, in laboratory space resources and faculty recruitment will occur in the next decade as a high priority.

Consistently ranked in U.S. News & World Report "Best Cancer Hospitals" for the past ten years, Moffitt Cancer Center and Research Institute is a free-standing, not-for-profit cancer center. Moffitt opened on the campus of Tampa's University of South Florida in October, 1986 and became Florida's only NCI designated Comprehensive Cancer Center in 2001. There is vast opportunity within our framework for information technology, scientific research, clinical treatment and quality of life studies, as we see over 15,000 new cancer patients each year. Our environment is one of multi-modality, patient-centered care requiring highly collaborative clinical research programs. Moffitt is committed to education through a wide range of residency and fellowship programs.

The Moffitt Research Institute is comprised of approximately 140 Principal Investigators, 58 laboratories, and 306,000 square feet of research space. The Cancer Center is comprised of a large ambulatory care facility, a 206-bed hospital, with a 36-bed blood and marrow transplant program, 12 state of the art operating suites, a 30-bed intensive care unit, a high volume screening program, and a basic science research facility.

The Moffitt Cancer Center is affiliated with the University of South Florida. Primary and secondary University appointments are available, as applicable. Academic rank is commensurate with qualifications and experience.

For inquiries about the position, contact Dr. Jeffrey Weber, Director, Donald A. Adam Comprehensive Melanoma Research Center, at 813-745-2007.

To apply, visit our website www.moffittcareers.org and refer to requisition number 4348.

MOFFITT CANCER CENTER

Moffitt Cancer Center provides a tobacco-free work environment, is an equal opportunity, affirmative action employer, and a drug free workplace.

Nontraditional Careers:

Opportunities Away From the Bench

Webinar

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists across different sectors and strategies you can use to pursue a nonresearch career.

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Participating Experts:

Dr. Lori Conlan
Director of Postdoc Services,
Office of Intramural Training and Education
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President
Cambridge BioPartners

Dr. Marion Müller
Director, DFG Office North America
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(German Research Foundation)

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Science Careers
From the journal *Science* AAAS

Autonomous Province of Trento
Provincial law of 2 August 2005, no. 14, article 22

European Union – 7th research framework programme
Marie Curie Action - COFUND

"Call for proposals 1 – post-doc 2009 – Incoming"
Abstract

The Autonomous Province of Trento has approved with decree n. 1604 dated 25th June 2009 the activation of the **"Call for proposals 1 – post-doc 2009 – Incoming"** aimed at funding research projects, according to article 22 of the provincial law of 2nd August 2005, no. 14, with the financial support of the European Commission, within the context of the 7th research framework programme for the period 2007-2013 - "People" specific programme - Marie Curie actions - COFUND - "Trentino - The Trentino programme of research, training and mobility of post-doctoral researchers" project.

The research projects must be presented by researchers with a PhD, obtained in Italy or abroad no more than three years before the deadline of this call, or to be achieved within the beginning of the project activities.

The research projects, for which the researchers take scientific and management responsibility, must be presented through the research bodies which have their registered office or operating centres inside Trento Province (host organisations).

"Call for proposals 2 – team 2009 – Incoming"
Abstract

The Autonomous Province of Trento has approved with decree n. 1604 dated 25th June 2009 the activation of the **"Call for proposals 2 – team 2009 – Incoming"** aimed at funding research projects, according to article 22 of the provincial law of 2nd August 2005, no. 14, with the financial support of the European Commission, within the context of the 7th research framework programme for the period 2007-2013 - "People" specific programme - Marie Curie actions - COFUND - "Trentino - The Trentino programme of research, training and mobility of post-doctoral researchers" project.

The research projects must be presented by researchers with a PhD, obtained in Italy or abroad at least five years before the deadline of this call, or with equivalent experience gained in qualified Italian or foreign research centres or laboratories.

The research projects, for which the researchers take scientific and management responsibility, must be presented through the research bodies which have their registered office or operating centres inside Trento Province (host organisations).

The researchers must not already belong to the personnel structure of these research bodies.

"Call for proposals 5 – post-doc 2009 – Reintegration"
Abstract

The Autonomous Province of Trento has approved with decree n. 1604 dated 25th June 2009 the activation of the **"Call for proposals 5 – post-doc 2009 – Reintegration"** aimed at funding research projects, according to article 22 of the provincial law of 2nd August 2005, no. 14, with the financial support of the European Commission, within the context of the 7th research framework programme for the period 2007-2013 - "People" specific programme - Marie Curie actions - COFUND - "Trentino - The Trentino programme of research, training and mobility of post-doctoral researchers" project.

The research projects must be presented by researchers with a PhD, obtained in Italy or abroad no more than five years before the deadline of this call, or to be achieved within the beginning of the project activities.

The researchers must have obtained an academic qualification in Trentino of at least secondary school level and have carried out research abroad, in qualified research centres or laboratories, for at least three years before the deadline of this call.

The research projects, for which the researchers take scientific and management responsibility, must be presented through the research bodies which have their registered office or operating centres inside Trento Province (host organisations).

"Call for proposals 6 – team 2009 – Reintegration"
Abstract

The Autonomous Province of Trento has approved with decree n. 1604 dated 25th June 2009 the activation of the **"Call for proposals 6 – team 2009 – Reintegration"** aimed at funding research projects, according to article 22 of the provincial law of 2nd August 2005, no. 14, with the financial support of the European Commission, within the context of the 7th research framework programme for the period 2007-2013 - "People" specific programme - Marie Curie actions - COFUND - "Trentino - The Trentino programme of research, training and mobility of post-doctoral researchers" project.

The research projects must be presented by researchers with a PhD, obtained in Italy or abroad at least five years before the deadline of this call, or with equivalent experience gained in qualified Italian or foreign research centres or laboratories.

The researchers must have obtained an academic qualification in Trentino of at least secondary school level and have carried out research abroad, in qualified research centres or laboratories, for at least three years before the deadline of this call.

The research projects, for which the researchers take scientific and management responsibility, must be presented through the research bodies which have their registered office or operating centres inside Trento Province (host organisations).

The guidelines for the formulation and the submission of the research project proposals, which must be edited according to a specific format, and any other aspect are reported in the call for proposals available at the internet site:

http://www.uniricerca.provincia.tn.it/bandi_ricerca.asp

Deadline for proposal submission is **5 p.m. of Monday September 14th, 2009.**

Head of the University and Scientific Department
- Fernando Guarino -

POSITIONS OPEN

EVOLUTIONARY BIOLOGIST TENURE-TRACK FACULTY POSITION

Augustana College, Sioux Falls, South Dakota, invites applications for a tenure-track **ASSISTANT PROFESSOR** in the Department of Biology beginning September 2010. A Ph.D. is expected; postdoctoral experience is preferred. The position carries both teaching and research expectations. A very generous research startup will be provided.

Salary is competitive and dependent upon qualifications; excellent fringe benefits are included.

Please visit our **website: http://www.augie.edu/jobs** for more information on the position along with the application procedure.

Augustana College is an Equal Opportunity/Affirmative Action/Title IX Employer. Qualified women and minority applicants are encouraged to apply. Applicants must comply with the Immigration Reform and Control Act and are required to submit official transcripts upon employment.

POSTDOCTORAL POSITION
Macrophage Signal Transduction
Beth Israel Deaconess Medical Center

A Postdoctoral position is available beginning August 2009 to study macrophage innate immune receptor–mediated signal transduction pathways. These NIH-supported studies focus on alveolar macrophage innate immune responses to opportunistic pathogens, and examine the influence of HIV-1 infection. M.D./Ph.D. applicants with at least two years of postdoctoral experience in macrophage cell and molecular biology, and with published experience or strong interest in HIV virology/cellular immunology and signal transduction pathways are encouraged to apply. Immunohistochemistry and confocal microscopy experience is desirable.

Applicants should submit a cover letter indicating your research interests and expertise, curriculum vitae, and three letters of reference to: **Souvenir D. Tachado, M.D., Division of Pulmonary and Critical Care Medicine, Dana 6, Room 652, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215. E-mail: stachado@caregroup.harvard.edu.** *BIDMC is an Equal Opportunity, Affirmative Action Employer.*

POSTDOCTORAL POSITIONS
University of Kansas Medical Center

Dr. Xiaobo Zhong in the Department of Pharmacology, Toxicology, and Therapeutics at University of Kansas Medical Center has two NIH-funded positions available to study epigenetic regulation of cytochrome P450 gene expression in liver development. Recent Ph.D. graduates with a background in epigenetics, fluorescence in situ hybridization, immunohistochemistry, confocal imaging, or bioinformatics are encouraged to apply. Applicants should provide a cover letter, curriculum vitae, and the names of three references. To review the position description and apply online, go to **website: http://jobs.kumc.edu** and search for **position M0202173.** *The University of Kansas Medical Center is proud to be an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOWSHIPS. NIH-funded positions are available in the laboratories of **Dr. Rajeshwar Tekmal,** Professor, and **Dr. Ratna Vadlamudi,** Associate Professor, Obstetrics-Gynecology, University of Texas Health Science Center at San Antonio. Projects involve studying estrogen receptor signaling in breast and ovarian cancer and in therapy resistance. Highly motivated candidates with training in molecular or cell biology and/or transgenic mouse models are desired. Applications including curriculum vitae and three references should be sent to: **Cissy Thorpe, e-mail: thorpe@uthscsa.edu.**

All postdoctoral appointments are designated as security sensitive positions. The University of Texas Health Science Center at San Antonio, Texas, is an Equal Employment Opportunity/Affirmative Action Employer.

POSITIONS OPEN

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To learn more and apply, visit **website: http://www.rti.org/job11323.**

We are proud to be an Equal Employment Opportunity/Affirmative Action Employer, Minorities/Females/Persons with Disabilities/Veterans.

POSTDOCTORAL POSITION
The University of Rochester
School of Medicine and Dentistry
Division of Endocrinology and Metabolism

Postdoctoral positions are available in the laboratory of **Stephen Hammes, M.D., Ph.D.,** Chief of Endocrinology and Metabolism at the University of Rochester School of Medicine. The laboratory studies steroid production and signaling in the ovary. The basic research focus is on nongenomic steroid signaling and G protein cross-talk with steroid and epidermal growth factor receptors. The translational focus is on diseases of androgen excess such as polycystic ovarian syndrome (PCOS) and androgen-dependent tumors such as prostate cancer. A highly motivated M.D. or Ph.D. with training in molecular biology and experience in handling mice is required. Please electronically transmit curriculum vitae and cover letter plus names of three references to: **Stephen Hammes, e-mail: stephen_hammes@urmc.rochester.edu.**

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

POSTDOCTORAL SCIENTIST
at the Institut Pasteur

The laboratory of Integrative Neurobiology of Cholinergic Systems at the Pasteur Institute of Paris has an opening for a young scientist:

Postdoctoral scientist, funded in the first instance by a two-year grant from the French National Science Foundation (ANR). The successful candidate will carry out slice electrophysiology in the laboratory. Prior experience in electrophysiology is required.

Interested candidates should contact: **Dr. Uwe Maskos, e-mail: umaskos@pasteur.fr,** and ask two references to send letters of recommendation.

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