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Education
& Technology
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Education & Technology

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COVER

As students become absorbed with technology-based games, educators grapple with how best to use technology. Immersive simulations represent one way in which new media can enhance traditional learning experiences. See the special section beginning on page 53.

Photo Illustration: Yael Kats; Images: Walter Bibikow/ Getty Images (desks); Alamy (girl)

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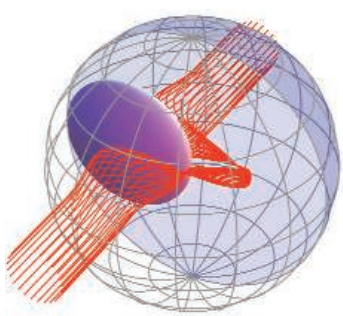

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S. Myong et al.
A host protein that recognizes invading viruses activates innate immunity defenses only when it senses both double-stranded RNA and a 5′ triphosphate.
10.1126/science.1168352

Neuronal Activity–Induced Gadd45b Promotes Epigenetic DNA Demethylation and Adult Neurogenesis
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Neural activity induces an immediate early gene that triggers epigenetic modifications needed for neurogenesis.
10.1126/science.1166859

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A selection of our favorite and most read stories.

A Whiff of Mad Cow
Normal form of protein linked to neurodegenerative conditions may aid sense of smell.

Is Morphine a Guy Drug?
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SCIENCEONLINE FEATURE

VIDEO: Education and Technology
An introduction to this week's special section about how education is changing in the face of technology.
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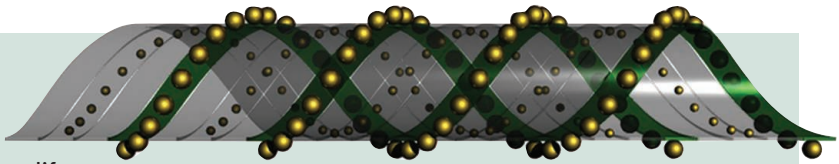
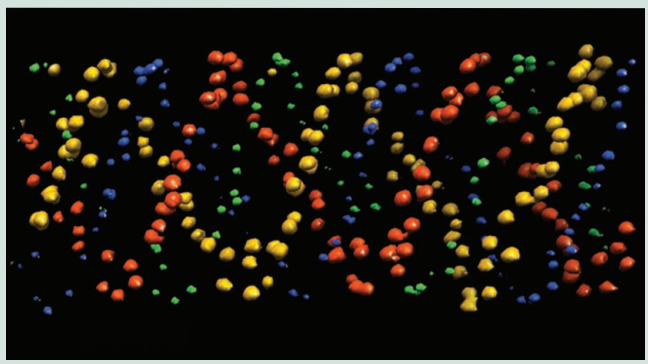
EDITED BY STELLA HURTLEY

Gold Nanoparticles on a Roll >>



The optical or electronic properties of nanoparticles can differ from those of the bulk material through confinement effects and through their interactions with one another through space. The possibilities for tuning and exploring such interactions would be enhanced if nanoparticles could be attached to frameworks so that they are arranged in three dimensions. **Sharma *et al.*** (p. 112) attached gold nanoparticles to single DNA strands and incorporated them into DNA networks that could curl up to form tubules. Electron tomography revealed that the nanoparticles were arranged on the surface of the tubules in a variety of spiral morphologies. In addition, through their own steric repulsions, the nanoparticles actively influenced the assembly process.



Keeping Fungal Invaders at Bay

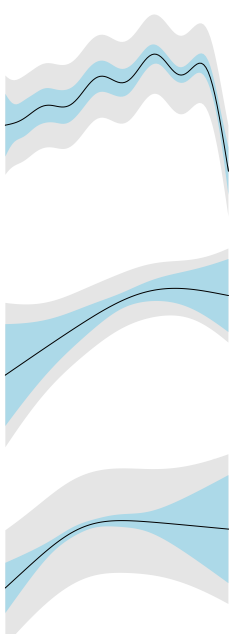
Plant cells mobilize a cell-autonomous response to the sites of attempted fungal penetration, resulting in the polarization of the peroxisome. The mechanism driving this polarization has remained unclear. Now **Clay *et al.*** (p. 95, published online 18 December) and **Bednarek *et al.*** (p. 101, published online 18 December) show that the pre-invasion resistance mechanism is due to the coordinated and infection-induced biosynthesis of a specific glucosinolate molecule. This molecule is made and delivered to the outside of the cell through the action of the peroxisomal proteins (PEN2 and PEN3) and is produced via a biochemical reaction that uses myrosinase products as a substrate. Bednarek *et al.* show that derived compounds of PEN2 play a key role as antifungal compounds and that this route of synthesis occurs in living cells and only in response to infection. Clay *et al.* have discovered how these myrosinases and their related breakdown products act as signaling molecules in the *Arabidopsis* defense response. Thus, a family of metabolites controls this nonhost resistance pathway.

Ultra Clean, Ultra Small

Carbon nanotubes have generally been found to fall into two categories—metallic or insulating. **Deshpande *et al.*** (p. 106) now report transport measurements on ultraclean individual single-walled carbon nanotubes. Even though the synthesized nanotubes are a mixture of nominally metallic and insulating types, all of the nanotubes were actually insulating, exhibiting an energy gap dependent on the radius of the tubes. This insulating behavior may be due to a Mott-insulating state resulting from electronic correlations in the tubes.

Adding Injury to Insult

Coral reefs worldwide are under terrible pressure from rising sea-surface temperatures, pollution, and overexploitation. Another emerging threat to their health is ocean acidification—the declining pH of surface seawater layers caused by the absorption of increasing amounts of atmospheric CO_2. Many studies have demonstrated recent declines in the coverage and numbers of live coral reefs, as well as reduced coral diversity, but few have examined how rates of coral calcification have been affected. **De'ath *et al.*** (p. 116; see the news story by **Pennisi**) examined growth patterns of 328 massive Porites corals from the Great Barrier Reef of Australia and found that their rates of calcification have declined by nearly 15% since 1990, to values lower than any seen for the past 400 years. The main causes of this continuing decline appear to be increasing water temperatures and ocean acidification.



A Warped Route to Cloaking

Transformation optics and metamaterials allow the manipulation of light with unprecedented control, giving rise to possibilities like invisibility, hyperlensing, and cloaking. However, most approaches so far have been based on mappings of Maxwell's equations in a Euclidean, flat space geometry, which has generally limited the extent to which these properties can be realized to monochromatic light. **Leonhardt and Tyc** (p. 110; see the Perspective by **Nicolet and Zolla**, published online 20 November) describe a theoretical approach based on mappings in a curved, or non-Euclidean, space that may open up applications to a broad range of wavelengths. Furthermore, the physical properties required of materials to achieve broadband invisibility need not be as extreme as in previous approaches.

Right on Target

Cotranslational protein targeting to membranes involves signal recognition particle (SRP) and its receptor. SRP contains a noncoding RNA, which catalytically accelerates the interaction of

CREDITS (TOP TO BOTTOM): SHARMA *ET AL.*; DE'ATH *ET AL.*

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SRP with its receptor. **Bradshaw *et al.*** (p. 127) now show that SRP RNA is a molecular switch that is triggered by signal sequences on secretory and membrane proteins. When activated in response to signal sequence binding, SRP RNA profoundly accelerates the forward rate of SRP binding to the SRP receptor. Previously, this effect was masked because detergent mimics the signal peptide, activating SRP RNA constitutively. Thus, cargo (the signal sequence) controls the SRP protein targeting machine.

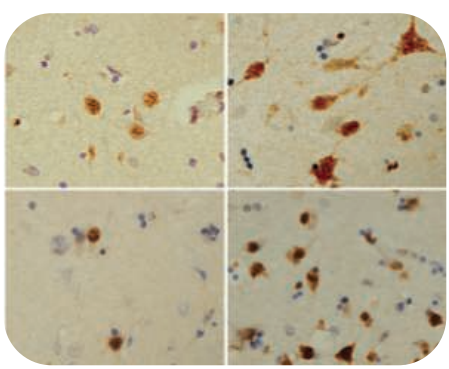
Bright Shiny Flowers

The study of flower color has primarily focused on chemical pigmentation. **Whitney *et al.*** (p. 130) explore how the structural features on the petal surface of a tulip flower generate color independently of chemical pigmentation via iridescence. Floral iridescence may result in an ultraviolet signal that is visible to insects. Bumblebees can learn to use information from iridescence to select among potential food sources. Thus, iridescence may contribute to plant-pollinator interactions.

Life's Too Short

Our ability to control scourges like dengue virus and the malaria parasite is undermined by a lack of vaccines and the evolution of drug and insecticide resistance. Control measures, which eliminate the older insects that have accumulated more parasites, not only reduce the transmission rate of the parasite but also remove the selection pressure for the evolution of resistance, because young adult insects are still allowed to reproduce. **McMeniman *et al.*** (p. 141; see the Perspective by **Read and Thomas**) studied the potential value of a life-shortening strain of *Wolbachia,* as a biological control agent. This bacterium was established in laboratory populations of the dengue-transmitting mosquito *Aedes aegypti* after a 3-year adaptation program. The *Wolbachia* did not eliminate egg production by the mosquito, but it did halve the adult's life span, which is probably enough to slow the transmission of a pathogen that takes several days to mature within its insect host.

Chaperone to Neurodegeneration

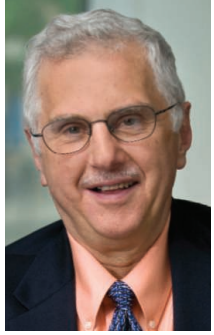


Although autophagy in general is known to play a role in the process of neurodegeneration, it is not clear what role, if any, chaperone-mediated autophagy, which selectively regulates the levels of specific cytoplasmic proteins, plays in cellular survival and death. Members of the MEF2 (myocyte enhancer factor 2) family are nuclear transcription factors involved in neuronal survival, differentiation, and synaptic function. Now **Yang *et al.*** (p. 124) provide in vitro and in vivo data from knockout mice, transgenic mice, and human brains to show that chaperone mediated autophagy directly targets MEF2 for lysosomal degradation. During the process, MEF2 is translocated from the nucleus to the cytoplasm. The chaperone-mediated autophagy of MEF2 is sensitive to the level of α-synuclein, increased levels of which can cause Parkinson's disease. Thus, chaperone-mediated autophagy can play a direct role in modulating the neuronal survival machinery, and may be involved in the process by which mutant and/or overexpressed α-synuclein can undermine neuronal viability.

Trading Toxins

Some bacterial viruses (phage) scoop up host DNA as they are packaged into particles. **Chen and Novick** (p. 139) show that this capacity can involve bacterial genes encoding virulence determinants, leading to their transfer to another species of pathogenic bacteria. Several temperate phage, able to integrate their DNA into bacterial chromosomes and reproduce without lysing their hosts, mediated the transfer of toxin genes from *Staphylococcus aureus* to *Listeria monocytogenes*. Both pathogens can occur simultaneously in bovine mastitis, and phage transduction of the pathogenicity determinants was seen to happen in raw milk. This veterinary condition is succumbing to antibiotic resistance, and phage therapy has offered an alternative bactericidal treatment; however, it may have the unwanted side effect of promoting the transfer of bacterial virulence factors among pathogens and thereby pose a risk to humans.

CREDIT: YANG *ET AL.*

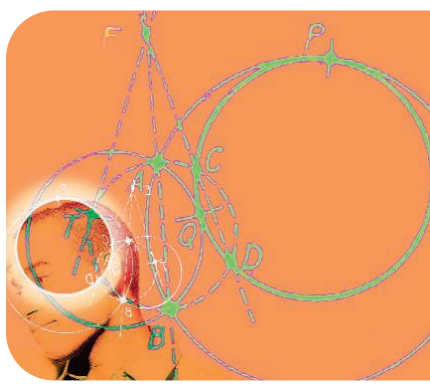


Bruce Alberts is the Editor-in-Chief of *Science*.

Making a Science of Education

FOR SUCCESS IN AN INCREASINGLY COMPLEX, CROWDED, AND DANGEROUS WORLD, A NATION must strive to be a meritocracy: Its education and social systems should be structured to select those with the most talent, energy, wisdom, and character as the next generation of leaders for each segment of society. When I was young, I was taught that providing equal opportunities for everyone was a matter of social justice—part of the social contract in the United States. Now, I believe that it is also a matter of national survival. Any country that fails to encourage and develop the talent in each individual through its public school system will suffer greatly, because the quality of a nation depends on the collective wisdom of both its leaders and its citizens.

An outstanding education system imparts values that support good citizenship, while empowering adults to be life-long learners and problem solvers who can make wise decisions for their families, for their communities, and for their workplaces. Such an education system must continually evolve to remain relevant to the interests and needs of each new generation. To achieve these ambitious goals, we will need much more emphasis on both science education and the "science of education." It is my hope that *Science* can help to promote progress on both scores.



In 2006, *Science* began a monthly Education Forum. We now plan to build on this strong beginning by recruiting high-quality articles on education from the world's best experts for every section of the magazine. Thus, we will be publishing important work in education as Perspectives, Policy Forums, Reviews, or as original Research Reports and Articles, while continuing to cover education in the News section. This first issue of 2009, with its focus on Education and Technology (see page 53), represents a start that will hopefully inspire many more articles to come.

As this special issue explains, the computer and communication technologies that have profoundly altered many other aspects of our lives seem to hold great promise for improving education as well. But technology is only a tool. To fulfill its promise for education will require a great deal of high-quality research, focused on its utilization and effects in both school and non-school settings. Only by collecting and analyzing data on student learning can we hope to sort out the many variables that determine effectiveness.

The same type of scientific research is also needed to explore, analyze, and improve each of the many other components of educational systems. For example, the most important element of any education system is a highly skilled teacher. Teacher recruitment, preparation, retention, and professional development all need to be informed by scientific research in education. Curricula, pedagogy, assessment, and school system management similarly require focused research. We hope that what scientists are learning about each of these important aspects of education will be reported and reviewed in *Science*.

Research in the social sciences is especially challenging because of the conditionality of its findings: The effects of an intervention are likely to depend on many variables that need to be studied and understood. Some readers may therefore question whether the science of education deserves a prominent place in this prestigious journal. For them, I offer the wisdom of Alfred North Whitehead, who wrote 80 years ago: "The art of education is never easy. To surmount its difficulties, especially those of elementary education, is a task worthy of the highest genius." [But] "when one considers…the importance of this question of the education of a nation's young, the broken lives, the defeated hopes, the national failures, which result from the frivolous inertia with which it is treated, it is difficult to restrain within oneself a savage rage. In the conditions of modern life the rule is absolute, [a country] that does not value trained intelligence is doomed."

The sense of rage is every bit as appropriate today. But we now recognize that we must look at the "art" of education through the critical lens of science if we are to survive.

–Bruce Alberts

10.1126/science.1169941

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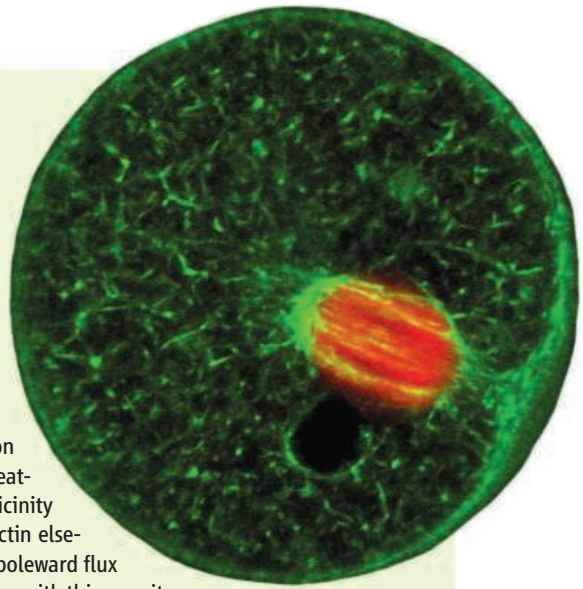
EDITED BY GILBERT CHIN AND JAKE YESTON

CELL BIOLOGY

Moving Toward Asymmetry



Asymmetric division intrudes at various stages of development in order to generate daughter cells with distinctly different fates. An extreme instance occurs during oocyte maturation when the oocyte expels half of its chromosomal capital by packaging them into a small polar body, leaving behind the vastly larger haploid egg. To pull off this feat the oocyte needs to move its centrally located mitotic spindle to the cell periphery, in a process that relies on the actin cytoskeleton.

Schuh and Ellenberg describe the role played by actin by watching spindle relocation in real time in live mouse oocytes. The actin cytoskeleton undergoes constant remodeling that relies on the activity of an actin-nucleating protein, formin-2. In particular, actin (shown at right, green) in the vicinity of the spindle (red) showed net flux toward the spindle poles, whereas actin elsewhere in the cell did not perform concerted directional movements. This poleward flux became especially apparent as the spindle approached the cell cortex. Along with this recruitment of actin, the activated form of the actin motor myosin-2 was also observed to accumulate near the spindle pole–associated actin filaments. Inhibition of myosin-2 motor activity interfered with the movement of actin toward the spindle poles and with spindle movement toward the periphery. Thus, spindle relocation depends on the interaction between the mitotic spindle poles and the actin cytoskeleton, which promotes the relocation of the spindle to the cell periphery and allows for the asymmetric division that produces egg and polar body. — SMH

Curr. Biol. **18**, 10.1016/j.cub.2008.11.022 (2008).

ASTROPHYSICS

A Star at Death's Door

Some stars die violent deaths. If they are born with masses above about eight times that of the Sun, they become unstable as they approach the end of their nuclear fusion lives and collapse under the force of gravity. As a result they explode as supernovae, producing a sudden radiation burst that can outshine an entire galaxy for a short period of time. Such events don't usually go unnoticed; astronomers have been observing them over the centuries. However, very few stars have been observed right before their death, given the need to presciently acquire high-resolution images of the galaxy in question at just the right time.

Mattila *et al.* used the European Southern Observatory Science Archive to find high-quality images of the pre-explosion site of supernova 2008bk, which was discovered in March 2008 in a galaxy 13 million light-years from Earth. By comparing the archive observations with a new image of the supernova they took using the adaptive optics system on the Very Large Telescope, they were able to unambiguously identify the star that exploded—an unfortunate red supergiant that started its life with a mass 8.5 times that of the Sun. The observation is consistent with theoretical models of supernovae, and provides further constraints on their progenitors. — MJC

Astrophys. J. **688**, L91 (2008).

IMMUNOLOGY

Finding Negatives Wherever

Many autoimmune diseases are associated with specific alleles of the major histocompatibility complex (MHC), but how a particular MHC allele contributes to a particular disease is often not known. The MHC class II allele HLA-DQ8 has been linked to celiac disease, an autoimmune disease driven by T cell responses against dietary gluten, as occurs in wheat, for example. In contrast to other MHC alleles, HLA-DQ8 lacks an aspartic acid residue in its peptide-binding pocket and thus preferentially binds to peptides and T cell receptors (TCRs) that carry a negative charge. In celiac disease, negatively charged gluten-derived peptides are generated by tissue transglutaminase (which converts glutamine to glutamic acid); however, how HLA-DQ8 contributes to disease is not clear because transglutaminase requires inflammation for its activation.

Hovhannisyan *et al.* demonstrate that anti-gluten T cell responses can be mediated by HLA-DQ8 bound to native (nondeamidated) gluten peptides. Recruited T cells contained negatively charged TCRs, which helped to stabilize HLA-DQ8 binding of nondeamidated peptide. Inflammation resulting from this response led to the activation of transglutaminase and to peptide deamidation. The T cells were highly cross-reactive and mounted stronger responses to deamidated gluten peptides due to the enhanced stability of TCR-peptide-HLA-DQ8 interaction. These studies suggest how HLA-DQ8 may drive an amplified T cell response to gluten in celiac disease. — KM

Nature **456**, 534 (2008).

OCEAN SCIENCE

Winter Carbonate Collapse

Anthropogenic fossil-fuel burning is increasing the concentration of CO_2 in the atmosphere, which in turn is causing more CO_2 to dissolve in

CREDITS (TOP TO BOTTOM): SCHUH AND ELLENBERG, *CURR. BIOL.* **18**, 10.1016/J.CUB.2008.11.022 (2008); ESO/S. MATTILA, S. SMARTT, M. CROCKETT, J. ELDRIDGE, J. MAUND, J. DANZIGER

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the ocean, thereby lowering the water's pH. Such ocean acidification in turn decreases the concentration of carbonate ion $(CO_3)^{2-}$, which makes it more difficult for calcifying organisms such as foraminifera, pteropods, and corals to build their skeletons. So far, most of the attention paid to this process has focused on the time-averaged chemistry of the ocean, but organisms actually experience seasonal carbonate and pH variations. McNeil and Matear examine these variations and show that anthropogenic CO_2 uptake is likely to induce winter aragonite undersaturation in some regions of the ocean when atmospheric CO_2 levels reach 450 parts per million. These findings underscore the importance of understanding the seasonal dynamics of marine carbonate chemistry, as natural variability could hasten the deleterious impacts of future ocean acidification. — HJS

Proc. Natl. Acad. Sci. U.S.A. **105**, 18860 (2008).

CHEMISTRY

Diverse Scaffolds

Chemical libraries are widely used in bioactivity studies and drug discovery, yet many such libraries cover only a limited range of chemical shapes, or molecular scaffolds. Morton *et al.* have now achieved a library of over 80 different scaffolds by exploiting the power of ring-closing metathesis, a process involving scission and redistribution of carbon-carbon double bonds that leads to the formation of ring systems. Using two types of building blocks and fluorous-tagged linkers, the authors prepared cyclic products in four or five steps using combinations of just six reaction types. The compounds prepared contained numerous structural features such as isolated, fused, and spirocyclic ring systems; intramolecular hydrogen bonding; unsaturation; and dense substitution that were reminiscent of natural products. Many of the scaffolds had not been previously synthesized. — JFU

Angew. Chem. Int. Ed. 10.1002/anie.200804486 (2008).

CELL BIOLOGY

Follow the Leader

Cell migration is fundamental, being an integral part of human development and a driving force behind tissue repair. Many cells possess the ability to migrate directionally in response to environmental signals, a process known as chemotaxis. When starving, individual *Dictyostelium discoideum* cells aggregate in a head-to-tail fashion to form a migrating stream. This coordination is achieved by transmitting an amplified cAMP signal to neighboring cells. Adenylyl cyclase, the enzyme that makes cAMP, is concentrated at the rear of the cell and is required for this collective migration. Kriebel *et al.* discovered that cyclase is present in intracellular vesicles and that vesicular trafficking via actin and microtubule networks is required to generate its asymmetric distribution. Migrating cells were also found to shed cyclase-containing vesicles from the back of the cell. This directional release of vesicles could form extracellular tracks along which other cells follow, in what may prove a general mechanism for coordinated migration processes. — HP*

J. Cell Biol. **183**, 949 (2008).

AGRICULTURE

Controlling Salt Intake

It's hard to know which we might run out of sooner, fresh water or fresh land. Most agricultural systems depend on a plentiful supply of both. The problem is that irrigation, especially in brackish environments, and intense land usage can increase soil salinity, degrading the productivity of the land. Decreasing agricultural productivity in the face of increasing community needs moves us in the wrong direction. Meanwhile, demand rises for feed crops used to support milk and meat production. Khan *et al.* have devised a feed crop growth system that can be managed in the midst of brackish waters. Surveys of Pakistani herdsmen and herbalists led them to investigate the grass *Panicum turgidum*, which can grow in brackish waters and salty soils, but avoids accumulating salt itself, which would make it unappetizing and harmful as animal fodder. Cocultivation within a grid of *Saueda fruticosa*, a salt-accumulating plant that is used locally to make soap, kept the soil salinity stable. Farmers in southwestern Pakistan may be willing to replace their standard maize feed with this system. — PJH

Panicum turgidum.

CREDIT: AJMAL KHAN

Agric. Ecosyst. Environ. 10.1016/j.agee.2008.10.014 (2008).

*Helen Pickersgill is a locum editor in *Science*'s editorial department.

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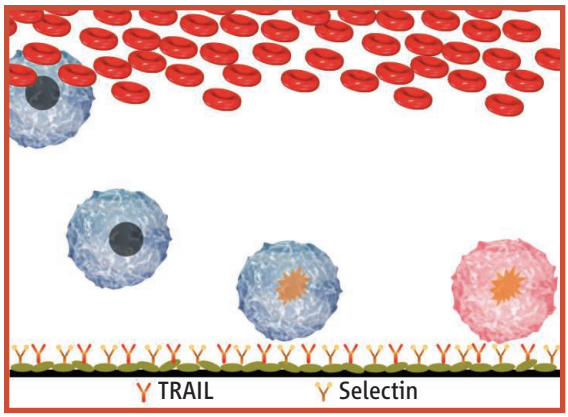
Information for Authors
See pages 634 and 635 of the 1 February 2008 issue or access www.sciencemag.org/about/authors



To Catch a Cell

Circulating tumor cells—the cause of cancer metastases—are the bane of oncologists. Cells can evade surgery, drugs, and radiation, moving through the bloodstream to set up shop elsewhere in the body. But Cornell University biomedical engineer Michael King and his colleagues have an idea to stop these cells in their tracks.

As the team will report in an upcoming issue of *Biotechnology and Bioengineering*, they have coated a small, coiled tube with two human proteins. One, called a selectin, attracts cancer cells, causing them to stick temporarily to the tube wall. The second, called TRAIL, then activates cell death, or apoptosis.

In the laboratory, a single pass through the tube killed nearly one-third of the cancer cells in a solution. The ultimate goal is to implant a protein-lined shunt into a cancer patient's arm that will reduce the number of malignant cells enough to allow the immune system to get rid of the rest.

"This approach is extremely novel," says Bryan Greene, chief scientific officer of BioCytics Inc., a biotechnology company in Huntersville, North Carolina. It's all very preliminary—the researchers have yet to show it will work in living animals. But, he says, "if these are indeed the cells that ultimately form distant tumors, then targeting [them] should lead to a dramatic patient response."

No Free Ride for Blubber

Looking for another good reason to keep that New Year's resolution to lose weight? How about saving the planet?

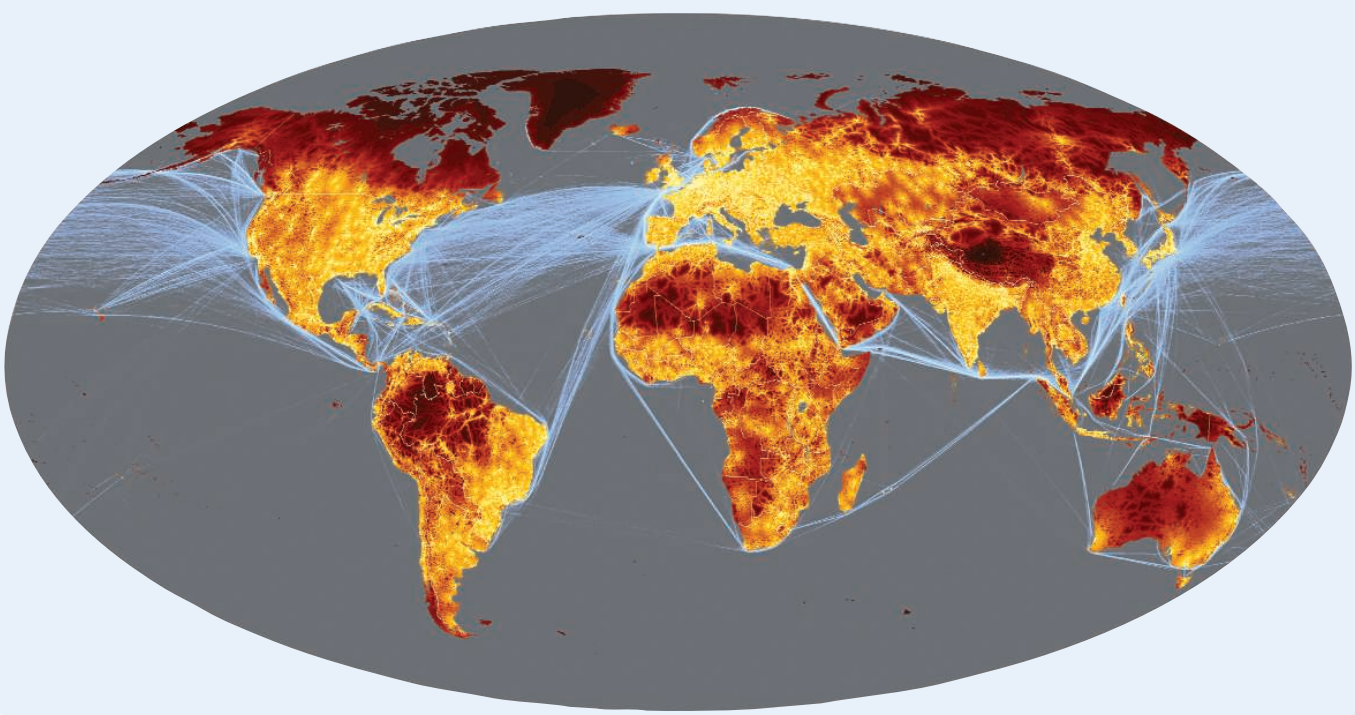
Operations researchers Sheldon Jacobson and Douglas King at the University of Illinois, Urbana-Champaign, calculate that trimmer U.S. drivers would burn about 3.8 billion liters less fuel per year. The estimate comes from combining driving data from the Department of Transportation, fuel-efficiency figures from the Environmental Protection Agency (EPA), and obesity numbers from the National Center for Health Statistics.

Cars and light trucks currently burn around 0.0045 liters per kilogram of cargo every 100 km, EPA says. With Americans traveling 7.2 trillion km per year, each extra half-kilo of flab requires using an additional 150 million liters of gas. The average adult is nearly 14 kg overweight, teens around 3.6 kg, and children 1.4 kg.

All this excess adds up to anywhere from 2.8 billion to 4.2 billion liters, the authors report online in *Transportation Research Part D: Transport and the Environment*. "We are essentially transporting a load of blubber around," says the journal's editor, Kenneth Button, an economist at George Mason University in Fairfax, Virginia. "We're damaging the environment by being fat."

GETTING THERE

The first Global Accessibility Map has been assembled by the European Commission and the World Bank. It depicts the time needed to travel by land or water—from less than an hour (pale yellow) to 10 days (dark brown)—from any location in the world to the nearest city of 50,000 people or more. It also indicates the density of shipping lanes. Only 10% of the world's land area is now "remote"—defined as more than 48 hours from a large city—according to the World Bank. The map was made for *World Development Report 2009* (see gem.jrc.ec.europa.eu/gam/index.htm).



CREDITS (TOP TO BOTTOM): KULDEEP RANA/CORNELL UNIVERSITY; ANDREW NELSON/WORLD BANK DEVELOPMENT RESEARCH GROUP

EDITED BY YUDHIJIT BHATTACHARJEE

People to Watch in 2009

The United Kingdom's new science minister, **Paul Drayson**, is the first to have a seat in the Cabinet and chair a new committee for science and innovation. Research funding fared well during the first decade of the Labour government, and researchers hope Drayson will fight for them in the current financial crisis. "We desperately need a champion like him in the run-up to the next spending review" in 2010, says Member of Parliament Phil Willis, chair of the House of Commons science committee.

For China's health minister, **Chen Zhu**, 2008 was framed by two disasters that observers say he handled well: the Wenchuan Earthquake in May and the tainted milk scandal that reared its head in September (*Science*, 28 November 2008, p. 1310). This year, the Paris-trained hematologist's primary challenge will be health care reform, including the creation of a specialized funding channel for medical research. Whether he prevails—and keeps his job—may depend on whether China can avoid another health crisis. "He's an ideal person to ask to fall on his sword if the need arises," says one scientist, noting that Chen is one of only two ministers who is not a member of the Communist Party.

The United Kingdom's biomedical community will be watching British biochemist and Nobelist **Paul Nurse** closely this year as he delivers a scientific plan for a controversial $1 billion laboratory that the government wants to build by 2013 (*Science*, 14 December 2007, p. 1704). The ambitious UK Centre for Medical Research & Innovation would include as many as 1000 scientists from University College London, biomedical charities the Wellcome Trust and Cancer Research UK, and the National Institute for Medical Research (NIMR), whose staff has resisted moving from its Mill Hill location to downtown London. Among Nurse's tough choices will be how many NIMR labs to relocate and whether the center should include biocontainment facilities to enable work with dangerous pathogens.

Expect **Svante Pääbo**, a paleogeneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, to make headlines this year by completing a very rough draft of the Neandertal genome. Two years in the making, the genome sequence signals how far the field of ancient DNA has come since Pääbo's team was able to isolate the first mitochondrial DNA from a Neandertal in 1997. Comparing the draft to the human and chimpanzee genomes would help determine which sequence differences likely define our species. But, as Pääbo pointed out in 2001, "it is a delusion to think that genomics in isolation will ever tell us what it means to be human."

Scientists and environmental activists are hoping that **Lisa Jackson**, nominated to be administrator of the U.S. Environmental Protection Agency (EPA), will reenergize the agency. They'd like to see her start by overhauling EPA's science advisory board and adding top scientists. "That makes a statement that science will be the important voice," says toxicologist Ellen Silbergeld of Johns Hopkins University in Baltimore, Maryland. Naming a world-class scientist to head the Office of Research and Development would also be a "huge boost to morale," says Linda Greer of the Natural Resources Defense Council. The community also hopes Jackson will undo administrative changes that aim to slow or reduce the use of research in regulations.

Can Singapore make a name for itself in information technologies and engineering the way it has in biomedical research? That's the challenge for **Lim Chuan Poh**, chair of Singapore's Agency for Science, Technology and Research (A*STAR), who must devote himself this year to turning the agency's just-completed Fusionopolis into a hub for public and private research in interactive media, physical sciences, engineering, and technology. Lim's military demeanor—he rose through the ranks of the Singapore Armed Forces—is quite a contrast from the fast-talking, wisecracking style of his A*STAR predecessor, Philip Yeo, who led the creation of Biopolis in 2004. The economic crisis poses a major hurdle for luring private sector labs to the development.

Got a tip for this page? E-mail people@aaas.org

As university endowments continue to plummet amid a deepening recession, the academic community is watching how two leading institutions—and Cambridge, Massachusetts, neighbors—are responding to the crisis. Harvard University President **Drew Faust** (left) has asked campus administrators to scale back the university's 2009 budget in the wake of the projected 30% decline of its $37 billion endowment. Harvard officials are reconsidering a plan to move two graduate schools to a new campus across the Charles River in Allston (*Science*, 11 July 2008, p. 190). Several kilometers down the road, Massachusetts Institute of Technology President **Susan Hockfield** (right) has called for a $50 million cut in the university's operating budget. Many universities have imposed hiring freezes, and the pain is expected to intensify.

CREDITS: (CLOCKWISE FROM CENTER) DORNON, MPI-EVA; AP; A*STAR; DONNA COVENEY/MIT; TONY RINALDO; THE ROCKEFELLER UNIVERSITY; AP; ADRIAN BROWN/BLOOMBERG NEWS/LANDOV

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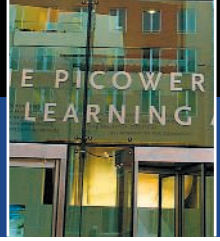

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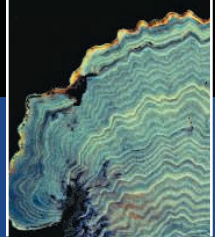

Coral growth slows 27

THE TRANSITION

Holdren Named Science Adviser, Varmus, Lander to Co-Chair PCAST

In 1997, Harvard physicist John Holdren co-chaired a report by a White House advisory panel that recommended transforming the federal science enterprise to "maintain [U.S.] leadership in the science and technology of energy." In the ensuing decade, Holdren's reputation as an international energy maven has steadily risen. But his pitch for more federally funded research, for everything from greater energy efficiency to solar and wind technologies, hasn't changed. In April 2008, for example, he told late-night TV host David Letterman that the government "should be quadrupling to ten-tupling the amount of money we're putting into energy research and development."

Now Holdren will have a chance to make the case for such increases from the inside. In a 20 December 2008 radio address, President-elect Barack Obama announced that he has picked the 64-year-old career academic to be his science adviser and director of the White House Office of Science and Technology Policy (OSTP). In that job, he will also co-chair the President's Council of Advisors on Science and Technology (PCAST), which issued the 1997 report. Obama also chose biomedical heavyweights Harold Varmus, president of the Memorial Sloan-Kettering Cancer Center in New York City, and Eric Lander, director of the Broad Institute in Cambridge, Massachusetts, to be the two outside co-chairs of PCAST, and Jane Lubchenco, a marine ecologist at Oregon State University in Corvallis, as administrator of the National Oceanic and Atmospheric Administration.

The U.S. research community is ecstatic with those selections and the previous announcement that Nobelist Steven Chu would be nominated as energy secretary (*Science*, 19 December 2008, p. 1774). All are already respected scientific leaders. (Holdren and Lubchenco are former presidents of AAAS, which publishes *Science*, and all five are members of the National Academy of Sciences.)

When combined with Carol Browner, who has been named to the new position of White House energy and climate czar, the appointments portend an aggressive effort to carry out Obama's campaign commitment to make energy "the number-one priority." In particular, they augur well for a massive government effort in renewable energy technologies and in setting tough limits on carbon emissions. "If you were going to launch an Apollo-like project [in energy], you would make appointments like the ones he's made," says former presidential science adviser Neal Lane. "They're superb." The crowd has also cheered the speed with which the next president has acted: President George W. Bush waited until June to name his science adviser, John Marburger.

Added energy. John Holdren brings expertise in energy, climate, nuclear proliferation, and other topics to his job as presidential science adviser.

But that euphoria will be tested in the weeks and months to come. The new president still has several important science slots to fill. The list includes the heads of the National Institutes of Health and the Food and Drug Administration. The betting is heavy that there will also be new leaders at NASA, the Centers for Disease Control and Prevention, and the Department of Energy's (DOE's) Office of Science, among other posts.

There's also the question of how Obama plans to deploy his stable of talent. Marburger says the plethora of energy expertise among top Obama officials will mean that OSTP will "have to be redefined in relation to these other centers of formulating policy." The divvying up of tasks has already begun. Last week, for example, it was Browner, not Chu, who briefed Vice President–elect Joe Biden on the outlines of a stimulus package expected to include both traditional construction projects and investments in green technology. Scientific groups have been lobbying heavily for research to be included in that package, which congressional Democrats hope to have ready for the president shortly after he's sworn in on 20 January.

Holdren runs the Woods Hole Research Center in Massachusetts, which studies how human activity affects the global environment. Trained as a plasma physicist, he's been an international energy guru for 3 decades at the University of California, Berkeley, and at Harvard. OSTP manages much more than just energy, of course. But colleagues at Woods Hole say he's up to tackling policy questions in biology, environmental science, and nuclear proliferation, his specialty.

The other appointees also boast impressive resumes. After winning the 1997 Nobel Prize in physics for his work on supercooled atoms, Chu put aside his academic career to develop the next generation of biofuels and alternative sources of energy. Since 2004, he has been director of DOE's Lawrence Berkeley National Laboratory. Lubchenco has devoted her professional career to studying the sustainable management of marine environments, including the impacts of climate change, and communicating that knowledge to the public.

Graham Allison, Holdren's boss at Harvard's Belfer Center for Science and International Affairs, predicts he will make a smooth transition from outsider to insider. "John is the very model of a policy-relevant scientist," says Allison, a former top defense adviser in the Administrations of Presidents Ronald Reagan and Bill Clinton. In practice, however, Holdren's effectiveness will be

CREDIT: KEITH SRAKOCIC/AP PHOTO

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shaped largely by factors that are out of his control (see p. 28). Although nobody is betting that DOE's research budget will grow 10-fold, for example, Holdren would be a hero if he helps Obama achieve his goal of a 10-year doubling of the budget for U.S. basic research, including a massive bump in the energy sciences.

Scientists could get a glimpse of the new Administration's approach to science policy when the rest of the members of PCAST are announced. In his December 2008 radio address, Obama promised "to remake PCAST into a vigorous external advisory council that will shape my thinking." Varmus, who chaired a similar but unofficial group of experts that advised the Obama campaign, told *Science* recently that he, Lander, and Holdren have begun talking about a "reconstituted PCAST."

Obama has already gotten some surprisingly candid advice about PCAST from Marburger and his co-chair, venture capitalist Floyd Kvamme. A recent self-assessment of the council's record included recommendations to make it smaller, with more working scientists, and to give it a more active role in interacting with the White House, other executive agencies, and Congress. Whether or not Obama grants PCAST a larger role, his decisions to date suggest that he is prepared at least to listen to many of the nation's top scientists.

–ELI KINTISCH AND JEFFREY MERVIS

A brief version of this story ran on Science's *policy blog,* http://blogs.sciencemag.org/scienceinsider

SCIENTIFIC COLLABORATION

Tehran Incident Threatens U.S.-Iran Project

A member of a U.S. scientific delegation headed by the president of the Institute of Medicine (IOM) was interrogated for 9 hours last month in his Tehran hotel, casting a pall over years of painstaking efforts to cultivate ties between the Iranian and U.S. scientific communities. In a statement last week (nationalacademies.org/morenews/20081226.html), the U.S. National Academies labeled the incident a "serious breach," and its three presidents declared that they "cannot sponsor or encourage American scientists to visit Iran unless there are clear assurances that the personal safety of visiting scientists will be guaranteed."

The incident involved Glenn Schweitzer, director of Eurasian programs at the academies. He was staffing a delegation led by IOM President Harvey Fineberg that was exploring opportunities for collaboration in the medical sciences. On 4 December 2008, three men who claimed to be security officers confronted Schweitzer and spent 3 hours grilling him in his hotel room. They returned 2 days later for another 6 hours of questioning. The men threatened to prevent Schweitzer from leaving Iran and told him that exchange visitors are not welcome.

"This really was a big surprise. It's a risk we did not expect at all," says William Colglazier, executive officer of the academies' National Research Council. One Iranian scientist told *Science* that two Iranian scientific academies have sent "official apologies" to Schweitzer, who was allowed to leave the country as scheduled on 7 December. But Schweitzer says he has not received the apologies, and Colglazier says the U.S. academies are still awaiting a formal response from the Iranian government.

In a separate incident last week, Carl Ernst, a professor of religious studies at the University of North Carolina, Chapel Hill, ran into trouble with immigration officials at Tehran airport. Ernst was sent back to Dubai and had to wait 12 hours before getting permission to enter Iran, where he received a prize from the Institute for Social and Cultural Studies in Tehran.

It's unclear whether the incidents are unrelated or opening salvos of a concerted effort to derail academic cooperation with the United States. "There are various interest groups who are unhappy about people-to-people relations such as S&T exchanges. As a result, there will always be attempts to jeopardize these exchanges," says one Iranian scholar. Others say that the risk of incidents is especially high in the run-up to Iran's presidential elections in June 2009. "Tension is seen as beneficial by many conservatives in Iran," says a second Iranian scholar. "Conservatives are mostly suspicious and some of them even dead set against the opening of Iran towards the West." In a promising development, *Science* has learned that organizers of a spring school on quantum many-body theory, to be held in May at the University of Tehran, were told this week by Iranian officials that "formal documents for security assurances" for visiting scholars have been sent to relevant institutes.

CREDIT: NAS

Grilled. Glenn Schweitzer was questioned for 9 hours in his hotel room.

Schweitzer has spearheaded the academies' 8-year effort to nurture scientific ties with Iran, including workshops on various topics and an exchange of science policy experts between the academies and Sharif University of Technology in Tehran. "Glenn has always operated in an absolutely open and transparent way," says Norman Neureiter, director of the Center for Science, Technology and Security Policy at AAAS (*Science*'s publisher). "It's most unfortunate he was treated this way. It doesn't bode well for the future of these relationships."

Schweitzer, who was traveling on a visa issued expressly for the meeting, says this was his first problem in 10 trips to Iran in the past decade. But it's soured him on future visits. "I hope this is more of a bump in the road rather than a derailment," he says. "But I won't go back. I'll let others pick up the mantle."

–RICHARD STONE

A brief version of this story ran on Science's *policy blog,* http://blogs.sciencemag.org/scienceinsider

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RESEARCH ASSESSMENT

U.K. University Research Ranked; Funding Impacts to Follow

Researchers and university officials across the United Kingdom were eagerly poring over detailed statistics last month, figuring out how their departments fared in the latest government rankings of research quality. The occasion was the release on 18 December of the results* of the latest Research Assessment Exercise (RAE), a massive evaluation of 159 higher education institutions that cost more than £10 million to conduct and occupied some 1000 scientists who spent up to a year on peer-review panels.

Although the RAE carefully avoided giving overall scores, U.K. newspapers and other publications immediately used the data to construct "league tables," and schools began celebrating or lamenting their rise or fall since the last evaluation in 2001. "We're really chuffed. It's been fantastic," says a spokesperson for Queen Mary, University of London, a relative unknown that unexpectedly shot up into the top 15 of the U.K.'s research universities in several ranking tables.

More is at stake than reputation. The results will affect the distribution over the next 5 years of some £1.5 billion in annual funding from the government. Although U.K. universities and specialty research institutions obtain about half their budgets from competitive grants and programs, industry, and educational charities, the RAE determines the annual governmental block grant each institution can depend on for an extended period. But how the RAE data translate into cash won't be announced until next spring. "This is an exercise in how you allocate scarce resources, and that part is still undecided," says Nick Dusic of the U.K.'s Campaign for Science and Engineering.

This is the 6th RAE since 1986 and the last in its current form. The government intends to switch from using peer-review panels to a more quantitative approach. There have been few complaints about those doing the peer review—primarily U.K. scientists but also some from abroad—yet each new RAE tends to spark a controversy. In 2001, a furor ensued when the Higher Education Funding Council for England (HEFCE) decided to concentrate funding allocations on institutions receiving the top grades (*Science*, 21 December 2001, p. 2448). In contrast, Scotland decided to spread its money more equally, which was made easier by the smaller number of research outfits there.

The 2001 RAE was one factor that led to the closure of many science departments, and universities have been preparing for the next RAE ever since. The University of Manchester and the University of Manchester Institute of Science and Technology reportedly even decided to merge to present a stronger case for research funds. There have also been grumbles that certain schools submitted only their best for evaluation, a stratagem that may raise their position in the league table but could backfire because funding allocations are usually based in part on the number of researchers whose work was submitted to RAE. The RAE "affects institutional behavior and individual behavior, though it's hard to tell how much gameplaying is going on," Dusic says.

A big change in the 2008 RAE is that overall grades weren't awarded. Instead, institutions received a "quality profile" for each discipline—the percentage of research submitted that peer reviewers rated as world-class (4*), internationally excellent (3*), recognized internationally (2*), recognized nationally (1*), or unclassified. That more detailed evaluation, say school officials, should help them evaluate their strengths and weaknesses better. It also led to a lot of boasting about the overall quality of U.K. science, with HEFCE officials noting that 87% of U.K. research is of international quality, i.e., the top three grades.

The next big controversy over the RAE concerns its death. In a new evaluation scheme, dubbed the Research Excellence Framework (REF), the U.K.'s higher education funding bodies plan to assess research quality using primarily measures such as competitive grants obtained, Ph.D.s granted, and citations received for papers. Many questions remain about whether this approach can truly evaluate research quality across disciplines that include music, midwifery, economics, philosophy, and all the traditional sciences.

In preparation for the first REF, likely in 2014, U.K. officials are now running a test with about 20 U.K. institutions. They've also commissioned several studies, one evaluating whether interdisciplinary work would be at a disadvantage when measured by such metrics rather than by peer-review panels. (The conclusion: essentially, no.)

Soothing some nervous institutions, those in charge of the REF have recently agreed that it will contain an element of peer review, although the exact role remains undefined. Rather than evaluating massive submissions of research results, a panel of scientists from each discipline may help pick the appropriate metrics—patents, performances, or citations, for example. "The REF will be much more of an evolutionary change than a radical departure from the RAE," predicts Dusic.

For now, with the 2008 RAE funding formula unknown and money allocations still up in the air until March, no one knows which science departments might close or who may be looking for a job. "There's not a lot they can do now except wait and see," says Dusic. The RAE "has real consequences on people. That's why people take it so seriously." **–JOHN TRAVIS**

RESEARCH FORTNIGHT'S RAE 2008 RANKINGS

Name	Rank 2008	Rank 2001	Staff
University of Oxford	1	1	2246
University of Cambridge	2	2	2040
University College London	3	3	1793
University of Manchester	4	4	1824
University of Edinburgh	5	5	1640
Imperial College London	6	6	1225
University of Nottingham	7	14	1388
University of Leeds	8	8	1270
University of Sheffield	9	12	1205
University of Bristol	10	9	1199
King's College London	11	7	1172
University of Birmingham	12	10	1175
University of Southampton	13	13	1098
University of Glasgow	14	11	1161
University of Warwick	15	18	966
Cardiff University	16	15	1030
University of Newcastle	17	16	932
University of Liverpool	18	17	958
University of Durham	19	21	759
Queen Mary, University of London	20	23	687

Who's number one? In its analysis of raw data, the journal *Research Fortnight* placed Oxford first. Others had Cambridge, the London School of Economics, and the Institute of Cancer Research at the top.

SOURCE: *RESEARCH FORTNIGHT*

*www.rae.ac.uk

CREDIT: 2005 RICK FRIEDMAN/CORBIS

Casualty. An MIT center financed by the Picower Foundation will survive, but some of its funding is now in jeopardy.

RESEARCH FUNDING

For Many Scientists, the Madoff Scandal Suddenly Hits Home

The alleged Ponzi scheme created by Wall Street investor Bernard L. Madoff has touched many scientists, including dozens who received funding from a respected charitable group that was decimated when the fraud collapsed. The Picower Foundation announced last month that it would "cease all grant making effective immediately" after 20 years of operation. On its 2007 tax return, the foundation reported assets of nearly $1 billion. Its endowment was managed by Madoff.

The Picower Foundation was especially generous to scientists, all of whom are now reeling from the sudden loss of millions of dollars in research money. "Of course, hearing on the news about Madoff and the people who lost millions or billions of dollars was a tragic thing … in abstract," says J. Timothy Greenamyre, a neurologist at the University of Pittsburgh in Pennsylvania. Then, he says, he received an e-mail from Barbara Picower informing him that his and his colleagues' funding was over, "and it was suddenly terribly tangible and just devastating."

Greenamyre was one of seven senior Parkinson's disease researchers whose labs made up a consortium, now 5 years old, formed and funded by the foundation established in 1989 by Barbara and Jeffry Picower. In 2007 alone the seven together received about $4 million. One recipient compared the money to a prestigious U.S. National Institutes of Health (NIH) Pioneer Award; another said its value exceeded that of an R01, the bread-and-butter NIH grant on which many researchers rely. As recently as October, the Parkinson's group conferred with Barbara Picower and discussed expanding the consortium to include additional scientists.

Along with its Parkinson's project, the foundation began financing a similar consortium in diabetes and obesity research just over a year ago, giving about $2.3 million in 2007 to five prominent researchers, including Jeffrey Flier, the dean of Harvard Medical School in Boston. All had been told that the funding would last at least 3 years; Flier, for example, was supposed to receive $1.5 million during that time. Now the funds have dried up, and researchers are trying to determine what, if anything, they have left over from grant money the Picowers already provided.

The Picowers also extended their generosity to the Massachusetts Institute of Technology (MIT) in Cambridge; in 2002 they gave $50 million to form MIT's Picower Institute for Learning and Memory. That money has been paid, but an additional $4 million pledged to the institute in May is in jeopardy. It was intended to launch the Picower Institute Innovation Fund (PIIF), to support institute faculty members, postdoctoral fellows, and students pursuing creative or high-risk neuroscience research.

The Picower Foundation had planned to provide $2 million per year for 2 years to PIIF, with the option of further funding if the science proved compelling, said Mark Bear, a neuroscientist and director of the Picower Institute for Learning and Memory, in an e-mail message. "The second $2 million installment is due in early 2009," he wrote. "I am extremely disappointed that this exciting and innovative program is at risk." It's not clear how or whether MIT can fill the money gap and keep PIIF running.

The Picowers were also donating $200,000 a year for graduate fellowships at MIT, money that will likely vanish along with the foundation. They made numerous smaller donations to various disease foundations, such as $250,000 in 2007 to a mantle cell consortium at the Lymphoma Research Foundation in New York City.

Consortia members say they were deeply distressed by the e-mail message they received on 19 December from Barbara Picower, in which she informed them of the calamity. They say it's not just the money they will miss but also the Picowers' rare commitment to high-risk research.

"The Picower money was really transformative for us," says D. James Surmeier, an electrophysiologist at Northwestern University in Evanston, Illinois, who was part of the Parkinson's group. The Picowers enabled his lab to chase a hypothesis that was "fairly speculative" about how Parkinson's disease damages neurons. Surmeier found that an influx of calcium into dopamine neurons makes the cells vulnerable in Parkinson's disease and more likely to die. A drug used to treat high blood pressure blocks this flow, and Surmeier thinks it may help Parkinson's patients, too. "They were interested in the big break," says Surmeier of the foundation. "The loss of that money now will stop that research dead in its tracks."

All members of the Picower-funded Parkinson's and obesity consortia with whom *Science* spoke agreed that the groups would likely disintegrate. However, a clinical trial of a possible drug therapy for Parkinson's based on Surmeier's work, organized without funding from the Picowers, is set to begin shortly. Another worry for these researchers, many of whom said the Picower money amounted to 20% or more of their funding, is that they may have to lay off members of their labs. Several were reluctant to discuss this possibility, noting that lab members were off for the holiday and didn't yet know that the Madoff fraud had touched them directly.

Barbara Picower, the foundation's president, did not respond to an e-mail message, and a foundation attorney declined to comment. But in a statement announcing that the foundation would close, Barbara Picower noted that it had distributed more than $268 million in grants since its inception in 1989 and expressed hope that its impact would endure. **–JENNIFER COUZIN**

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PLANETARY IMPACTS

Did the Mammoth Slayer Leave A Diamond Calling Card?

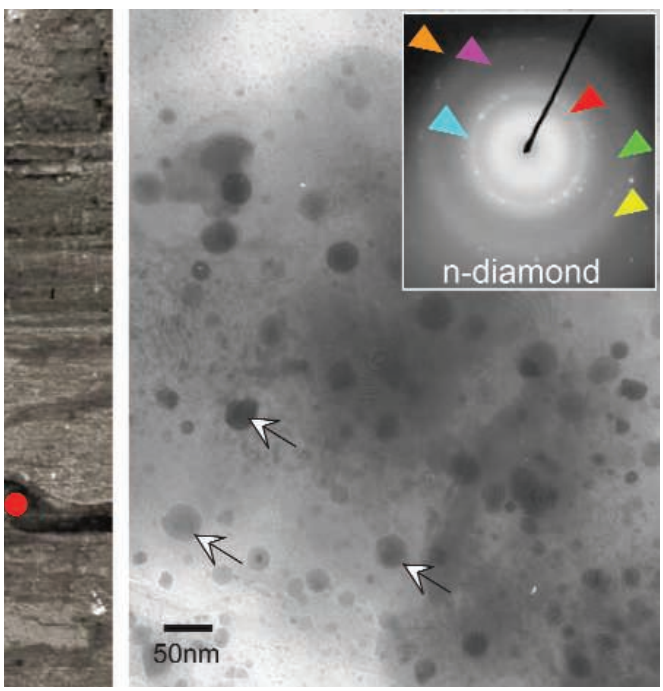
In this issue of *Science* (p. 94), a group of nine researchers presents the latest evidence for a cosmic catastrophe just 12,900 years ago. Last year in the *Proceedings of the National Academy of Sciences* (*PNAS*), six of these authors—along with 20 others—made a wide-ranging case for a shower of exploding comet fragments over the North American ice sheet. Such a cataclysm could have wiped out mammoths and other large mammals, abruptly ended the Paleo-Indian Clovis culture, and triggered a millennium-long return to near-glacial cold. The idea met with skepticism in the community of impact researchers.

Now, this group of nine is presenting the kind of evidence impact researchers have been asking for: transmission electron microscopy showing nanodiamonds from the geologic moment of the putative catastrophe. Unlike the last time, impact researchers are intrigued by the new evidence. But they aren't persuaded yet. "It may be a very great discovery, [but] I can't say this is wrong or right. We need a bit more evidence," says high-pressure mineralogist Falko Langenhorst of the University of Bayreuth in Germany.

Diamond has long been associated with impacts. Fragments up to a few tens of nanometers in size have been found in debris from the impact that killed off the dinosaurs. Impacting asteroids can carry diamonds that formed around distant stars. Impactors can shock graphite to diamond; diamond may also condense from carbon vapor in impact clouds. The *PNAS* paper claimed to detect such impact nanodiamonds using nuclear magnetic resonance, but specialists in the method roundly rejected that NMR evidence as erroneous (*Science*, 7 March 2008, p. 1311).

The transmission electron microscopy (TEM) evidence is faring better, up to a point. In the *Science* paper, geoarchaeologist Douglas Kennett of the University of Oregon, Eugene, and his colleagues report the detection of nanodiamonds at six locations widely spread across North America. The nanodiamonds appear in a thin layer of sediment radiocarbon-dated to 12,900 years ago, often at the base of a distinctive "black mat" of organic-rich sediment. The black mat has been associated with the beginning of the millennium-long Younger Dryas cold interval, the end of the Clovis culture, and the last of the mammoths.

The nanodiamonds described in the paper were identified using TEM and electron diffraction analysis. They were usually encased in millimeter-size, honeycombed spherules made largely of carbon that the group and others have found at the base of the Younger Dryas boundary layer but not above or below it. At the fall meeting of the American Geophysical Union in San Francisco last month, four of the *Science* authors reported finding abundant nanodiamonds at a total of 16 sites across North America and western Europe at the Younger Dryas boundary, but not just above or below it.



Sky with diamonds. Rather than stardust, nanometer-size diamonds may be a product of the newborn sun.

Opinions vary among nanodiamond experts. "The chances are good they have nanodiamonds," says spectroscopist Peter Buseck of Arizona State University, Tempe. Physicist Tyrone Daulton of Washington University in St. Louis, Missouri, is less convinced. "Maybe they found diamonds, maybe they didn't," he says. "It looks interesting, [but] there's not enough information in this paper to say whether they found diamonds." Langenhorst agrees. The measured electron diffraction "d-spacings" used to infer a diamond crystal structure are not unique, he notes: "There are many materials that have that structure. It could be just a metal."

Even those persuaded that nanodiamonds have been found have reservations. "I'm convinced they're nanodiamonds," says geochemist Iain Gilmour of the Open University in Milton Keynes, U.K., but "that doesn't necessarily make a connection to impacts. The fundamental thing is tying down where they come from." Researchers analyzing impacted rock and ejecta find plenty of diamonds, many of which have been strewn far and wide to accumulate in geologically quiet places like lake bottoms, swamps, and the deep sea floor. But few researchers have searched for diamonds in such accumulation zones away from known impacts. That has left the natural history of nanodiamonds largely a blank.

One exception is microscopist Dominique Schryvers of the University of Antwerp in Belgium and his colleagues. They found carbon spherules in almost every one of 70 samples from apparently undisturbed forest topsoils, grasslands, and swamps across Europe. The millimeter-size spherules—in soil estimated to be 1 or 2 millennia old—bear a striking resemblance to those found at the 12,900-year-old Younger Dryas boundary. In an early 2008 paper in *Diamond and Related Materials*, they describe nanodiamonds found in spherules from near Burghausen, Germany, and Spa, Belgium, though similar diamonds were found in selected samples from other areas. "We have no idea where the material is coming from," says Schryvers. "There's no real proof of an impact. They could come from anywhere."

So once again the advocates of a Younger Dryas impact are hearing that they have yet to make their case. "They're a long way from being able to use [the nanodiamonds] to justify an impact," says impact mineralogist Bevan French of the Smithsonian National Museum of Natural History in Washington, D.C. "I don't think you've got a unique impact marker" in nanodiamonds. But then, it took the better part of the 1980s for proponents of the dinosaur-killing impact to win that argument. **–RICHARD A. KERR**

CREDIT: D. J. KENNETT *ET AL.*, *SCIENCE*

CORAL REEFS

Calcification Rates Drop in Australian Reefs

Wall Street isn't alone in suffering a steep downturn. A large-scale study in Australia's Great Barrier Reef has revealed that the rate at which corals absorb calcium from seawater to calcify their hard skeletons has declined precipitously in the past 20 years, slowing coral growth. The report, on page 116, provides empirical data that fuels concerns that increased carbon dioxide in the air is putting these diverse marine ecosystems at risk (*Science*, 4 May 2007, p. 678). "This study has provided the first really rigorous snapshot of how calcification might be changing" as the ocean temperature and acidity rise, says marine biologist Ove Hoegh-Guldberg of the University of Queensland in Australia. "The results are extremely worrying."

Corals start out as soft-bodied larvae that settle on hard surfaces, take on algal partners, and begin pulling dissolved calcium compounds out of the water to lay down a hard skeleton. A reef arises from whole colonies of corals laying down these secreted skeletons. To keep ahead of erosion, they must calcify quickly enough to make up for what's being lost due to wave action and other forces. But some coral experts worry that the expected doubling of the carbon dioxide in the atmosphere over the next 50 years—and the subsequent ocean acidification—could make keeping up next to impossible. Several laboratory studies have suggested that as seawater pH declines, so do coral calcification rates, although one recent experiment has not shown this effect.

Glenn De'ath, an ecological modeler at the Australian Institute of Marine Science (AIMS) in Townsville, and his colleagues looked at archived coral samples for signs of such a slowdown. Between 1983 and 1992 and between 2002 and 2005, AIMS researchers had collected coral cores from 69 reefs spanning the length and breadth of the Great Barrier Reef. The coral sampled, *Porites*, grows over many decades, even centuries, into 6-meter-tall mounds. It lays down annual growth bands, making it possible to count back to specific years and correlate growth with sea surface temperature and other environmental data for the same period.

The researchers sliced up cores and used x-rays and a technique called gamma densitometry to measure annual growth and skeletal density; from those two parameters they calculated annual calcification. In their first pass, De'ath, AIMS coral biologist Katharina Fabricius, and AIMS climatologist Janice Lough found a decline in calcification rates since 1990 in 189 colonies from 13 reefs. They then broadened the analysis to include a total of 328 colonies spanning coastal to oceanic locations, including 10 cores that dated back centuries.

They found that calcification rates increased 5.4% between 1900 and 1970, but have dropped 14.2% from 1990 to 2005, mainly due to a slowdown in growth from 1.43 centimeters per year to 1.24 centimeters per year. "The very fact that the effect is seen on inshore as well as offshore reef sites rules out [the chance that] the observed decline has been due to declining coastal water quality," says Hoegh-Guldberg.

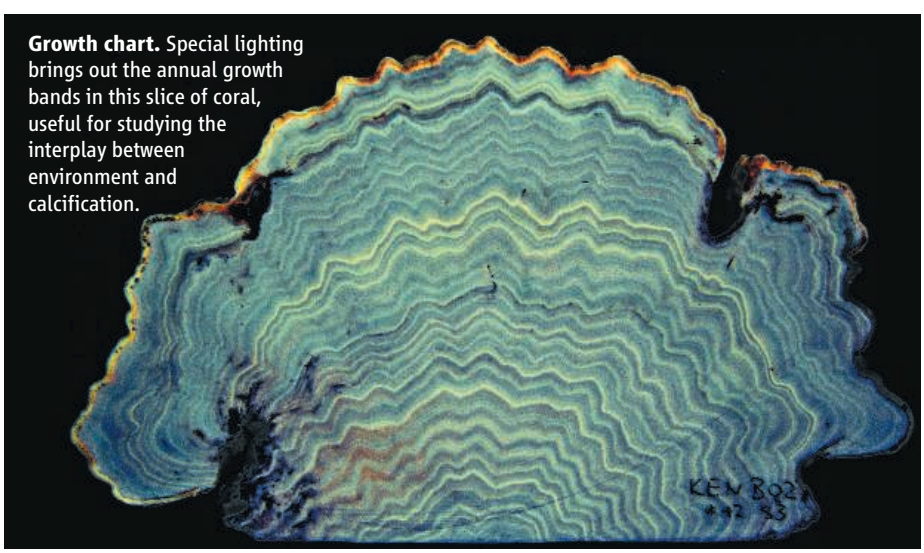
In the 1990s, researchers had predicted that ocean acidification might one day become a problem for corals, and at the 11th International Coral Reef Symposium (ICRS) in July 2008, global experts suggested that humans had about a decade to "get our act together" to reduce carbon dioxide emissions, says Clive Wilkinson, global coordinator of the Global Coral Reef Monitoring Network in Townsville. But calcification decline is "here and now and over the past decade, not some time in the future, as we predicted," he notes. "This has been happening under our noses."

Problems are showing up beyond the Pacific. Anne Cohen, a marine biogeochemist at the Woods Hole Oceanographic Institute in Massachusetts, and her colleagues are using computed tomography scans to look at growth and calcification in Bermuda brain corals. There's been a 25% decline in both since 1959, they reported at ICRS. "On Bermuda, the change appears unprecedented at least through the past century," says Cohen.

However, the role of acidification in this decline is far from settled. In the laboratory, Alina Szmant, a coral physiological ecologist at the University of North Carolina, Wilmington, and her colleagues found that neither low pH nor a lowered calcium carbonate concentration (which results from increased acidity and is considered key to calcification) slowed coral growth. Instead, calcium bicarbonate proved key, her team reported at ICRS. She faults previous lab studies because they used hydrochloric acid, not carbon dioxide, to lower the pH of the water in the calcification studies. Hydrochloric acid and carbon dioxide have different effects on seawater chemistry and bicarbonate concentration, she says. Her conclusion: "It's not clear that carbon dioxide enrichment will have negative effects on calcification rates."

Cohen also has some reservations. Commenting on De'ath's work, she says "the timing is later, and the magnitude and rate of the calcification decline is greater than one might expect if seawater saturation state [acidification] were the primary driver." Temperature and other environmental factors likely come into play.

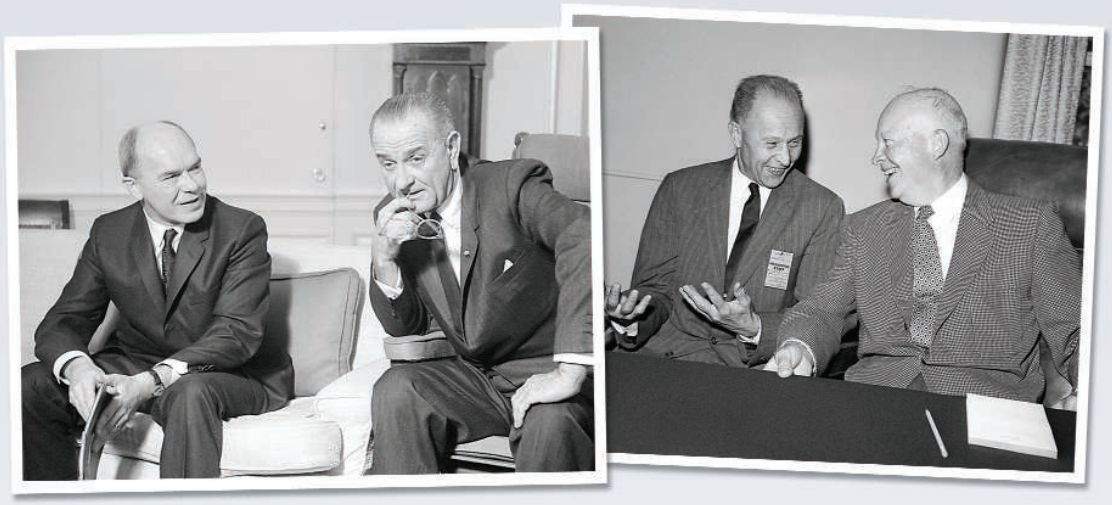
But whatever the cause, the new data are grounds for concern. In December, Wilkinson's group released a 4-year assessment, *Status of Coral Reefs of the World: 2008*, which said that 45% of the world's reefs were healthy with no significant threats from human activity on the horizon. But looks can be deceptive, says Cohen: "Even though the corals *look* healthy, some pretty dramatic changes are occurring beneath the surface." **–ELIZABETH PENNISI**



Growth chart. Special lighting brings out the annual growth bands in this slice of coral, useful for studying the interplay between environment and calcification.

CREDIT: GLENN DE'ATH

Published by AAAS



Truth to power. Donald Hornig with Lyndon Johnson, and George Kistiakowsky with Dwight Eisenhower.

Bending the President's Ear

A science adviser is only as effective as the president wants him to be, say the men who have held the job since Sputnik

On 7 August 1996, Jack Gibbons was facing a crisis. Gibbons, President Bill Clinton's science adviser, had been out of the country when news leaked that federal and academic scientists had discovered what looked similar to fossilized bacteria-like organisms in a meteorite that had fallen from Mars. NASA officials were recommending caution. But Dick Morris, an influential White House political adviser, was urging Clinton to announce that the United States would send astronauts to the Red Planet to search for signs of life.

Gibbons quickly sent word to the president to let "science run its course." Clinton agreed, and in a hastily arranged press briefing the chief executive emphasized robotic missions and the "fundamental" questions that remained about the meteorite's significance. Today, most Mars scientists believe it contains no signs of biological activity. Gibbons's intervention, says his press aide, Rick Borchelt, was key to "talk[ing] us off the ledge that Dick Morris wanted us on."

In discussing how decisions are made in the White House, Gibbons likes to quote Victor Hugo's adage that "science has the first word on everything, but the last word on nothing." Even so, the Mars rock episode showed that the science adviser can influence national policy. To stay abreast of fast-moving events, Gibbons relied on a strong network within the West Wing. His team of scientific experts provided rapid analysis of NASA's views. Gibbons also had enough credibility among Clinton's senior advisers to get his message delivered in time. Those qualities, say former officeholders, are exactly what an adviser needs to be effective.

Last week, President-elect Barack Obama selected John Holdren to be his science adviser and head of the Office of Science and Technology Policy (OSTP) within the White House (see p. 22). In doing so, Obama also pleased science mavens by saying that he would elevate Holdren to the position of assistant to the president, a title held by Gibbons but not by the current adviser, John Marburger. "Under Bush, the position has been marginalized," says Rodney Nichols, former president of the New York Academy of Sciences and author of one of three recent reports urging the next president to take such a step.

At the same time, however, insiders say that title is only one factor in determining a science adviser's effectiveness. It's just as important to be a savvy politician, an effective manager and convener, and a figure respected by both senior White House officials and the scientific community. "It requires grace and intelligence and sharp elbows," Nichols says.

Those qualities must be exercised within an environment set by the president's agenda and influenced by day-to-day events. D. Allan Bromley, adviser to George H. W. Bush, was given the opportunity to create the cross-agency climate science program. Marburger was asked to defend the policy adopted by President George W. Bush early in his term to limit federal research on embryonic stem cell lines. John F. Kennedy told his science adviser, Jerome Wiesner, exactly what would happen if funding fertility research at the National Institutes of Health, as Wiesner had urged, came back to haunt him. "I'll blame it on you," Wiesner quoted the president in a 1980 memoir.

Former officeholders also stress the importance of being a member of the president's team. Bringing one's scientific expertise to bear on an issue is not the same thing as arguing on behalf of the scientific community, they say. As George Keyworth, science adviser to President Ronald Reagan, put it in a 2006 speech, a new adviser must avoid at all costs the perception that he comes "with an agenda that might differ from the president's."

Ties that break

Unlike the national security adviser or a Cabinet secretary, the science adviser holds little inherent power. But circumstances can give the officeholder an inside track to power. At the request of President Franklin Roosevelt, Vannevar Bush, director of the Carnegie Institution of Washington, famously proposed expanding federal support for science at the end of World War II, setting in motion a process that led to the creation of the National Science Foundation in 1950. After Sputnik, President Dwight Eisenhower appointed electrical engineer James Killian as the first full-time presidential science adviser.

Early advisers served crucial roles in the rapidly evolving U.S. military and space enterprise. But many observers say the position has declined in influence over time. Roger Pielke Jr. of the University of Colorado, Boulder, wrote last year in *Nature* that modern presidents are "hardly in need of more science advice," now that the government receives guidance each year from an estimated 18,000 outside experts.

So what makes a science adviser effective? Former White House staff members say personal ties, like Gibbons's link to Vice

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President Al Gore, can help. Likewise, Bromley, a physicist at Yale University, Bush's alma mater, served on various White House advisory committees while Bush was vice president under Ronald Reagan. When Bush later asked him to become his science adviser, Bromley extracted a promise of support and Oval Office access when necessary. "Because of Bromley's proximity to the president, Cabinet-level people came to his meetings," says Damar Erb, his executive assistant.

In contrast, Lee DuBridge had been a respected adviser to the Pentagon before being tapped by President Richard Nixon. But Nixon's inner circle saw him as a water carrier for the scientific establishment and excluded him from budget meetings. That wasn't his only liability, however. In 1970, IBM physicist Richard Garwin, a member of the advisory group that preceded the President's Council of Advisors on Science and Technology, spoke out against the Administration's backing of research into supersonic flight. DuBridge's public support of the program was deemed by White House aides to be insufficiently forceful, says former *Science* reporter Dan Greenberg, and he was forced to resign months later.

Edward David, his replacement, also had a short and rocky tenure. "Ed David … [says] just to give another half-billion to the National Science Foundation. That isn't going to do a goddamn bit of good," Nixon told his advisers in a conversation recorded in June 1971 and described by Greenberg in his 2001 book, *Science, Money, and Politics*. After Nixon's landslide reelection the following year, he eliminated the post of science adviser, its staff, and advisory committee. (Congress passed legislation to reinstate the office in 1976, a move led by President Gerald Ford.)

But staying in the president's good graces isn't always enough, either. To be effective, a science adviser also needs chutzpah, says Clinton adviser Neal Lane, who recalls a meeting to discuss upcoming presidential initiatives to which he wasn't invited. Lane showed up anyway and persuaded an aide to put a name card for him on the table. "It was a very important meeting," says Lane, who recalls Clinton asking him about a proposed nanotechnology initiative that ended up in the president's next budget request to Congress.

Former Marburger staffer Pat Looney says Marburger earned a reputation in the White House as an "honest broker" on issues including coordinating astrophysics experiments and competitiveness legislation. He did it by not "telling [agencies] how to run their business" and working well with White House budget officials.

But that influence only extended so far. Last year, when Julie Gerberding, head of the Centers for Disease Control and Prevention, submitted congressional testimony on climate change to the White House for review, it was watered down. Marburger's staff said their views had been ignored, and the culprit was later revealed to be Vice President Dick Cheney. "Having the OSTP comments just be one among many seems to not be giving scientific input the priority it deserves," says Michael MacCracken, a former White House climate change official.

Former advisers differ on how broadly they should try to spread their tentacles. Bromley, regarded as one of the most effective science advisers in recent times, described in a 1994 memoir how he focused on a "small number of areas … that were of particular national importance." Bromley restructured the Federal Coordinating Council for Science Engineering and Technology and won the president's support for a rule stating that agencies had to send their top officials "or they were not represented at all." The council helped Bromley coordinate federal climate research and materials science as well as launch initiatives on international science and science education.

Gibbons greatly expanded the council, however, arguing that "you have to coordinate somehow." But Professor Christopher Hill of George Mason University in Arlington, Virginia, believes Gibbons went too far. "The agencies were just overwhelmed with committee work," Hill says.

There's no question that a successful science adviser needs a strong staff. But experts disagree on just what that means. The report that Nichols co-authored, from the Woodrow Wilson International Center for Scholars, recommended the traditional complement of four Senate-confirmed deputies. That's double the number Marburger believed sufficient when he set up his office in 2001. Eliminating an environmental deputy weakened the White House's ability to coordinate climate change research, says Richard Moss, the White House climate program director under Clinton and, until 2006, for Bush (*Science*, 10 October 2008, p. 182). But Marburger blames the timidity of the program itself, saying that OSTP and the White House budget office "were willing to support a much bolder reallocation of resources than [the program] ever recommended."

Obama's pledges and appointments to date raise the hopes of science policy watchers that their concerns will be addressed in his administration. But if history is any guide, Holdren's effectiveness will also be determined by his ability to navigate the political winds swirling about the White House.

–ELI KINTISCH

PRESIDENT	SCIENCE ADVISERS	MAJOR ISSUES
Dwight Eisenhower	<< James Killian, George Kistiakowsky	<< Space exploration, National security
John Kennedy	Jerome Wiesner	Arms control
Lyndon Johnson	Donald Hornig	Environment
Richard Nixon	Lee DuBridge, Edward David, Guyford Stever	Antiballistic missiles, supersonic transportation
Gerald Ford >>	Guyford Stever	Energy, Environment
Jimmy Carter	Frank Press	Energy, Environment
Ronald Reagan	George Keyworth, William Graham	<< Missile defense, Competitiveness
George H. W. Bush	D. Allan Bromley	International science, Competitiveness
Bill Clinton >>	John Gibbons, Neal Lane	Biomedical research, << International Space Station
George W. Bush	John Marburger	Homeland security, Bioweapons

CREDITS (TOP TO BOTTOM): NASA; NASA; DAVID HUME KENNERLY/WHITE HOUSE; USMDA; WIKIPEDIA; NASA

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BRAIN CANCER

A Viral Link to Glioblastoma?

Circumstantial evidence hints that cytomegalovirus, a common herpesvirus, may play a role in the aggressive brain cancer, but big questions remain

SAN FRANCISCO, CALIFORNIA—Neurosurgeon Charles Cobbs is trying to remove as much of a brain tumor as possible without damaging tissue important for speech. He needs the patient's cooperation, so the anesthesiologist dials down the propofol anesthetic and places a laptop computer on a stand near the patient's head. The 77-year-old man, who has a glioblastoma near the crease that separates the frontal and temporal lobes, slowly wakes up.

Images of familiar objects flash on the screen, and the patient names them while Cobbs electrically stimulates his exposed cortex with a hand-held probe. "Cow, car, hairbrush," the man says. He sounds groggy, but the words are unslurred. Cobbs turns up the current for stronger stimulation and does another pass as the man counts to 50. Satisfied that the tumor can be safely resected, the surgical team here at the California Pacific Medical Center puts the patient back under, and Cobbs scoops out a bloody glob of tissue, most of which goes into a vial to be sent to the lab.

After the surgery, Cobbs is optimistic. Glioblastoma is the worst grade of malignant glioma, a relatively uncommon but deadly class of brain tumors that kills 97% of those diagnosed within 5 years. But Cobbs says the material he removed had the rubbery feel of dead tissue rather than the "grape jelly" consistency of a growing tumor. That would be good news for the patient, and Cobbs thinks it may also bode well for his own unorthodox hypothesis about malignant glioma.

Cobbs thinks the cancer may be caused, or at least spurred on, by cytomegalovirus (CMV), a common herpesvirus. Several years ago, Cobbs reported that malignant gliomas are rife with CMV, and he says work by other researchers supports his idea that the virus may be a potent promoter of these cancers. His patient has been taking an antiviral drug called Valcyte, made by Roche Pharmaceuticals, in addition to receiving standard radiation and chemotherapy. Cobbs thinks drugs and other therapies that target CMV may extend the lives of patients who otherwise have few options.

The idea is controversial and unproven. Why would such a common virus cause cancer in only a small subset of those infected? And why have test tube experiments so far failed to show that CMV transforms healthy cells into cancerous cells? These and other nettlesome questions beg for answers before Cobbs's theory can gain widespread acceptance.

Yet several researchers and clinicians who attended a meeting Cobbs organized in Boston last October say there is enough circumstantial evidence in support of the idea to merit serious attention. "It's a promising concept, and it gives us hope of new treatment strategies for this horrible disease," says Cecilia Söderberg-Nauclér of the Karolinska Institute in Stockholm, Sweden. She and others have already begun small clinical trials with drugs and immunotherapy directed at CMV.

> ***"Your job as a scientist is to think for yourself and question what the herd is thinking."***
>
> **—CHARLES COBBS, CALIFORNIA PACIFIC MEDICAL CENTER**

Counter to the mainstream

At age 45, Cobbs still has the rangy build of a collegiate rower, which he was at Princeton University. Soft-spoken and bespectacled, he doesn't come across as a rebel, although his thinking about malignant glioma has run counter to the mainstream. Cobbs did research as a medical resident suggesting that these tumors were "boiling cauldrons of inflammatory molecules," and he wondered whether this inflammation might somehow trigger the cancer or help it grow. It wasn't a fashionable idea at the time, and for a young investigator, it was a risky avenue to pursue.

Reading *Surely You're Joking, Mr. Feynman!*, a memoir written by the iconoclastic physicist Richard Feynman, boosted his confidence. Cobbs says the message he took away from the book is that "your job as a scientist is to think for yourself and question what the herd is thinking." Cobbs had a hunch that CMV might be just the sort of virus to lurk around and cause the kind of chronic inflammation he suspected of contributing to cancer. The virus infects for life, and mouse studies have found that it infects the neural stem cells thought to give rise to glioblastomas. To investigate, he and colleagues tested 27 malignant glioma samples for CMV proteins and genetic material. All were positive, the researchers reported in *Cancer Research* in 2002. Samples of noncancerous brain tissue tested negative.

Not everyone was convinced by the findings. In 2005, a team of researchers at City of Hope Hospital in Duarte, California, reported in *Modern Pathology* that they'd failed to find CMV in glioma samples. "I was kind of distressed and heartbroken," Cobbs recalls. A team at Duke University Medical Center in Durham, North Carolina, wasn't finding CMV in its samples, either. "We called [Cobbs] and said, 'What's the deal?'" says

CREDIT: LACY ATKINS/*SAN FRANCISCO CHRONICLE*

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Delicate operation. Charles Cobbs removes a brain tumor at California Pacific Medical Center.

CREDIT: LACY ATKINS/SAN FRANCISCO CHRONICLE

Duane Mitchell, a neuro-oncologist at Duke. Cobbs suspected that the other researchers were using less sensitive methods and offered to visit both teams to demonstrate his technique. Only the Duke team took him up on it.

Since then, there have been several confirmations. Last February, Mitchell and colleagues reported finding CMV in more than 90% of glioma samples they examined; a team at M. D. Anderson Cancer Center in Houston, Texas, published similar findings a few months later; and Söderberg-Nauclér and colleagues have a forthcoming paper as well. "Without a question, the virus is there," says Söderberg-Nauclér. But is it a bystander or an agent provocateur?

A suspicious resume

CMV is one of eight human herpesviruses; it infects at least half of the population in developed countries and nearly everyone in developing countries, where poor sanitation and hygiene encourage its transmission. Although it generally doesn't cause problems in healthy adults, CMV is a common cause of birth defects, and it can cause a host of serious problems in immunocompromised people.

How it might trigger or abet cancer is not known, but there's no shortage of possibilities, says virologist Jay Nelson of Oregon Health and Science University in Portland. One way it could do this "is by preventing the immune system from taking the tumor out," he posits, because CMV is adept at manipulating signaling molecules on cell surfaces that would otherwise flag infected cells for destruction. Another possibility is that the virus helps sustain tumors by promoting angiogenesis, the growth of new blood vessels. Nelson's lab reported last year that CMV-infected heart cells churn out a slew of growth factors and other compounds that spur angiogenesis.

The virus also makes proteins that interfere with cellular machinery that normally prevents tumors, says Robert Kalejta, a cancer biologist and virologist at the University of Wisconsin, Madison. In 2003, Kalejta and colleagues reported that a CMV protein called pp71 helps break down retinoblastoma protein (Rb), a potent tumor suppressor. More recently, they identified a CMV enzyme called UL97 that inactivates Rb (*Science*, 9 May 2008, p. 797). "Clearly, these are two proteins that can do what's required" to transform healthy cells into tumor cells, Kalejta says. "The question is, are they really doing that in these cancers?"

Kalejta says he's intrigued but not yet convinced. He notes that unlike proven cancer-causing viruses such as human papillomavirus and Epstein-Barr virus, CMV has not been shown to transform healthy cells into cancerous cells. The lack of direct evidence that CMV can cause cancer remains an important caveat, Kalejta says.

Liliana Soroceanu, a neuroscientist who collaborates with Cobbs at the California Pacific Medical Center, hopes to gain clues about how CMV might contribute to malignant glioma by culturing cells from tumor tissue Cobbs has removed from patients. She plans to use a CMV gene chip developed by Nelson's team to examine which genes the virus turns on in infected glioma cells—and to see whether turning those genes off, using RNA interference, for example, slows the growth of cultured tumors.

Partners in crime? Cobbs suspects that malignant gliomas like this one may be aided by cytomegalovirus.

Bedside stories

Evidence of a link between CMV and glioma has come from the bedside as well as the lab bench. One serendipitous finding comes from a clinical trial with glioblastoma patients run by Robert Prins and colleagues at the University of California, Los Angeles. They have been testing a vaccine intended to train patients' immune systems to attack their tumors. To do this, they grow immune cells from each patient in culture, expose them to proteins from that patient's tumor, and inject them back into the patient.

As it happens, one patient responded to the vaccine by mounting a massive immune response against a CMV protein, the researchers reported last July in *The New England Journal of Medicine*. That patient has now survived nearly 6 years without a recurrence of the cancer, longer than 99% of patients who receive standard care, Prins says. (All patients in the trial received standard care—surgery, radiation, and chemotherapy—in addition to the vaccine.) Although it's impossible to know if the vaccine is the reason, given the slim odds of surviving that long with glioblastoma, Prins thinks it's highly likely.

At Duke, Mitchell and colleagues are pursuing a similar approach more tightly focused on CMV. They are vaccinating glioblastoma patients with immune cells exposed to CMV proteins. The trial is intended to assess the safety of the treatment, but there are signs that it is having the desired effect. At November's meeting of the Society for Neuro-Oncology, the researchers reported that the vaccine had enhanced immune responses against CMV, and the 21 patients in the trial had a median survival time of more than 20 months. The median survival time so far for such patients is typically less than 15 months, Mitchell says.

At the Karolinska Institute, Söderberg-Nauclér and colleagues have just completed a 2-year trial with Valcyte, the antiviral drug Cobbs's patient received. (Cobbs prescribed the drug off-label, not as part of a clinical trial.) The 42 patients with glioblastoma began taking Valcyte after surgeons had removed their tumors and researchers confirmed that the tumors were infected with CMV. Researchers used magnetic resonance imaging to look for signs that tumors grew back. The study is double-blinded, so the researchers don't yet know which patients received the drug and which received a placebo. Even so, Söderberg-Nauclér is optimistic. "We have patients that looked extremely bad at the beginning that have survived 2 years," she says. The trial concluded just before Christmas, and the team should know soon whether those patients received the drug—or just a remarkable stroke of good luck.

Cobbs says he's hopeful that if the Swedish trial goes well, Roche will fund a larger one. He insists he doesn't want to hype the evidence for a link between CMV and glioma but says he's gratified that other researchers are starting to take the idea seriously. "It may be a house of cards, but my gut tells me it might pan out to be something big time."

–GREG MILLER

POLAR SCIENCE

A Death in Antarctica

The death in 2000 of a young Australian astrophysicist at the U.S. South Pole station raised many troubling questions. Eight years later, there are few answers

On the last day of his life, Rodney Marks woke up vomiting blood. The 32-year-old postdoc was wintering over at the U.S. Amundsen-Scott South Pole Station, where he was operating AST/RO (Antarctic Submillimeter Telescope and Remote Observatory) for the Harvard-Smithsonian Center for Astrophysics. Over the next 10 hours, Marks made three trips to see Robert Thompson, the station's doctor, becoming increasingly anxious, disoriented, short of breath, and pained. Then he went into cardiac arrest. After attempting to resuscitate Marks, Thompson pronounced him dead at 6:45 p.m. on Friday, 12 May 2000.

Outside, the temperature was –62°C. The station would remain in the throes of the brutal Antarctic winter and its 24-hour nights for another 5½ months. Addressing the 48 scientists, construction workers, and service personnel at a hastily called meeting, Thompson explained that Marks had died of unknown but natural causes. With no way out until November and with plenty to do, each of the winterovers mourned the sudden loss of someone who had enriched the tight-knit community with his keen intellect, bohemian ways, and outgoing personality. Then they went back to work, leaving their fallen comrade to be preserved in storage by the inhuman cold.

On 30 October, after flights resumed between Antarctica and New Zealand, Marks's body was taken out of storage and flown to Christchurch, New Zealand, on its way to burial in his native Australia. In mid-December, Martin Sage, a forensic pathologist in Christchurch, delivered another shocker: Marks, in apparent good health, had died of methanol poisoning. In dispassionate prose, Sage described how Marks had consumed approximately 150 milliliters of a colorless and slightly sweet-tasting liquid, commonly known as wood alcohol, under unknown circumstances. By the time Marks visited the base's rudimentary medical center, his system had converted the methanol—used routinely at the pole to clean scientific equipment—into formic acid, leading to the acute acidosis that caused his symptoms. The source of the methanol, Sage reported, "is not apparent from the accounts given to date," adding that "there is a distinct possibility" Marks may not have known that he was drinking methanol.

The new information in the autopsy was a revelation to colleagues, who had assumed his death was caused by a massive stroke or heart attack. It spawned a fresh set of troubling questions. Had Marks drunk the methanol intentionally? If so, why would he have wanted to kill himself? If the ingestion was an accident, how had it happened? If deliberate, had someone spiked his drink or switched glasses without his knowledge?

"I can't imagine how he could have drunk it," says Antony Stark, an astronomer at the Smithsonian Astrophysical Observatory and principal investigator for AST/RO, which is funded by the U.S. National Science Foundation (NSF). "I cannot believe he committed suicide. He had friends. He had a fiancée; the work was going well; the instrument was doing fine."

It would be nearly 8 years before the New Zealand government, in the person of coroner Richard McElrea, would deliver an official statement about what had happened. However, the coroner's report, published in September 2008, answered none of those questions—and raised several troubling new ones. "I formally record that Rodney David Marks … died as a result of acute methanol poisoning, the methanol overdose being undiagnosed and probably occurring 1 to 2 days earlier, …" McElrea begins the last paragraph of his 50-page report, echoing Sage's autopsy. Then the coroner jammed all the outstanding issues, still unresolved, into the last half of that grammatically challenged sentence. "[Marks] being either unaware of the overdose or not understanding the possible complications of it, the medical assistance to him being compromised by an Echtachem [*sic*] blood analyzer being inoperable, death being unintended."

The report was packed with fresh details from dogged police work and hearings held in 2000, 2002, and 2006. But it leaves

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Into the darkness. The setting sun in late March heralds 6 months of winter for those at South Pole's Amundsen-Scott Station.

unanswered why a healthy and apparently happy young scientist consumed a lethal amount of a known poison. One reason for the continuing mystery is the nature of the care that Marks received. A report by a team of physicians who reviewed Thompson's medical notes weeks after Marks died concluded that "additional laboratory investigation" and other analyses "were warranted" in treating Marks that day. The review, led by chief medical adviser Gerald Katz of Raytheon Polar Services (RPS) Co. of Centennial, Colorado, NSF's polar logistics contractor, also stated that several tests not performed "were available at the time and would have been helpful in narrowing the diagnostic possibilities."

Another contributing factor was the isolated locale. McElrea's report acknowledges the "limitations" posed by conducting an investigation from a distance of 5000 km and with little firsthand evidence available. Still, he says, there were steps that could have been taken. "The scene could have been preserved for photographs … and initial statements could have been obtained from all relevant personnel," he writes. "Very little of this process happened," he notes. He also cites "legal, diplomatic, and jurisdictional hurdles" erected by the U.S. government that delayed his inquiry. In most cases, the relevant agency was NSF, which is responsible for all U.S. scientific activity on the frozen continent, and RPS.

Paul Marks, Rodney's father, thinks that the U.S. government assigned the case a low priority because of his son's Australian citizenship. "If it had been one of yours, a U.S. citizen," Marks told *Science* recently, "I can't believe that the FBI wouldn't have been involved from the start and that no stonewalling would have occurred."

A final obstacle was the 7-month gap between the astronomer's death and the autopsy. Although the report from the team of physicians, submitted in July 2000, noted that "there is no evidence to point to homicide, accidental poisoning, environmental toxicity, or infection," McElrea says its conclusion was premature because the autopsy finding methanol as the cause wasn't released until December. "I respectively [*sic*] disagree that accidental poisoning and even foul play can be adequately disregarded without a full and proper investigation … with proper protocols for preservation and recording of evidence."

A scientific life. Australian Rodney Marks worked on the SPIREX infrared telescope in 1997–98 and returned 2 years later to operate AST/RO.

CREDITS: D. A. HARPER; (INSET) CENTER FOR ASTROPHYSICAL RESEARCH IN ANTARCTICA

Popular in purple

Rodney Marks grew up in a small coastal town in the southern state of Victoria. By age 7, he was doing crossword puzzles with the help of a thesaurus. A scholarship to a prestigious private school in nearby Geelong fed his budding interest in math and science, which he pursued at the University of Melbourne. In 1993, he enrolled in a Ph.D. program in astronomy at the University of New South Wales.

By all accounts, Marks enjoyed shattering stereotypes. Astrophysicist Gene Davidson, a New Zealand native who wintered over to operate another telescope the same year Marks died, recalls meeting the bearded, rangy, 6'2" free spirit in the mid-1990s at a session for graduate students during the annual meeting of the Astronomical Society of Australia. "He didn't look like a typical scientist. He had long hair and [dressed] Goth, with black fingernails. He stood out," says Davidson, now a scientist at Australia's research nuclear reactor in Sydney. Marks, who had dyed his hair purple during the winterover, also played guitar in a heavy metal band, The Changelings, that performed from the South Pole during a global celebration on 1 January 2000 marking the new millennium.

Marks first wintered over in 1997–98, caring for an infrared telescope called SPIREX and using some of the data in his thesis. But he also liked sharing his passion for science. On Wednesday evenings, for example, Marks gave a series of 1-hour introductory lectures on astronomy to the entire base. In addition to being educational, the talks helped bridge the gap between scientist and layperson. "Rodney was a very popular person in the community," recalls Darryl Schneider, a physicist who was wintering over that year to maintain the Antarctic Muon and Neutrino Detector Array.

But science wasn't the only thing in Marks's life. On many nights, he'd hang out in the galley, socializing first with the nonsmokers, who left early, and then with the smokers, who tended to arrive later and linger until the wee hours. In his spare time, he provided free French lessons. (Michael Ashley, his thesis adviser, had recommended a short-term project at the University of Nice that required a knowledge of French. Marks, who spoke not a word of the language, told him, "Okay,

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sounds interesting; I'll do it." Within a few months, Ashley recalled at a memorial service, Marks was fluent.)

Marks's winterover in 2000, his third tour at the pole, required him to coordinate experiments being done remotely on AST/RO and to collect data on viewing conditions. "He was running the whole instrument, and he was doing a very good job," says Stark, who has made 21 trips to Antarctica but never wintered over at the pole. "All our winterovers have gone on to good academic jobs, and I'm sure that Rodney would have gotten one, too. He was an excellent scientist."

Stark and others can't imagine that Marks would have jeopardized such a bright future by knowingly ingesting methanol, and Stark says the toxic liquid wouldn't have been just lying around. It was typically used in January when the cryogenic parts of the telescope were being cleaned and reassembled, he notes, not after the austral winter had set in and the instrument was in use. In any event, the bottles containing methanol were clearly marked and kept in a locked cabinet.

The coroner's report offers no eyewitness accounts of how Marks swallowed the methanol. But it contains speculation from those who knew him about whether his drinking habits were to blame. Will Silva, a Seattle, Washington–based physician who has wintered over at the South Pole three times and had gotten to know Marks the previous season, testified that Marks "was a steady sort of bloke who drank to excess on occasion" but who had a "high tolerance for alcohol." (Silva was working at Palmer Station on the Antarctic peninsula the year Marks died and was one of the doctors who reviewed Thompson's notes.) Davidson says that his friend "tended to be a binge drinker, but so were a lot of people. Rodney certainly wasn't an alcoholic. He didn't need alcohol to get through the day." Dr. Thompson testified in November 2000 that he "was strongly leaning toward alcohol withdrawal and anxiety as contributing factors" when Marks came to the clinic on the day he died.

A mechanical mystery

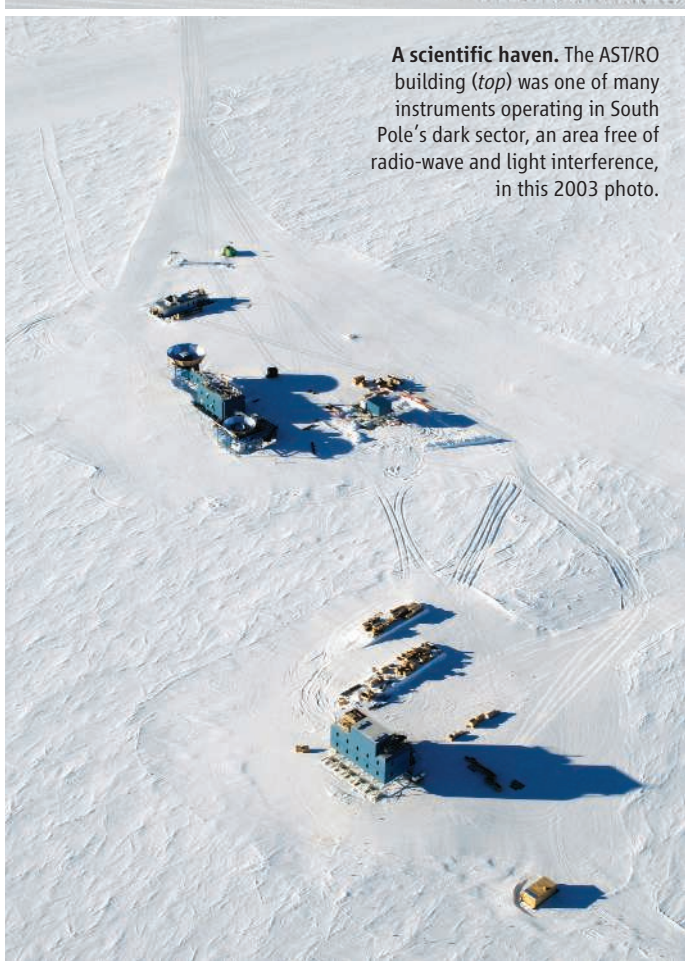
Thompson's initial diagnosis never raised the possibility of methanol poisoning. In part, that's because he didn't perform a test that might have tipped him off. And the reason for that omission is another element of the case that troubles Marks's friends and relatives.

In setting up the clinic after he arrived in November 1999, Thompson found that a machine called an Ektachem, which can measure a patient's blood chemistry, needed to be recalibrated every time it was turned back on. "It was an 8- to 10-hour process once it went down," he testified, and Thompson said he "was too busy providing critical care to Rodney" once Marks arrived on 12 May to take the time needed to do it.

What Thompson didn't know is that the problem was due to the failure of a lithium battery that allows the machine to maintain its electronic memory after it's turned off. Had the Ektachem been kept running, it would have been available for immediate use after Marks showed up even though its battery was dead. (The battery didn't affect how the machine performed once it was calibrated.)

Thompson also testified that the machine was difficult to use, unreliable, and that the contractor was responsible for maintaining it. Not so, says Silva. Operating and maintaining the machine "is quite straightforward," he told the coroner in 2006. He also explained that the manufacturer, Ortho Clinical Diagnostics, offers comprehensive online and free telephone technical support to deal with any problems. The coroner tried unsuccessfully to contact Thompson to invite him to respond to Silva's testimony on this and other points; the physician's current whereabouts are unknown.

Whether the machine could have saved Marks is debatable. Sage, the forensic pathologist, testified that Marks's "chances of survival would have been considerably greater" with a timely diagnosis. Silva is less sanguine, having testified that "he very much doubted" that the standard treatment of infusing a 10% ethanol solution "could have succeeded given the magnitude of Rodney's intoxication." The coroner's report sides with Sage, concluding that "the ektachem analyzer, if operational on the day, could well have led to an analysis of methanol poisoning, with the chances of his survival being considerably enhanced." And McElrea blames Thompson for its unavailability. "It was his responsibility to keep it calibrated," he writes. Leaving aside whether the treatment would have worked, Paul Marks and others argue that fingering methanol immediately would also have likely triggered a more thorough investigation at the scene.



A scientific haven. The AST/RO building (*top*) was one of many instruments operating in South Pole's dark sector, an area free of radio-wave and light interference, in this 2003 photo.

CREDITS (TOP TO BOTTOM): THOMAS NIKOLA/CORNELL UNIVERSITY, DEPARTMENT OF ASTRONOMY; SGT. LEE HARSHMAN/U.S. AIR FORCE/NSF

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Silva, who no longer works for the Antarctic program, was one of two persons whom the coroner singled out for praise in his report. (The other is Harry Mahar, a former NSF health and safety officer now working at the U.S. State Department.) Both men provided "meaningful evidence" on several matters that NSF officials had declined to discuss, the report notes.

Explaining why he agreed to testify, Silva told *Science* that "Rodney was one of our mates. … I did what I did because I think it's important to shed light on what happened when something goes amiss. I fancy that had NSF or RPS done some investigation and made it available to the authorities, the coroner probably would not have felt the need to become involved. [But] it appeared to us that there had been no substantive investigation."

Hurdles to clear

Why did the coroner's investigation take so long? McElrea says he gave it his best shot. "The New Zealand police carried out as effective an investigation as was possible given the legal, diplomatic, and jurisdictional hurdles that arose over a number of years," McElrea writes.

McElrea's report notes that NSF never gave him a copy of the July 2000 Katz report that reviewed Thompson's medical records. (McElrea eventually obtained it, however, and attached it to his report.) In addition, McElrea describes how it took his chief investigator, Detective Senior Sergeant Grant Wormald, nearly 3 years to obtain information from Marks's co-workers at the pole after he sought the cooperation of NSF. NSF finally agreed to distribute a voluntary questionnaire to them but attached several strings. NSF officials vetted the content of the questionnaire "to assure ourselves that appropriate discretion has been exercised." Once the questionnaire passed muster, it was mailed out by RPS, with a note saying that participation was voluntary. The police heard back from only 13 of the 49 co-workers.

McElrea also says that unspecified "procedural reasons" foiled repeated efforts by Wormald to contact Thompson for a follow-up interview. In addition, the report includes Wormald's testimony in 2006 that "despite numerous requests, [he] was not entirely satisfied that all the information about investigations made by RPS or NSF has been disclosed to the New Zealand police or coroner."

McElrea's report doesn't address why those hurdles were thrown in his path, and he has declined further comment. "My role as a judicial officer is complete on the giving of findings and it is not appropriate that I discuss or comment on the case further," he e-mailed *Science* in late November.

However, it's possible that McElrea's own workload may have contributed to the slow pace of the investigation. Until last year, the coroner's job was a part-time position for McElrea, a partner in a large law firm in Christchurch. McElrea was also in the midst of writing a book when he claimed jurisdiction over the case after Marks's body was flown to Christchurch. Oddly enough, the book describes the heartbreaking saga of a group of men trapped in Antarctica while laying in supplies for Ernest Shackleton's aborted land crossing in 1914. *Polar Castaways* was published in 2004.

NSF officials say the foundation has shared all appropriate materials with New Zealand authorities. Karl Erb, director of NSF's Office of Polar Programs, wrote in September 2005 to Neville Mathews, New Zealand's police representative at its embassy in Washington, D.C., that the Katz report dealt "only with medical aspects of the case in an effort to determine the cause of death and whether any action to protect other personnel at South Pole Station was required." (The report, issued 5 months before the methanol poisoning became known, concluded that "no definitive diagnosis could be ascertained from the available data.") The review, he added, "[is] therefore of little value to your inquiry." Erb insists that NSF was attentive to requests from New Zealand for help, pointing to the coroner's statement in his report that he "acknowledge[s] the cooperation of NSF with the inquiry over several years."

As to whether additional evidence should have been gathered at the scene, Erb told *Science* this fall that the South Pole "is a working environment. It would not have been practical to cordon off the area. It's a very small place, and every part of it is in constant use."

Erb says he deeply regrets what happened: "It's a tragic, tragic event. And I have so much sympathy for his parents and family." However, he doesn't think that NSF could have done anything differently. "If the coroner had had any reason to suspect foul play, he would have told us, and we would have contacted the Justice Department," Erb says. "But we were assured 8 years ago that there was no evidence of foul play."

No resolution

Eight years after the tragedy, residents at the South Pole live and work in a new $150 million station that was dedicated earlier this year. The medical quarters where Marks spent his last hours have been replaced by a modern medical facility, with telecommunications equipment that allows specialists in the United States to guide the station physician in carrying out diagnostic and therapeutic procedures. The living quarters, although hardly plush, are decidedly roomier. The science is booming: AST/RO and its 1.7-m mirror, for instance, has been succeeded by the 10-m South Pole Telescope, the largest ever deployed at the pole, which began making millimeter-wavelength observations last year.

CREDIT: PHOTO BY SAM KITTNER FOR NSF

"If the coroner had had any reason to suspect foul play, he would have told us, and we would have contacted the Justice Department."

—KARL ERB, DIRECTOR, OFFICE OF POLAR PROGRAMS, NSF

As a decade once filled with promise for his talented son winds down, Paul Marks doesn't hold out much hope of getting to the bottom of what transpired. "After so long, it's probably impossible to ever know what happened and if he died by sinister means or by accident," he says. "That's something we have to live with."

McElrea's report says that Marks's death points to a flaw in a system that governs the behavior of all nations that operate in Antarctica. In his sole recommendation to the New Zealand government, he says that the "partial outcomes" in this case "point to an urgent need to set comprehensive rules of investigation and accountability for deaths in Antarctica on a fair and open basis."

Paul Marks also believes that there are lessons to be learned from his son's death. "The overall management system, and the way NSF and Raytheon behaved that allowed this to happen, that's something that should be addressed," he says. "People will find ways to do bad things. But things should never have reached the point at which somebody could drink a tainted liquid."

–JEFFREY MERVIS

LETTERS

edited by Jennifer Sills

Literature Citations in the Internet Era

J. A. EVANS'S REPORT "ELECTRONIC PUBLICATION AND THE NARROWING OF SCIENCE AND scholarship" (18 July, p. 395) suggests that (i) the average age of citations to scientific papers dropped over the years as more electronic papers became accessible and (ii) the citations are concentrated on a smaller proportion of papers and journals. Such conclusions are not warranted by Evans's data.

To measure the evolution of the average (or median) age of the references contained in papers, one has to look at all the references in all published papers and observe the evolution of their age over time. As we have shown using Thomson Reuters's Web of Science data for the period 1900 to 2004 (for a total of 500 million references in 25 million papers), the average (and median) age of all references began to decrease in 1945 but has increased steadily since the mid-1960s. This trend is visible in all sciences, including the social sciences and the humanities (*1*, *2*). The median age of references in fields of science and engineering moved from 4.5 years in 1955 to more than 7 years in 2004, and in medical sciences it increased from 4.5 to 5.5 during the same period (*1*). In fact, Evans's conclusions only reflect a transient phenomenon related to recent access to online publications and to the fact that the method used does not take into account time delays between citation year and publication year. Our data also show that in disciplines in which online access has been available the longest (such as nuclear physics and astrophysics), the age of references declines for a number of years in the 1990s but then increases from 2000 to 2007, the last available year of our data set. We have also measured the concentration of citations (and journals) by three different methods, including the one used by Evans. All three measures clearly show that concentration is in fact declining for papers as well as for journals (*3*). Although many factors affect citation practices, two things are clear: Researchers are increasingly relying on older science, and citations are increasingly dispersed across a larger proportion of papers and journals.

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Narrower Focus May Be More Efficient

IN HIS REPORT "ELECTRONIC PUBLICATION and the narrowing of science and scholarship" (18 July, p. 395), J. A. Evans expresses dismay that electronic publication narrows scholarship. He found that more recent articles included fewer, more recent citations. However, Evans gives us no reason to believe that this is actually detrimental to science. Indeed, he suggests that the integration of science in the days of paper-only journals may have been an unintended consequence of poor indexing. Contrary to what Evans claims, we may find that scientists' narrower focus on the literature is a good sign.

Science has frequently been compared to evolution by natural selection (*1*). In some species, relatively few offspring reproduce. Similarly, in science, relatively few papers affect subsequent scholarship. The citation norms that are emerging as a result of the growth of electronic publication may be making the scientific community more efficient. Scientists may be spending less time reading literature that is extraneous to their research. Before we bemoan the changes in citation patterns that Evans has discovered, we should examine more carefully the causes and effects of such changes.

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To Each Citation, a Purpose

THE REPORT BY J. A. EVANS ("ELECTRONIC publication and the narrowing of science and scholarship," 18 July, p. 395) claims that electronic publication "may accelerate consensus and narrow the range of findings and ideas built upon." But do the currently available data support this chilling conclusion?

Evans's argument is based on evidence that with electronic access, fewer papers and fewer older papers are cited, and that cited papers are less broad and diverse. To understand these

Designing cloaks with warped space

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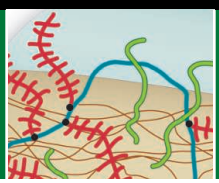

Cartilage lubrication

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trends, one needs to consider the diverse purposes of citations as well as the mindset and motivation of authors when they decide which papers to cite (*1*). By expanding the range of papers known to authors, a more complete grasp of current literature helps them to select more appropriate citations. If the citations are more relevant and focused, the observed trends toward fewer citations may be a positive development.

But why are cited papers less broad and less diverse? Current citation indices do not distinguish the purposes of citations, which may serve as confirmation, refutation, background, technical details, or another role. Evans does not know whether narrow citers are more likely to converge with prevailing opinion than broad citers. Given the lack of evidence, it is speculative to equate narrow citing with hastening of consensus. Validation of this assumption requires a more refined analysis that is not available in Evans's databases: classification of citations (*2*). Disappointingly, Evans fails to consider such a roadmap, which would move the analysis to the next, more conclusive level.

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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.

A Cycle of Tradition and Innovation

IN HIS REPORT ("ELECTRONIC PUBLICATION and the narrowing of science and scholarship," 18 July, p. 395), J. A. Evans states that "[b]y enabling scientists to quickly reach and converge with prevailing opinion, electronic journals hasten scientific consensus," and he warns that there may be a cost of this development. Findings that do not become part of the consensus quickly may be forgotten quickly, and interesting findings and ideas may be overlooked if the selection of literature is narrow in scope and time.

This warning is backed by classic arguments in the philosophy of science. Thomas Kuhn was the first to argue that the development of science builds on an essential tension between the convergent activity of normal science and divergent activity of scientific revolutions (*1*). On one hand, during phases of normal science, the confident and continuous use of accepted theories enables science to move faster and penetrate deeper than if dramatic theory changes were needed all the time. Thus, convergent thought often increases the effectiveness and efficiency with which scientific problems are solved. On the other hand, if this was the only mode of conducting science, there would be no fundamental innovation. Often, new discoveries lead to major rearrangement of the intellectual equipment as researchers discard elements of old theories, develop new theories, and establish new relationships between known theories. This requires flexibility and open-mindedness.

The consequences of the new patterns in the use of journal literature may differ for different phases of science. During phases of normal science, it is important to exhaust the full potential of the accepted theory to make sure that it is not abandoned too quickly. During such phases, a narrow focus on closely related findings will increase the effectiveness and efficiency with which existing theory and observation are adjusted and brought into closer and closer agreement. On the contrary, when anomalous results are encountered that question the adequacy of the previously accepted theory, alternatives must be developed. In these cases, it is important to distribute risk between several different approaches (*2*, *3*), and a narrow focus will substantially impede progress.

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Response

THE LETTER BY Y. GINGRAS *ET AL*. ALLOWS ME to clarify the scope of my research. I demonstrated the narrowing influence of online availability on the depth and breadth of attention in science. Each of my models controlled for year to test whether the availability of deeper online archives was associated with more shallow and narrow subsequent references to available journals than would have occurred had they not gone online. I acknowledged the historical trend and am relieved that Gingras *et al*. find the same. Citations deepen as more research lives in the past; articles cited are more numerous and less concentrated as more authors and universities produce research.

Gingras *et al*. question but do not test my claim that electronic availability narrows citation trends. They anecdotally note that online access came first to the physical sciences, where citations are deepest and deepening. Consider, however, that each field possesses distinctive features that confound comparison. For example, the physical sciences have recently received less funding and experienced slower growth than the biological sciences, increasing the relative importance of past research. I compared each journal and each subfield only to itself as its online availability shifted. The narrowing influence of online availability remained robust in analyses with varied time of online availability and citation years and for nearly all broad subfields. Indeed, under many of these alternative specifications, online influence appeared larger than recorded in my Report.

K. B. Wray and C. S. von Bartheld *et al*. express doubt that increased search efficiency and faster consensus could be anything but positive for scientists and science. However, we have no evidence that

online availability gives researchers more proof or better judgment. We do know that scientists working with online journals have more exposure to what others find important as they hyperlink through citations and select references from the highest-impact journals in their search lists. Moreover, there is strong evidence from economics (*1*, *2*), sociology (*3*, *4*), and business (*5*)—and for varied markets, including those trafficking scientific ideas (*6*)—that the most popular products attract disproportionately more attention as (i) markets grow, (ii) the marginal cost of reproducing and distributing products is low, and (iii) consumers gain more exposure to others' choices. As articles become available online, each of these properties is enhanced.

Von Bartheld *et al*. appropriately observe that my Report counts references without identifying their purpose. Some meticulous classifications of this type have been performed (*7*), but problems remain (*8*). Most references, for example, are censored—an idea is mentioned but not formally cited. To move forward, we need the distribution of statements across articles themselves to understand the degree to which online availability influences scientific search, consensus, and advance (*9*, *10*). Emblematic is a recent study that analyzes millions of extracted, sequenced statements about molecular interactions from thousands of articles (*11*). It demonstrates that a skeptical interpretation about the truth value of scientific resolutions is at least as likely as a confident one, and so cautions us against assuming that quick resolutions are optimal.

H. Andersen draws on Thomas Kuhn's imagery of the "essential tension" to suggest that the shift to online information search optimizes "normal science" but undercuts revolutionary discovery. I agree with Andersen that recent patterns of online usage likely promote elaboration of recent ideas over the generation of new ones. It is unclear, however, whether these developments make normal science more effective. Scientific paradigms require generations for a research community to work out in detail. Consider the molecular science and engineering disciplines that have arisen in the wake of the discovery of DNA. Recent research that extracted molecular interactions from chemical and biological journals estimates that over one billion molecular interactions have been published in the past 25 years, but their number and the age and diversity of articles in which they appear make them practically inaccessible to individual researchers (*12*). Current patterns of online search will likely exacerbate the speed of forgetting. In other words, scientists find Internet information search more efficient for producing their articles (*13*) and it appears to hasten consensus within subfields; but this may not be optimal for normal science, which demands that myriad dispersed details be organized to complete a paradigm.

For this reason, I concur with Andersen's conclusion that other approaches to finding information should be cultivated. As we consider which, we must recognize that scientists will not return to bricks-and-mortar libraries (*14*). Electronic publication could support multiple research paradigms, but only when computer-assisted approaches move beyond searching, ranking, and summarizing. As we begin to computationally extract (*10*), compare, and link together claims from online literature into probabilistic inferences (*11*)—as we use computation to read and reason rather than simply shelve and retrieve volumes—online availability could enhance both normal and revolutionary science.

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CORRECTIONS AND CLARIFICATIONS

Research Articles: "Optical images of an exosolar planet 25 light-years from Earth" by P. Kalas *et al*. (28 November 2008, p. 1345). In the abstract, the phrase "matching predictions of its location" was inadvertently added to the end of two different sentences during final corrections. The phrase correctly appears at the end of the third sentence, whereas the fifth sentence should read "Dynamical models of the interaction between the planet and the belt indicate that the planet's mass is at most three times that of Jupiter; a higher mass would lead to gravitational disruption of the belt."

News Focus: "Reaching for the stars in Romania" by M. Enserink (21 November 2008, p. 1183). Romanian psychologist and former education and science minister Mircea Miclea, who chaired a presidential advisory committee on education and science, is not a member of Ad Astra, as reported on page 1185.

Brevia: "Magmatically triggered slow slip at Kilauea Volcano, Hawaii" by B. A. Brooks *et al*. (29 August 2008, p. 1177). The beginning data points associated with the HOLE site in Fig. 1B should have been medium and gray, identical to the rest of the data points, rather than small and purple. There is no change to the data.

Research Articles: "Draft genome of the filarial nematode parasite *Brugia malayi*," by E. Ghedin *et al*. (21 September 2007, p. 1756). The name of an author, Brian P. Anton, was inadvertently omitted after Owen White's. The author's affiliation is Division of Restriction Enzymes, New England BioLabs, Inc., 240 County Road, Ipswich, MA 01938, USA.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Log or Linear? Distinct Intuitions of the Number Scale in Western and Amazonian Indigene Cultures"

Jessica F. Cantlon, Sara Cordes, Melissa E. Libertus, Elizabeth M. Brannon

Dehaene *et al*. (Reports, 30 May 2008, p. 1217) argued that native speakers of Mundurucu, a language without a linguistic numerical system, inherently represent numerical values as a logarithmically spaced spatial continuum. However, their data do not rule out the alternative conclusion that Mundurucu speakers encode numbers linearly with scalar variability and psychologically construct space-number mappings by analogy.

Full text at www.sciencemag.org/cgi/content/full/323/5910/38b

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Stanislas Dehaene, Véronique Izard, Pierre Pica, Elizabeth Spelke

The performance of the Mundurucu on the number-space task may exemplify a general competence for drawing analogies between space and other linear dimensions, but Mundurucu participants spontaneously chose number when other dimensions were available. Response placement may not reflect the subjective scale for numbers, but Cantlon *et al*.'s proposal of a linear scale with scalar variability requires additional hypotheses that are problematic.

Full text at www.sciencemag.org/cgi/content/full/323/5910/38c

Comment on "Log or Linear? Distinct Intuitions of the Number Scale in Western and Amazonian Indigene Cultures"

Jessica F. Cantlon,[1,3]* Sara Cordes,[1,2] Melissa E. Libertus,[1,2] Elizabeth M. Brannon[1,2]

Dehaene *et al*. (Reports, 30 May 2008, p. 1217) argued that native speakers of Mundurucu, a language without a linguistic numerical system, inherently represent numerical values as a logarithmically spaced spatial continuum. However, their data do not rule out the alternative conclusion that Mundurucu speakers encode numbers linearly with scalar variability and psychologically construct space-number mappings by analogy.

The Mundurucu language bears an unusual linguistic phenomenon: The language includes number words only for the numbers one to five, whereas numerical values greater than five are labeled with approximate quantifiers like "some" or "many" (*1*). Consequently, native speakers of Mundurucu present a rare opportunity to study the nature of human numerical concepts in the absence of a robust verbal numerical system. Dehaene *et al*. (*2*) tested Mundurucu- and English-speaking participants on the number-line estimation task developed by Siegler and colleagues (*3*, *4*). In (*2*), participants positioned numerical values, presented either as nonsymbolic values (dot arrays or tones) or as symbolic, spoken number words, on a line anchored at both ends with fixed numerical values. Both groups positioned the numerical values ordinally, from small to large, along the length of the line. English speakers positioned the symbolic number words at linear intervals, but they positioned most of the nonsymbolic numerical values at logarithmically spaced intervals. In contrast, Mundurucu speakers positioned both the nonsymbolic and symbolic numerical values logarithmically. The authors thus concluded that a linear numerical code is unique to cultures that engage in formal education and that space-number mappings like those reported for Western societies (*5*–*7*) are culturally universal. Here, we offer an alternative account for each of these conclusions.

Dehaene *et al*. (*2*) tested Mundurucu and English speakers' numerical performance against the predictions of a precisely linear numerical code (Fig. 1A) and a logarithmic numerical code (Fig. 1B). Based on goodness of fit, the authors concluded that, unlike English speakers, Mundurucu speakers psychologically encode both symbolic and nonsymbolic numbers logarithmically. However, a third possibility was not tested: the possibility that Mundurucu speakers psychologically encode numbers linearly with scalar variability (Fig. 1C) (*8*).

The logarithmic code (Fig. 1B) and the linear-scalar code (Fig. 1C) predict similar outcomes in numerical performance. Both codes predict that smaller numbers are easier to distinguish than larger numbers. Both codes also predict that the midpoint between two numerical anchors is at the geometric mean rather than the arithmetic mean. Thus, Dehaene *et al*.'s finding that the Mundurucu indicate that the "middle of the interval 1 through 10 is 3 or 4, not 5 or 6" (*2*) is consistent with either code. Finally, both codes predict responses on the number-line task that conform to a logarithmic function over and above a precisely linear function. A logarithmic behavioral response function would emerge from a logarithmic code because of the compressed scaling of numbers in psychological space. Under a linear-scalar code, a logarithmic response function would emerge from noise that increases proportionally with number, combined with a ratio comparison process between the anchor and intermediate probe values.

Unfortunately, the behavioral predictions from the logarithmic and linear-scalar codes are virtually impossible to distinguish from the subjective scaling data obtained by Dehaene *et al*. (*2*) and previous studies (*3*, *4*) that made similar claims (*9*, *10*). In fact, some have argued that the only class of experimental data that can disambiguate the underlying nature of approximate numerical representations is one derived from arithmetic operations (*8*, *9*, *11*).

As in previous studies that employed the same subjective scaling paradigm (*3*, *4*), Dehaene *et al*.'s report actually contrasts an approximate numerical code (either a logarithmic code or a linear code with scalar variability) with an exact numerical code (a precisely linear code). Their findings are consistent with previous research on Mundurucu speakers (*1*) that showed that both small symbolic numbers and nonsymbolic numerosities are represented approximately. The data are not informative as to which approximate numerical code

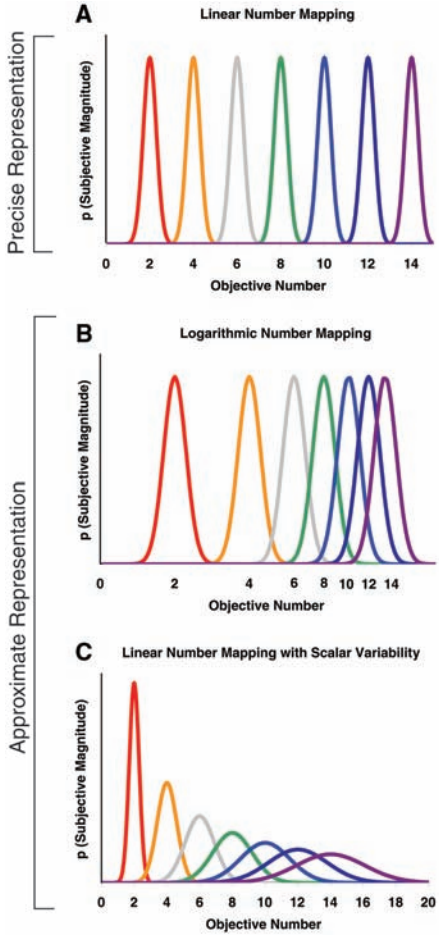

Fig. 1. The three hypothetical psychological codes underlying numerical representation. A precise linear code (**A**) posits equal spacing between values on the subjective number line and little variability. This code yields an exact mapping between objective and subjective number and thus allows one to appreciate that 99 and 100 differ by the same amount as 9 and 10. Under the logarithmic code (**B**), numerical values are psychologically compressed logarithmically with a constant amount of noise. Under this system, numerical representations become increasingly less distinct as objective number increases because they become closer together in psychological space. The linear numerical code with scalar variability (**C**) represents numerical values with equal psychological distances between adjacent values, and the amount of noise in the numerical representation increases proportionally with its value. Like the logarithmic code, the linear-scalar code predicts that confusion between neighboring values increases with magnitude, not because of the subjective spacing of the values but because of the increased variability with which each value is represented. Whereas the precisely linear code (A) represents objective numbers precisely, the logarithmic (B) and linear-with-scalar variability (C) codes represent numbers approximately.

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underlies nonsymbolic numerical judgments: logarithmic or linear with scalar variability. In this sense, the question of "log or linear?" remains unanswered.

Dehaene *et al*. (*2*) also reported that nonliterate Mundurucu speakers represent numbers on a spatial continuum. Such a finding would indeed be remarkable given that a psychological mapping between numbers and space has never been demonstrated in a nonliterate human or nonverbal animal. Previous work has shown that educated adults map numbers onto a one-dimensional spatial continuum that is strongly influenced by the culturally defined direction of reading (*5*–*7*). For example, when required to make a parity judgment, adults who read from left to right are faster at responding with their left hand to small values but, with their right hand to large values suggesting that the mapping of numbers onto space is defined by reading direction (*5*).

A critical feature of previously demonstrated spatial-numeric mapping effects is that the spatial property of participants' numerical responses emerged implicitly—that is, under circumstances in which a space-to-number mapping was not overtly required. In contrast, Dehaene *et al*. (*2*) presented subjects with the overtly spatial task of positioning numbers on a line between two numerical anchors. Under these conditions, the spatial property of participants' numerical responses may be only superficially similar to the number-space mapping evidenced in literate adults.

Spatial responses in the number-line estimation task need not reflect an inherent space-number mapping. Humans are masters of analogy, even early in development. For example, by 3 years of age, children can map the concepts "daddy," "mommy," and "baby" onto a large, a medium, and a small flower pot (*12*). Yet, we would not conclude from this finding that children's underlying psychological representation of a family is fundamentally mapped to three different-sized flower pots. Similarly, adult humans are skilled at mapping between unidimensional properties. For example, adults can adjust the loudness of a sound, the length of a line, or the size of a numerical value to match the brightness of a light (*13*). However, the ability to map between these dimensions does not imply that the psychological foundation of brightness perception is loudness, length, or number. Instead, mappings between dimensions that all have something in common can be accomplished analogically, in this case using the property of unidimensionality. Thus, the results of Dehaene *et al*. provide evidence that Mundurucu speakers can map between the unidimensional properties of length and number, but this is not evidence that Mundurucu number representations are deeply, fundamentally, or intuitively spatial.

In short, Dehaene *et al*.'s (*1*, *2*) extraordinary studies of the Mundurucu mind offer a provocative set of hypotheses regarding the universal underlying nature of human numerical representation. However, there is still room for debate as to whether a linear numerical continuum is strictly a cultural invention and whether the mind inherently maps numbers onto space.

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Response to Comment on "Log or Linear? Distinct Intuitions of the Number Scale in Western and Amazonian Indigene Cultures"

Stanislas Dehaene,[1,2,3,4]* Véronique Izard,[1,2,4,5] Pierre Pica,[6] Elizabeth Spelke[5]

The performance of the Mundurucu on the number-space task may exemplify a general competence for drawing analogies between space and other linear dimensions, but Mundurucu participants spontaneously chose number when other dimensions were available. Response placement may not reflect the subjective scale for numbers, but Cantlon *et al.*'s proposal of a linear scale with scalar variability requires additional hypotheses that are problematic.

Our study (*1*) investigated how the Mundurucu, an Amazonian indigenous people with little or no formal education, map numbers onto space. We agree with Cantlon *et al.* (*2*) that the performance of the Mundurucu on our number-line task could exemplify a more general capacity for analogical reasoning that allows mappings between space and other linear dimensions. The mapping of number to space is surely not the only mapping available to the human mind; indeed, one of our earlier studies provided evidence that the Mundurucu spontaneously relate large-scale three-dimensional spatial layouts to small two-dimensional geometric forms, using the latter as literal maps (*3*). Humans may well possess a generic capacity to think of all quantities, be they distances, object sizes, or any other continuous dimension, as fundamentally commensurate and assessable by a single measurement system (real numbers). Nevertheless, the appeal to analogy raises the crucial question of whether some stimulus dimensions are privileged when mapping stimuli onto space. In our study (*1*), the target sets varied on multiple dimensions, including element size, brightness, average area, and number, and each of these dimensions could have been mapped onto space. The two training trials provided insufficient instruction or feedback to fully distinguish between these possible mappings. Still, the Mundurucus spontaneously selected number as the main dimension underlying their pointing responses. This systematic pattern provides evidence that the mapping of number to space is intuitive and privileged.

Is the subjective scale of number logarithmic or linear? On this issue, Cantlon *et al.* (*2*) propose an alternative interpretation of our findings. Although the number-line responses of the Mundurucu are logarithmically spaced, their internal representation of number would be linear, with equal psychological distance between adjacent numbers but with linearly increasing variability. Cantlon *et al.*'s suggestion runs counter to the simplest construal of number-line placements as indicators of psychological distance. On this standard construal (*4*, *5*), participants evaluate the size of the numbers and place them at spatial distances relative to the endpoints that are proportional to their psychological distances from those endpoints. The challenge for the hypothesis of a linear code with scalar variability is to formulate what it would mean to possess a linear psychological distance metric and yet respond logarithmically in a test as simple as ours.

Cantlon *et al.* rise to this challenge by supposing that the number-line task is not as simple as it seems but involves "a ratio comparison process between the anchors and the probe values." This means that participants do not report the psychological distance between each probe number and the anchors (i.e., their difference, which would be linear) but the similarity between them (which takes into account their internal variability and hence varies with their ratio). In brief, on this account, the Mundurucu would base their spatial responses on perceived number similarity, all the while possessing a linear sense of psychological distance.

This account, however, is problematic for several reasons. First, it depends on the assumption that perceived similarity can be finely evaluated quantitatively, not only at threshold but also well above threshold (since quantities such as 3 are easily discriminable from both 1 and 10, even for infants). Second, this account posits two internal metrics, one of similarity and a distinct one of psychological distance, with the assumption that, despite what the term "distance" implies, the second one is not easily mapped onto space. Third, this account provides no explanation for the changes in task performance that occur with intercultural contact or education, without additional assumptions that are either highly implausible or demonstrably false. In our experiments, Mundurucu participants who could count in Portuguese showed a linear response with Portuguese number words but a logarithmic response with dot stimuli and with Mundurucu number words. In other experiments using this task (*4*, *5*), young children in U.S. elementary schools showed linear performance with a number line scaled from 1 to 100 but logarithmic performance at larger scales. To account for these performance patterns, Cantlon *et al.* (*2*) might propose that bilingual Mundurucu adults and U.S. school children learned to construe the number-line task differently. However, if these participants somehow learned that the task required mapping of psychological distance when the stimuli were Portuguese words or small numbers, and if they were endowed with a linear sense of numerical distances, then why did they fail to apply this mapping more broadly? Alternatively, Cantlon *et al.* could propose that the similarity relations among numbers change during development, as the linear code with scalar variability is replaced by a linear code with fixed variability. Although such a developmental change may occur, a large amount of data from numerosity discrimination (*6*), nonverbal arithmetic (*7*, *8*), magnitude estimation (*9*), and subjective similarity reports of symbolic numerals (*10*) shows that even in educated adults, number similarity still varies with numerical ratio or, equivalently, logarithmic distance. We therefore stand by the original hypothesis (*1*, *4*, *5*): Young children begin with a logarithmic sense of number, and education subsequently provides an additional linear representation, suitable for mapping numbers onto space, but which does not totally supplant the logarithmic representation in all tasks (*1*, *11*, *12*).

Since Fechner (*13*), Stevens (*14*), and Krueger (*15*), the issue of mapping from objective to subjective quantity has become increasingly technical. Cantlon *et al.* correctly point out that the logarithmic code and the linear code with scalar variability often make identical behavioral predictions, because both predict ratio-based numerical discrimination. The two models are not, however, empirically indistinguishable. A subtle but distinctive prediction concerns the shape of the internal noise: According to the logarithmic hypothesis, it should be Gaussian on a log scale, and therefore the distribution should be rightward skewed when plotted on a linear scale. Conversely, in some carefully designed situations (departing from mere discrimination), the linear model predicts a Gaussian distribution of responses on a linear scale, and therefore a leftward skewed distribution on a log scale. Several studies have attempted to characterize the noise distribution for number, either behaviorally

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(*12*) or, most crucially, with methods that directly probe the neural code for numerosity in monkeys and humans (*16*, *17*). All results so far support the logarithmic model.

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EDUCATION

The Gamers' Advancement of Learning

John Willinsky

What Video Games Have to Teach Us About Learning and Literacy
2nd ed.
by James Paul Gee
Palgrave Macmillan, New York, 2007. 253 pp. Paper, $16.95, C$19.75, £11.99. ISBN 9781403984531.

Young people, especially boys, from among discretionary-income classes owe a considerable debt of gratitude to James Paul Gee, a professor of literacy at Arizona State University. With a trip to the library, perhaps, those given to video-game playing can place this book on the kitchen table and earn from their parents many extra hours of playing time. And those parents, worried about the hours spent gaming, will form their own sense of indebtedness to Gee. For according to *What Video Games Have to Teach Us About Learning and Literacy*, a whole lot of learning is going on with every kill scored and level ascended in each new release of good video games.

For Gee, the gamers' debt is covered in full by the "life-enhancing experience" he has found in gaming, originally inspired by his Piaget-like interest in his own son's playing. The result of Gee's absorption in the game world is a highly readable book, made all the more engaging by its own double edge: part gamer-confessional, part learning-sciences lecture. Gee conveys the ways of serious gaming, unabashedly playing for the thrill of the kill. At the same time, this well-respected scholar (whose previous works contributed to paradigm shifts in linguistics and literacy) spells out no less than 36 principles by which a game like Castle Wolfenstein or Shadow the Hedgehog "facilitates learning in a good way."

The learning principles are not add-on enrichments, like vitamin D in chocolate milk. They are integral to what is good in the most popular games within this $9.5-billion industry (double the size of the U.S. school textbook business). Gee makes it clear that in facing System Shock 2's deadly cyber-assassins, the choice is between active, very active, critical learning (learning principle #1) or near-instant death. As for school, Gee has little trouble finding examples of the passive, uncritical learning that is all too often asked of students.

His point is not that students should be playing video games in school. Rather, his stand is that schools, as well as workplaces, could greatly profit by paying attention to what it is about games that so absorbs players in learning their way through terrains of such challenging and deadly problems. The book does not address in any substantive way how we might extract and transfer those principles to these other settings, as it is principally given over to taking readers inside the games and discussing how they exemplify different learning principles.

Yet as Gee's book progresses through its own ascending levels of difficulty—introducing theories of semiotic domains, competence regimes, and design grammars—it makes its own case for the active critical learning that such works sustain without virtual body counts and endless reincarnation cycles. Gee allows, with a parenthetical qualification, that "games cannot (yet?) be, in a sense, as deep or rich" as good books and movies. But he can also make rather extreme learning claims for good video games, as when he states, for example, that good games "make players think like scientists," because players must "hypothesize, probe the world, get a reaction, reflect on the results, reprobe to get better results."

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The problem with such a claim is not that he left out of the cycle the focus-hypothesis-on-likely-grant-and-apply stage. Nor is it that the game's instant gratification of kill or be killed and reborn seems mildly unscience-like. The issue is that Gee's own intricate theory of learning is itself about the limits of such transfer in the face of local contexts, specific genres, and traditional patterns. Which is only to say that it is hard to see how players will move their learning beyond the next game. Gee has a transfer principle (#29), but it involves "adapting and transforming that earlier game" into a new game. He points to gamers who become computer scientists and to nongaming studies of transfer to be found in bibliographical notes, but it will take another book to apply what games have taught us to nongame settings.

As someone who has decidedly not got game, I can see the real classroom potential in Gee's lively descriptions of players' establishing social (learning) networks and game information services, as well as offering hacked and rebuilt games, grounded in historical fact. The knowledge-based culture that has grown up around the games could well serve as a model for how students can help others learn what the world values knowing about the world. Here was a way for students to find intellectual agency in Wikipedia-like efforts to improve the public store of knowledge, whether about local communities, new forms of gaming, or public science projects such as Galaxy Zoo and Folding@Home.

The question for Gee, which connects to his valuable earlier work on new literacies and new capitalism, is what would it mean to have all of that youthful energy and painstakingly accumulated expertise (not to mention computing power and dollars spent) invested in learning that produced something of value to those who live beyond the World of Warcraft game-iverse. Could applying this learning, in all of its principles, to a form of public information service not also create among students gamelike elements of agency, expertise, commitment, patience, and curiosity, as well as the "critique of the game"? They might then follow Gee's own example of stepping away from the game long

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enough to help others make sense of what otherwise lies beyond their reach and experience.

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EDUCATION

Inside the Schooled Mind

Elsbeth Stern

Despite the everyday use of electrical appliances and technical devices and travel by cars and airplanes, only a minority of people living today has deeper insight into the scientific foundations of modern life. As a consequence, concerted efforts have been made in many modern societies to improve the level of public education in science, technology, engineering, and mathematics (STEM) and to increase the number of university students in these fields. As part of these efforts, psychologists, educators, and researchers from STEM disciplines have collaborated in attempts to explain why the human mind so often seems impervious to conventional instruction and how these barriers can be overcome by designing learning environments that are better adapted to our cognitive functioning. *Applying Cognitive Science to Education* provides an excellent example of the contributions that scientists, provided they are willing to cross some disciplinary boundaries, can make to solving important real-life problems.

Applying Cognitive Science to Education
Thinking and Learning in Scientific and Other Complex Domains
by Frederick Reif
MIT Press, Cambridge, MA, 2008. 489 pp. $38, £24.95. ISBN 9780262182638.

Discussing physics with laypeople, Frederick Reif (an emeritus professor of physics and education at Carnegie Mellon University and the University of California, Berkeley) found a topsy-turvy world. He was told that objects have an "internal force" of motion, conveyed to them by being pushed; that objects move because they are driven by this force; and that they finally stop moving because it gets "used up." A growing body of research literature in science education and cognitive psychology documents that such explanations (absurd as they may appear from an expert's point of view) are quite common, even among otherwise well-educated people. As Reif notes, students may successfully complete introductory physics courses without acquiring conceptual knowledge about how the world works that is compatible with scientific physics. Many teachers are terribly disappointed when after all those lessons their students still believe in internal force as the cause of motion.

Some teachers blame their students for this failure, bemoaning a lack of intelligence, interest, or motivation. Others make desperate efforts to introduce the concepts even more systematically. They strictly keep to a logical sequence, and they present very clear and precise definitions (preferably based on mathematical formulas). When tests require students to reproduce definitions and to figure out quantitative information, performance often seems satisfactory. However, a serious lack of conceptual understanding remains, as students cannot transfer the insights they should have gained to problems that differ from those dealt with in the classroom.

What has gone wrong? Often teachers hold the "direct transmission" view of learning, according to which successful classroom practice is seen as teachers providing information that students memorize and retell. Reif describes why such learning environments may, at best, help students accumulate facts or acquire simple skills but will not support them in building up the conceptual knowledge they need to model new and complex situations, as required in science and mathematics. He emphasizes that the main barrier that keeps students from learning science and mathematics is not so much what they lack, but what they have—namely, naïve scientific knowledge that often works well in everyday life but largely differs from and even contradicts scientific explanations.

Thus, to support students' learning, teachers must diagnose students' initial understanding of the content at hand. Knowledge not conforming to scientific views should not be dismissed as the sad result of deficiencies in previous instruction but, rather, be recognized as an inevitable step in learning. Effective teaching requires presenting students with questions and problems that stimulate processes of knowledge reorganization and thereby help them overcome their bounded or deficient beliefs. By referring to well-established cognitive theories of human information-processing, Reif explains in detail how different kinds of knowledge can emerge through learning and under what conditions this knowledge may be recalled for problem solving. For instance, he discusses why all students have to learn the use of multiple representations for the same situations as well as the translations among these representations.

The numerous examples and the sophisticated way they are embedded in theories of cognitive science make Reif's book a veritable gold mine for all those who teach physics or mathematics at high-school or college level. They will find introductory questions for activating students' prior knowledge about standard curricular content as well as stimulating ideas for engaging students in meaningful activities, e.g., through reciprocal teaching or written self-explanations. However, the book's merits go far beyond practical advice. A broad range of academics will find *Applying Cognitive Science to Education* intellectually stimulating.

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SCIENCE EDUCATION

A Handbook for Hands-On Learning

Robert A. Lue and Richard Losick

How do undergraduate students become engaged in and excited about science? For many scientists, the formative event was joining a laboratory for an inquiry-based project that had the elements of ownership and discovery. Indeed, rewarding undergraduate research experiences played a critical role in both of our career trajectories. In recent years, undergraduate research activities have grown from a cottage industry into a national movement. Programs now range from summer and term-time courses to engaging students in multiyear projects. This intense interest in undergraduate research has prompted serious thought about how young scientists should be mentored and how the effectiveness of undergraduate research programs should be assessed. Thus *Creating Effective Undergraduate Research Programs in Science*, a collection of essays edited by Roman Taraban (a psychologist at Texas Tech University) and Richard L.

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Blanton (a plant biologist at North Carolina State University), is particularly timely.

Scattered across stylistically disparate chapters, common themes are sometimes difficult to discern. But the book accomplishes two important things: First, by describing a range of activities at diverse institutions, it provides a blueprint for establishing and improving undergraduate research programs. Second, it offers insights into how one can assess the impact of undergraduate research experiences. Melding these two elements forms the basis for developing successful programs and the oft-overlooked but essential process of making revisions over time. Collectively, the programs reveal how an ensemble of approaches can be integrated at a single institution. And although the book does not cover highly successful summer research programs outside universities (such as that at Cold Spring Harbor Laboratory), the range of programs described is more than illustrative.

Creating Effective Undergraduate Research Programs in Science
The Transformation from Student to Scientist

Roman Taraban and Richard L. Blanton, Eds.

Teachers College Press, New York, 2008.
264 pp. Paper, $42.95.
ISBN 9780807748770.

For example, Angela Locks and Sandra Gregerman discuss the University of Michigan's Undergraduate Research Opportunity Program, which serves over 1000 students. This comprehensive program (which includes mentoring, peer advising, and pathways into research laboratories) emphasizes the benefit of enlisting freshmen and sophomores. Interestingly, it was initially aimed at underrepresented minorities but was later broadened to include all students—what works well for minorities and women also benefits all students. At the other extreme are course-embedded research experiences, such as those described by Carol Trosset, David Lopatto, and Sarah Elgin. In one example, Washington University students help finish and annotate the DNA sequence of a *Drosophila* chromosome. They tackle the biological problem of why the same genes are tightly packaged in chromatin in one species and not in another (heterochromatin versus euchromatin) and thereby make their own individual contributions to the literature and databases.

The book would be a valuable resource for faculty even if it had merely laid out a series of successful undergraduate research programs. However, it goes substantially further by highlighting several programmatic and institutional components that facilitate successful undergraduate research experiences. One essential component that recurs throughout the book is the importance of mentoring by both faculty and peers. Susan Russell notes that undergraduates' most common suggestions for improving their research experience are to increase faculty guidance and to make it more effective. The latter point (reflected in the surveys of students described by CarolAnne Kardash, Michael Wallace, and Linda Blockus) speaks to the unevenness of faculty mentoring. Those same surveys indicate the importance of better preparing faculty for mentoring.

The contributors do not dodge the challenging problem of assessment. As scientists, we are trained to have exacting controls for our experiments. Educational assessment is confounded by the subjects being humans and the general unavailability of control groups of students. Instead, we must rely on comparisons and surveys of students' perceptions of their experiences. Moreover, science educators generally have little training in the social sciences. Refreshingly, the book provides both a roadmap for carrying out assessment and a frank evaluation of the inherent difficulties in doing so.

Several chapters describe comprehensive survey tools for assessing student and faculty attitudes toward undergraduate research. In their discussion of course-embedded research, Trosset, Lopatto, and Elgin use a survey-based approach that Lopatto (in a separate chapter) applies to summer research. The authors establish the importance of initially defining the gains desired by individual students and by the faculty. In addition, their research skills evaluation form encompasses a thought-provoking roster of learning outcomes tied to the research experience. We hope that the assessment tools described in the book will give institutions data needed to better allocate resources. Such data should also foster debates among faculty and with students, leading to a transparent and effective planning process for undergraduate research programs.

Evidence for the beneficial effects of undergraduate research appears throughout the book. Students who participate in research demonstrate academic gains such as enhanced technical and problem-solving skills and an improved understanding of science. Hands-on experience enhances students' awareness of and commitment to science and increases their self-confidence in doing their own research. Russell highlights the important role research plays in the career trajectories of all students, and Locks and Gregerman emphasize the positive impact of research on the retention rates of underrepresented minorities.

CREDIT: HELEN K. WHITE/COURTESY ALAIN VIEL

Clearly hands-on research increases student interest in science, technology, engineering, and medicine careers and is often a formative undergraduate experience in the lives of future scientists. That said, we would go further and argue that the experience of tackling an inquiry-based research project is of central value to science education, and all students deserve to have access to this experience. A deep and nuanced understanding of science is a fundamental component of any educated individual's intellectual formation, and we can't imagine students achieving an adequate baseline of science literacy without some exposure to research. Therefore, the need for more research experiences—whether

embedded in courses, over the summer, or during the term in a lab—goes far beyond building a pipeline for future scientists. It speaks to a fundamental aspect that is sorely lacking in higher education today: the need to produce well-educated citizenry with the wherewithal to understand basic scientific ideas and to act accordingly.

As a last word on the benefit of undergraduate research experiences, the volume presents a chapter of testimonials from alumni of such programs. In it, Robin Henne recalls,

> My undergraduate research molded my way of thinking into that of a scientist. … I learned how to ask questions, design experiments, analyze data, and read scientific papers. …
>
> … It gave me the opportunity to explore the possibility of becoming a research scientist and led me to my passion of helping to improve society as a liaison between scientists and the public.

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EDUCATION

What's Wrong with Inferences from Test Scores?

Edward Haertel

Educational testing and test reports are ubiquitous. By and large, people who hear reports that scores on state tests have increased since last year, that the black-white achievement gap is closing, or that 81% of Massachusetts fourth graders are proficient readers think they understand pretty well what those reports mean. The logic of using test scores to evaluate schools, inform educational decisions, and influence curriculum and instruction is rarely questioned. The principal outcome of schooling is student learning; tests measure student learning; so test scores can show which students are meeting learning expectations and which schools are most effective. Test scores are objective, scientific, and easy to understand—so what's the problem?

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It turns out that there are a lot of problems and that we would do well to try and understand them better. Daniel Koretz's *Measuring Up* is an excellent place to start. The book is hard to classify. It is too sophisticated to be called a primer. There are no equations, so it can't be a measurement book. (Also, it is entertaining to read.) It says good things about testing and test use and takes apart some arguments of testing opponents, so it can't be an anti-testing book. But, it raises profound challenges to the interpretation of score trends on high-stakes tests, to the meaning of achievement trend and gap reports in terms of "percent proficient," to the interpretation of crossnational achievement comparisons, and to popular assumptions about testing of students in special populations (including some assumptions written into law). So, it can't be a pro-testing book, either.

Measuring Up
What Educational Testing Really Tells Us

by Daniel Koretz

Harvard University Press, Cambridge, MA, 2008. 361 pp. $29.95, £22.95, €27. ISBN 9780674028050.

The book is based on a course Koretz (a professor of education at Harvard) has offered to help nonspecialists become more intelligent consumers of tests and testing. He does a great service by clarifying measurement principles in the context of widespread testing uses and misuses. The book is rich with examples and helpful analogies. In addition to covering the basics of reliability, validity, standard setting, scaling, and score equating, it nicely explains the (sometimes flawed) chains of reasoning from examinees' test responses to various test score interpretations and educational policies.

Measurements are used throughout the sciences, and many readers will be familiar with ideas like sampling error, measurement error, or scale conversions. But social measurements with stakes attached are a different matter. Achievement tests are used deliberately to influence schooling, as when reformers press for rich, complex "performance assessments" to focus instruction on "higher-order skills." Predictable responses to high-stakes testing, such as a narrowed instructional focus on just the material tested, can lead to score inflation, undermining intended inferences from test scores. Some advocates of high-stakes testing as an educational reform tool have argued that even if there is some score inflation, tests cover material students should know, and so rising scores must signify some degree of real improvement. Koretz offers persuasive rebuttals, supported with empirical evidence.

The book is rich with concrete examples. Pointing out that even large score differences may show inconsistencies from one test to another, Koretz discusses the 2003 eighth-grade mathematics gap between the United States and Norway. That year, the Trends in International Mathematics and Science Study placed the U.S. students far ahead of their Norwegian counterparts, while the Programme for International Student Assessment placed Norway ahead of the U.S. by a statistically significant margin. Explaining selectivity bias, he describes an influential study that contributed to the popular idea of algebra as a "gatekeeper" course, showing how that study's conclusions were obviously unwarranted. In clarifying within-group versus between-group variation in test scores, he presents the surprising statistic that if the average differences in test scores between the major racial and ethnic groups in the United States could somehow be eliminated (making each group's score distribution match the current distribution for non-Hispanic white students), the total variability in scores would shrink by less than 10%. (His estimates, using different subject-grade combinations, ranged from 0.5% to 9%.)

A major theme, reflecting Koretz's own research over the years, is score inflation on high-stakes tests. Optimistic reports of year-to-year improvement on state tests under No Child Left Behind should not be trusted. When the same score trend is estimated using a low-stakes "audit" test (e.g., the National Assessment of Educational Progress) and a high-stakes test (e.g., a state test), the audit test tends to show smaller gains over time, sometimes much smaller. When a school district changes from one test to another and then after several years the earlier test is readministered, it has been found that the performance gains shown over time on the new test were in fact accompanied by a corresponding decline in performance as measured by the old test.

The last chapter offers reasoned advice on appropriate test use and cautious test interpretation. Koretz concludes:

> In all, educational testing is much like a powerful medication. If used carefully, it can be … a very powerful tool for changing education for the better. Used indiscriminately, it poses a risk of various and severe side effects. Unlike powerful medications, however, tests are used with little independent oversight. Let the buyer beware.

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ENVIRONMENT

"True" Conservation Progress

Conservation performance in securing biodiversity can be evaluated better with metrics based on the concept of a conservation balance sheet.

Eve McDonald-Madden,[1] Ascelin Gordon,[2] Brendan A. Wintle,[3] Susan Walker,[4] Hedley Grantham,[1] Silvia Carvalho,[1,5,6] Madeleine Bottrill,[1] Liana Joseph,[1] Rocio Ponce,[1] Romola Stewart,[1] Hugh P. Possingham[1]

The field of biodiversity conservation is hampered by weak performance measurement and reporting standards (*1*). In other areas, such as the corporate world, weak reporting of performance is considered bad practice, if not illegal (*2*, *3*). Although various evaluation frameworks for conservation programs have been suggested (*4*–*7*), few simple measures for unbiased reporting have been developed (*8*).

Credible performance measures should connect conservation outcomes to goals for public investment in conservation. Gains and losses must both be presented as an auditable conservation balance sheet (*8*), revealing the net benefit of conservation actions and policies reported against losses.

A major conservation performance metric in government state of the environment reports (*9*–*14*) is the size of the physical area protected, or the change in area protected. For example, South Africa reported that 6% of terrestrial habitat was contained within protected areas in 1999 (*9*); in 2001, North America reported an increase in land within reserves over time (*13*). However, these numbers provide no information on loss of habitat outside (or inside) reserved areas, or conservation opportunity costs of securing areas for conservation (*15*). Even when habitat loss is reported (*11, 12*), it is rarely possible to evaluate net conservation outcomes.

Performance Evaluation Metrics

Our performance evaluation metrics, F_{it} and M_i, may be used to assess the state of any conservation asset, such as vegetation types, habitat types, or threatened species distributions. The proportions of an individual asset (i) secured or lost at time t (relative to some historical reference point) are denoted as s_{it} and l_{it}, respectively. Here, secured means that an action is implemented that maintains the biodiversity asset (e.g., legislated reservation, or actions that secure biodiversity, such as threat mitigation or habitat restoration). The term "lost" means biodiversity is degraded or destroyed (e.g., by land clearing, weed invasion, or waterway nutrient enrichment). Loss can occur on "secured land" if biodiversity components decline [e.g., (*16*)]. The area of asset i remaining available for conservation or loss at time t is given by $A_{it} = 1 - s_{it} - l_{it}$.

F_{it} gives a static measure of the net positive change in an asset relative to all changes that have occurred in that asset:

$$F_{it} = \frac{s_{it} - l_{it}}{s_{it} + l_{it}}, \; -1 \le F_{it} \le 1$$

If the amount of the asset secured is greater than that lost, F_{it} is greater than zero (see table, p. 44, asset B). F_{it} will be negative if the reverse is true (table, asset C). Overall conservation performance can be assessed from the average value of F_{it} across all assets:

$$\bar{F}_{it} = \frac{1}{N}\sum_{i=1}^{N} F_{it}$$

N is the total number of assets considered.

M_i measures a rate of change between two time points:

$$M_i = \frac{\left(s_{it_2} - s_{it_1}\right) - \left|\left(l_{it_2} - l_{it_1}\right)\right|}{\left|\left(s_{it_2} - s_{it_1}\right)\right| + \left|\left(l_{it_2} - l_{it_1}\right)\right|}, \; -1 \le M_i \le 1$$

M_i is positive if an asset is protected at a greater rate than it is lost (table, asset B), and negative if loss exceeds protection (table, asset C). The average of M_i across all assets is

$$\bar{M}_i = \frac{1}{N}\sum_{i=1}^{N} M_i$$

F_{it} and M_i provide different information about conservation achievement. A limitation of having "simple" interpretable metrics is that a single metric may not cover all facets of conservation performance. For example, if no loss has occurred for a given asset relative to some historical reference point, $F_{it} = +1$, even if a small amount of the asset is secured (see table, p. 44, asset G). Likewise, M_i gives a score of +1 if there is a gain in area secured without loss, irrespective of the magnitude of that gain (table, assets D, E, and G); it will also give a value of –1 if there is loss without gain, irrespective of the magnitude of that loss (table, assets F and H).

Presenting a single metric may fail to differentiate these and other situations; however, presenting both F_{it} and M_i along with A_{it_2} enables differentiation and thus honest and comprehensive reporting of all scenarios.

Case Study from Queensland, Australia

We demonstrate the utility of our metrics in expressing overall outcomes of conservation action (or inaction) within Queensland between the years 1997 and 2003. We use statistics on ecosystem loss through land clearing and areas secured through reservation as reported by the Environmental Protection Agency (*17*). Assets identified are 86 "land zones," where a land zone is an area delineated by characteristic geology, soil, and vegetation. We show the average for each metric across all land zones (blue bar in chart, p. 44, bottom) and individual metrics for a representative sample of 20 land zones (green bars).

Conservation areas were equivalent to ~5% of the available land in 2003 [(D) on chart, p. 44, red bar]; this total is the standard global metric. This measure, although small, provides a positive impression of the conservation of indigenous habitats. However, when metrics are used that account for both loss and reservation, they tell a markedly different story. They reveal that, overall, Queensland has lost more habitat than has been reserved [(A) on chart, $F_{it} \approx -0.7$ in 2003], and reservation had exceeded loss in only 37% of all land zones in 2003 [(B) on chart]. On average, loss had exceeded reservation in 2003 and had occurred at a higher rate between 1997 and 2003, across all land zones [chart (A) and (B)].

Although new reserves were established in 89% of land zones, further investigation by means of M_i indicates that loss rate exceeded rate secured within 55% of the land zones. To

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HYPOTHETICAL SCENARIOS

Asset (i)	Gain or loss (%)				F_{it_2}	M_i	A_{it_2} (%)
	s_{it_1}	s_{it_2}	l_{it_1}	l_{it_2}			
A	5	10	5	10	0.00	0.00	80
B	5	20	5	10	0.33	0.50	70
C	5	20	5	40	–0.33	–0.40	40
D	5	10	5	05	0.33	1.00	85
E	5	10	80	80	–0.78	1.00	10
F	0	0	5	20	–1.0	–1.00	80
G	5	6	0	0	1.0	1.00	94
H	5	5	5	10	–0.33	–1.00	85
I	10	5	5	5	0.00	–1.00	90
J	5	5	10	5	0.00	1.00	90

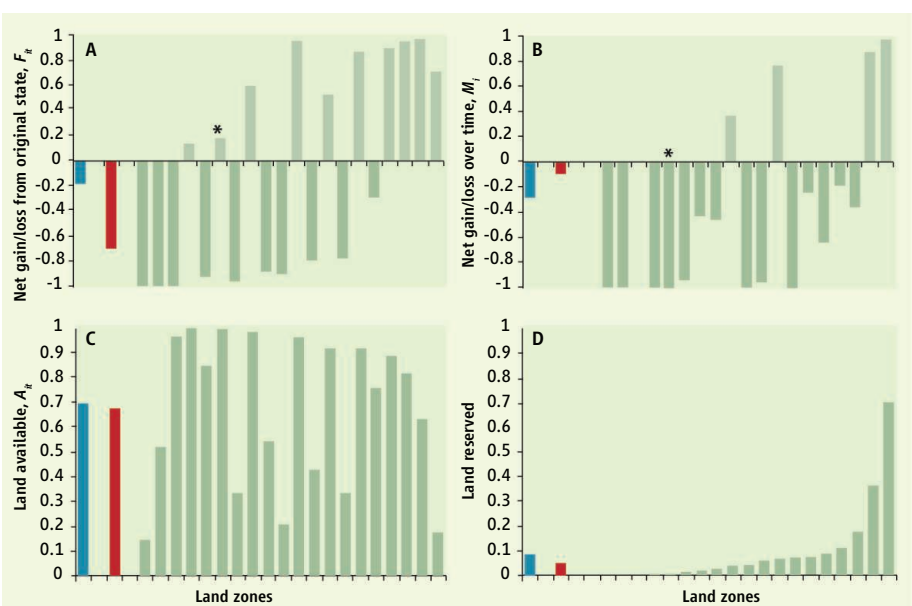
Hypothetical scenarios illustrating use of the metrics and the current proportion of land available for future conservation or loss.

further highlight the utility of reporting both F_{it} and M_i, we identify one land zone in Queensland where F_{it} and M_i show different net outcomes [see asterisk on chart, below, (A) and (B)]. F_{it} indicates that, overall, less loss has occurred than reservation for this land zone by 2003; however, M_i shows that loss has increased between 1997 and 2003, while reservation has remained unchanged. Large proportions of unprotected habitat persist in multiple land zones [chart, (C)], representing substantial opportunity for Queensland to improve conservation performance. Future changes in these metrics will indicate success or failure of the *Vegetation Management and Other Legislation Amendment Act of 2004*, which aims to phase out broad-scale vegetation clearing in the state (*18*).

Honest Reporting

We do not claim ours to be the best or only metric that could be developed: We merely aim to demonstrate that honest reporting is possible, can be simple and informative, and the current global standard of reporting gains, but not losses is unjustified and potentially misleading. We have demonstrated our metrics using a simplistic example where reservation indicates gain and habitat clearance indicates loss. These metrics could also be applied to other forms of conservation gain (e.g., covenants or areas under sustained pest control) and degradation (e.g., invasion of a weed into a reserve). It is also possible to apply this to a situation where loss and gain are not absolute and information is available on change in asset quality (*19*). However, substantial extra effort would be required to coherently report on change in quality over large areas. Our metrics could also be extended to conservation prioritization by incorporating costs of recovery and probabilities of success of conservation actions. Incorporation of nonconservation objectives, such as local livelihoods (*20*), would require modification of these metrics.

Honest metrics of conservation achievements are essential to inform conservation shareholders about the performance of their investments. In failing to mention the losses and opportunity costs of conservation investments, agencies reporting on conservation achievements are disclosing revenue rather than net profit and are being economical with the truth.



Conservation performance in Queensland, Australia, based on reservation and land clearing between 1997 and 2003 (*17*). (**A**) F_{it} calculated for 2003, (B) M_i between 1997 and 2003, (**C**) the proportion of land available in 2003, and (**D**) the proportion reserved in 2003. The values for 20 land zones (green bars) are summarized by the mean (blue bar) and the value of each measure based on the total reservation and loss across all land zones in Queensland (red bars, combined to allow comparison with the standard metric of the total reservation area). *Examples in which the metrics lead to different conclusions.

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ASTRONOMY

How Old Is That Star?

Despite being fundamental in determining its physical state, a star's age cannot be measured directly, and estimation methods are imprecise.

David R. Soderblom

Determining how long it has been since a star formed is a lot harder than it seems like it ought to be, and many very basic questions hinge on stellar ages. For instance, we'd like to know the ages of stars that have planets. We hope to detect signs of life on planets around other stars, but if we do, knowing the star's age is central to interpreting what is observed. Among the youngest stars, we'd like to know how long it takes for planetary systems to form and evolve. On a grander scale, the ages of stars and clusters of stars are needed to infer the history of our Milky Way galaxy and the pieces from which it was built. Did the halo of our galaxy form on its own or from fragments of captured satellite galaxies? Did the thick disk (*1*) form after the halo or contemporaneously? Has the thin disk (of which the Sun is a member) formed stars continuously, or in episodes? Many very basic questions can only be addressed if we can establish reliable ages. A recent symposium (*2*) brought together astronomers to examine the problems involved in estimating the ages of stars and groups of stars.

The physical state of a star—its size, temperature, and total energy output—are determined mainly by its mass, chemical composition, and age. Age is relevant because as a star gets older, nuclear reactions in its core alter the composition, leading to changes in the overall structure. We can directly measure the masses of stars that have companions, and we can directly determine the surface composition of a star through careful analysis of its emission spectrum. But we can measure a precise and exact age for just one star—the Sun—and that's because we can analyze solar system material in the laboratory, something we can do for no other star. That makes the Sun's age fundamental, and the Sun's case is particularly important because it presents a well-constrained situation that can then be modeled to gain insight into the detailed physics of other stellar interiors.

By calibrating models against the Sun, we can comprehend stars that are both more and less massive. Our understanding of the evolution of stars is closely tied to studying star clusters, groups of hundreds to thousands of stars that were formed together and so share the same composition and age. Or do they? Some of the most exciting astrophysics from the Hubble Space Telescope has been the discovery of multiple populations within single globular clusters (*3*), which are some of the oldest components of the Milky Way. Given what we now know about stellar physics, the available explanations include multiple ages (i.e., several epochs of star formation spread well apart in time), very different compositions of the cluster's members, or both. Neither alternative satisfactorily explains the observations, and a very basic conundrum has been exposed.

CREDIT: ANGLO-AUSTRALIAN OBSERVATORY/DAVID MALIN IMAGES

Aging stars. The image shows a region of our galaxy in the constellation of Scorpius. Some of the stars in the foreground (at a distance of about 400 light-years) have been formed and have emerged from their enshrouding dust and gas within the past 1 to 2 million years, making them among the youngest stars. In the lower left is Antares, illuminating material thrown out earlier from its own wind. The blue star at the top is ρ Ophiuchi, a massive, young star. The globular cluster in the lower right, Messier 4, is much further away (7200 light-years) and yet is one of the closest globular clusters. It happens to lie in the same line of sight, and at an age of about 13 billion years, it is among the oldest objects. Each of these kinds of stars and clusters poses particular problems in determining an age.

Most stellar ages are model dependent and generally have large uncertainties (at least 10 to 20%) with poorly understood systematic effects. Relative ages are more believable, which is why the globular clusters with multiple populations have been so intriguing. Some of the oldest stars in our galaxy have ages estimated from the decay of thorium or uranium (*4*). In these cases, age determination is very well understood, but the derived age is that from when the isotopes were formed, not necessarily the age of the star, and in any case, the initial abundance of the element remains uncertain.

Other age-estimation methods are empirical: We can see a consistent relation between a physical quantity and age, but we do not understand the underlying physical relation, even though we may have at least a reasonable scenario. For instance, stars like the Sun lose angular momentum with time. Convective stars (like the Sun) generate magnetic fields through the interaction of that convection with rotation. That magnetic field can grip an ionized wind (like the solar wind) to a considerable distance, transferring angular momentum and leading to spin-down, a process we witness on the Sun. Barnes (*5*) has used clusters to calibrate the rotation-age relations for stars of different colors (a proxy for mass on the main sequence), resulting in a "gyrochronology." A star's magnetic field manifests itself as activity in various forms that can be observed, and activity can also be seen to decline with age and can be calibrated too (*6*). A fundamental problem with these age indicators is that they are best calibrated against clusters of stars because clusters offer many stars with well-determined ages, but older clusters are rare (due to galactic forces that tear them apart), and it is the older stars whose ages we most want to determine. Asteroseismology may offer a solution. The European space mission CoRoT (*7*)

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and NASA's soon-to-be-launched Kepler mission (*8*) will both obtain ultraprecise measurements of variations in the brightness of solar-type stars at a level good enough to detect stellar oscillations. On an unresolved star (unlike the case for the Sun), only the lowest-order modes can be seen, but those modes penetrate the core of the star, the part that changes the most as a star ages, and its core fuses hydrogen into helium. That makes asteroseismology a potentially powerful technique for deriving the ages of older stars. Such ages also depend on stellar models, but we believe those models are sound for solar-type stars, and the model dependence is very different than for other techniques.

Overall, the situation for determining stellar ages is still sobering, and progress has been slow. It has reached the point where cosmologists claim better precision for their measurements than we can for the ages of the nearest and brightest stars. The challenge of determining an accurate age for a star therefore remains outstanding.

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PHYSICS

Cloaking with Curved Spaces

Designs based on the mathematics behind curved space-time may lead to invisibility cloaks.

André Nicolet and Frédéric Zolla

Who could have imagined that ideas underlying the theory of general relativity would help to render objects invisible? Einstein's theory tells us that the mass of objects curves space, so that light no longer travels a straight line in their vicinity but is bent by gravity. On page 110 of this issue, Leonhardt and Tyc (*1*) improve the design of invisibility cloaks by showing that curved spaces can be used to hide objects by bending light around them.

In 2006, Pendry (*2*) proposed that a geometric transformation of space could distort light beams so that they avoid a bounded region, and the region (and whatever it contains) becomes de facto invisible to observers. It can be shown that any geometric transformation of space can be translated into optical properties, and this approach provides a new way to design photonic devices. A very active field of research has emerged from this idea, but the important question remains: How can one build materials with the required properties, such as negative refractive index or magnetic behavior at optical frequencies (*3*).

The answer will likely come through the design and construction of metamaterials, which differ from ordinary materials in terms of the scale at which their properties arise. The physical properties of ordinary materials are based in their molecular structure, at a scale below 1 nanometer (nm). In metamaterials, the optical properties are determined both by these molecular-scale properties and by structure at a larger scale, around 100 nm. The use of metamaterials allows optical effects like cloaking to emerge that are unknown in classical optics.

Hiding in curved places. Einstein's ideas of curved space-time can be used to create invisibility cloaks. As curved space bends light rays to dodge a "hole" inside the cloak, objects and people may become invisible to observers.

A well-known example of a natural metamaterial is the blue color of Morpho butterflies. The colors of light correspond to various wavelengths (the visible spectrum ranges from 400 nm for violet to 700 nm for red). For most butterfly species, the wing colors are caused by pigmentation, the presence of substances with chemical structures that absorb light at particular wavelengths. But the wing of the Morpho butterfly is not pigmented; rather, it has a complex structure at the scale of the wavelength of light. The interaction of light with this structure determines the color (*4*). One hint that an ordered structure is present is that the wing is iridescent; its color depends on the angle at which we view it. The challenge in optical metamaterials is to design manufactured microstructures of this kind to obtain the properties demanded by theory.

The recipe for building an invisibility cloak is, at least formally, simple: Translate the proposed space transformation for bending light into optical properties, and design the necessary metamaterials. Unfortunately, the required optical properties are not simple, and thus the design of the corresponding metamaterials is actually very challenging. Perhaps the most vexing problem is that on the inner boundary of the cloak, the optical properties have singularities—that is, some of the optical constants must diverge to infinity.

The presence of a singularity arises from Pendry's geometric transformation, which must turn an ordinary space that propagates light into a space with a hole. Tearing a hole in space changes its topology and comes at the price of having singularities for the optical properties. Many theoretical efforts have focused on getting rid of this problem, and some attempts are being made that would generalize Pendry's transformation (*5*). However, these efforts have not led to completely satisfactory solutions.

The approach of Leonhardt and Tyc is reminiscent of Einstein's general relativity, but it does not use mass to curve space-time.

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However, the transformations of general relativity provide the mathematical tools that we need to curve space. The space transformations that have been used previously to design cloaks can locally compress and stretch space in all directions, but despite this huge design freedom, the result is still a flat space without any intrinsic curvature. The use of curved spaces allows new designs to be explored.

To introduce a curved space in the design of a cloak, it must be mapped on a three-dimensional physical space. A two-dimensional example of such a map is the Mercator projection of Earth on a flat sheet of paper. This kind of operation also results in equivalent optical properties but with a much greater variety of designs for cloaks. Leonhardt and Tyc have proposed a very skillful transformation from a carefully chosen curved space where a natural cloaking effect exists that results from the sophisticated paths that light takes around the hidden region. Translated into equivalent properties in Euclidean space, this scheme allows an invisibility cloak to be designed without singularities in the optical properties of the materials. Nevertheless, the required optical properties are still demanding.

Although the use of metamaterials solves many problems in cloaking, their properties are sensitive to the wavelength. Realizing the required optical properties across the entire visible spectrum presents an additional hurdle, especially when extreme values must be reached. Indeed, such values require resonances, that is, phenomena tuned to specific frequencies. Thus, bringing the material properties to moderate values is a necessary step for a cloak to work for all colors.

Theoretical work on cloaking has shown that, in principle, an invisibility device may be possible, but how will engineers design and manufacture such a device? The development of a real cloak will likely involve numerical modeling (*6*), as well as trial-and-error optimization, with little reference to the initial ideas. Concerning applications, fictional literature has made us acquainted with some frightening aspects of invisibility. However, the theory of cloaking can be translated to other kinds of waves encountered everywhere in physics. Indeed, the first practical applications of invisibility may well be the protection of small islands against dangerous sea waves (*7*).

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CHEMISTRY

Repair or Replacement—A Joint Perspective

Insights into the molecular origins of cartilage lubrication could lead to more efficient tissue repair and to longer-lasting prostheses for hip and knee joints.

Jacob Klein

The ultralow friction coefficients between the articulating cartilage surfaces in human hips or knees cannot be duplicated even by the most sophisticated technological means. Breakdown of this lubrication can lead to wear of the cartilage and to osteoarthritis. As human longevity increases, more than 50% of population may eventually suffer from debilitating osteoarthritic pains or joint breakdown due to such cartilage wear. In adults, damaged or worn cartilage has little capacity for self-healing, largely due to the low intrinsic density of chondrocytes (the cells responsible for synthesis of cartilage components, including the type II collagen from which its network is formed). Recent research into regeneration or replacement of damaged joints (*1*) points to directions that could improve prospects for osteoarthritis sufferers.

Efforts to repair cartilage often introduce chondrocyte cells, or stem cells that can differentiate into chondrocytes, to the damaged region, where they can regenerate tissue. The matrix (scaffolding) containing such cells, which is implanted in the tissue, must resist removal by friction as the cartilage surfaces slide past each other at high pressures. This underlines the importance of lubrication for articular cartilage repair. Better lubrication can also greatly increase the lifetime of hip or knee prostheses when joint replacement becomes necessary.

In a classic treatment for cartilage regeneration, known as microfracture, mesenchymal stem cells are released from marrow in the underlying bone to permeate the damaged cartilage area (*2*). There the stem cells undergo chondrogenesis (differentiation to chondrocyte-like cells) to accelerate tissue healing, although the resulting tissue is fibrillar, in contrast to the low-friction, smooth hyaline cartilage (see the figure). More recently, for early-stage osteoarthritis, chondrocytes harvested from a healthy cartilage region have been culture-expanded in vitro and transplanted into damaged regions to promote tissue regeneration (*2*). Synthetic biology may hold considerable future promise for such approaches. In one approach, somatic cells may be programmed to dedifferentiate to pluripotent stem cells (*3*), which could then be embedded in the cartilage to undergo chondrogenesis. Other approaches, still in their infancy (*4*), would seek to reprogram cells to their regenerative format as native chondrocytes. Ideally, these should, with the appropriate external cues, regenerate the native hyaline cartilage in damaged tissue.

Tissue engineering (*5*, *6*) for cartilage regeneration generally uses a combination of scaffolding, cells, and signaling molecules to induce chondrogenesis. Scaffolding matrices are implanted, self-assembled, or gelled within cartilage lesions; implanted scaffolds may also be glued to the tissue. Hydrogels of synthetic or naturally occurring macromolecules (including components of cartilage), self-assembling nanofiber networks, or plugs of healthy transplanted cartilage have been used as scaffolds, and several of these are undergoing clinical trials. The incorporated cells are typically mesenchymal stem cells; signaling molecules for chondrogenesis are often growth factors or genes (*5*).

An optimal scaffold environment would be biocompatible, have mechanical and permeation properties similar to those of healthy cartilage (*7*), not be broken down prematurely by tissue enzymes (*8*), and eventually biodegrade and be removed, in parallel with the

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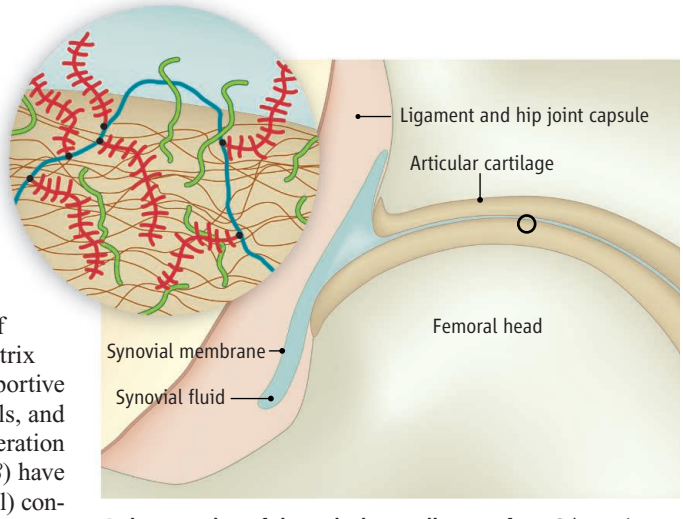
growth of new tissue. Recent attempts to optimize some of the properties of scaffolds include the following examples.

Moutos *et al*. (*7*) have described a scaffold woven from polyglycolic acid yarn and consolidated with a biocompatible hydrogel. The permeability and mechanical properties of this matrix mimic those of healthy articular cartilage. This matrix should therefore provide a better supportive environment for the incorporated cells, and thereby improve their cartilage-regeneration properties. Gonen-Wadmany *et al*. (*8*) have developed protein-poly(ethylene glycol) conjugates for creating hydrogel scaffolds; poly(ethylene glycol) protects the protein backbone against the enzymatic breakdown that often affects scaffolds consisting of proteins alone. Capito *et al*. (*9*) vary the traditional scaffolds: They create closed sac-like containers made of robust membranes through self-assembly of hyaluronic acid and peptide amphiphiles. The sacs are permeable to signaling molecules but not to cells. Sacs encapsulating mesenchymal stem cells and cultured in appropriate media readily undergo chondrogenesis, as revealed by expression of type II collagen. A different, promising approach uses sophisticated scaffolding matrices designed to recruit existing cells from the surrounding tissue. The cells then cause scaffold breakdown and release of signaling molecules previously stored in the matrix; these in turn stimulate the cells to more vigorous regeneration of new, normal tissue (*10*).

A crucial requirement for cartilage repair is that the scaffolding is attached at the cartilage lesions and integrates with the tissue; the attachment must be sufficiently robust to resist being torn off as a result of stress at an articulating joint. Wang *et al.* (*11*) have developed an adhesive based on chondroitin sulfate [a major component of the bottlebrush-like macromolecules, called aggrecans, that permeate the native cartilage (see the figure)]. The adhesive can be readily applied to glue (and integrate) scaffolding directly to the cartilage lesion. The resulting interfacial shear strength is ~50 kPa; for typical pressures of ~5 MPa at human hips or knees, this requires the friction coefficient at the scaffold surface during articulation to be <0.01 to avoid detachment of the scaffold. Similarly, high friction, and hence wear, may arise in regenerated tissue if it does not sufficiently mimic the healthy, smooth native cartilage closely (as happens, for example, with the microfracture technique). Efficient lubrication is therefore crucial not only in healthy joints, but also for tissue engineering of cartilage, and it is very important to understand its molecular origins.



A close-up view of the articular cartilage surface. Schematic section through part of a hip joint. The friction coefficients between the articular cartilage layers, compressed to 50 atmospheres or more in a hip joint, can be as low as 0.001 (*18*). (**Inset**) The detailed structure at the outer cartilage surface is thought to include charged macromolecules (*16*)—mainly hyaluronic acid (blue), to which are attached aggrecans (red) and lubricins (green)—that extend from the surface to form a brushlike layer. Synthetic charged brushes lead to low friction similar to that in articular cartilage (*12*), although, to date, only up to much lower pressures than in human joints.

Recent efforts to elucidate these molecular origins have focused on nanotribological studies of surface-attached molecules in aqueous media, seeking to emulate those at the cartilage surface (see the figure). Raviv *et al.* (*12*) showed that synthetic polyelectrolyte brushes attached to opposing surfaces can provide remarkable lubrication when mutually compressed to moderate pressures and made to slide past each other, with friction coefficients similar to those in healthy joints. Briscoe *et al.* (*13*) found that boundary lubrication under water was far superior to that in air or oil, and mediated by the hydrated surfactant headgroups. These and other studies (*14*, *15*) emphasize the importance of hydration layers surrounding charges in aqueous media as a basic lubrication element (*16*).

These nanotribological studies do not mimic the actual cartilage surface or the macromolecules emanating from it (see the figure), but rather provide insight into the origins of the very efficient lubrication in living joints (*16*). One difficulty is that at pressures of ~5 MPa, normal in hips or knees, friction coefficients attained in the laboratory to date have been much higher than the values of ~0.001 typical of human joints. Nonetheless, the insights gained from nanotribology can have immediate benefits for improved prostheses. For example, Moro *et al.* (*17*) achieved a massive reduction in wear-generated debris particles of the concave plastic cup of a hip prosthesis when they grafted polymer brushes to the plastic surface. Such debris particles are a major cause of failure of prosthetic implants through bone softening and consequent loosening of prostheses; their reduction through brush lubrication is thus a substantial potential benefit.

Future materials challenges will be to design scaffolds that provide optimal environments for the progenitor cells that they bring to the damaged tissue, or to stimulate indigenous cells; and to develop bioadhesives that promote tissue integration and prevent scaffold detachment during joint articulation. In replacement strategies, surface treatments that suppress the wear leading to implant failure may allow a closer approach to "lifetime" prostheses for the most widely used polymer-metal implant combinations. Thus, both for repair and replacement, a better understanding of the molecular mechanisms underlying the remarkable lubrication afforded by healthy articular cartilage at the high pressures in human hips and knees remains an urgent goal.

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GEOCHEMISTRY

A Matter of Preservation

Differences in the preservation potential of crustal rocks may explain peaks in crustal ages previously attributed to enhanced crust formation.

Chris Hawkesworth,[1] Peter Cawood,[2] Tony Kemp,[3] Craig Storey,[1] Bruno Dhuime[1]

The fossil record is well known to be biased by the unevenness of the geographical and stratigraphical sampling effort and inequality in the rock record available for sampling (*1*). There is increasing evidence that a similar unevenness biases the geological record of the generation and evolution of the continental crust. In a recent study, Campbell and Allen (*2*) reemphasized the link between peaks in the distribution of the uranium-lead (U-Pb) crystallization ages of the mineral zircon (which reflect the ages of the parent igneous rocks) and the development of supercontinents. It is not clear, however, why the development of supercontinents should be associated with the generation of unusual volumes of igneous rocks. Instead, peaks of crystallization ages in the continental record are likely to reflect biases in preservation.

The bulk composition of continental crust is similar to that of rocks generated in subduction-related settings (*3*). Globally, subduction is continuous, which in turn suggests that the processes of crust generation should result in a continuum of ages. However, the preserved geological record is marked by peaks of crystallization ages that indicate peaks of magmatic activity (*2*) and by peaks of juvenile (mantle-derived) crust formation (*4*, *5*) (see the first figure). If these peaks truly reflect the geological record, the implication is that continental growth is episodic on a global scale (*6*). However, the mechanism for this remains speculative (*7*). Alternatively, the peaks of both magmatic activity and juvenile crust formation may be a function of preservation, reflecting the unevenness of the rock record available for sampling. To what extent do magmas generated in different settings have different preservation potential? How does this preservation potential in turn shape the rock record now available for analysis?

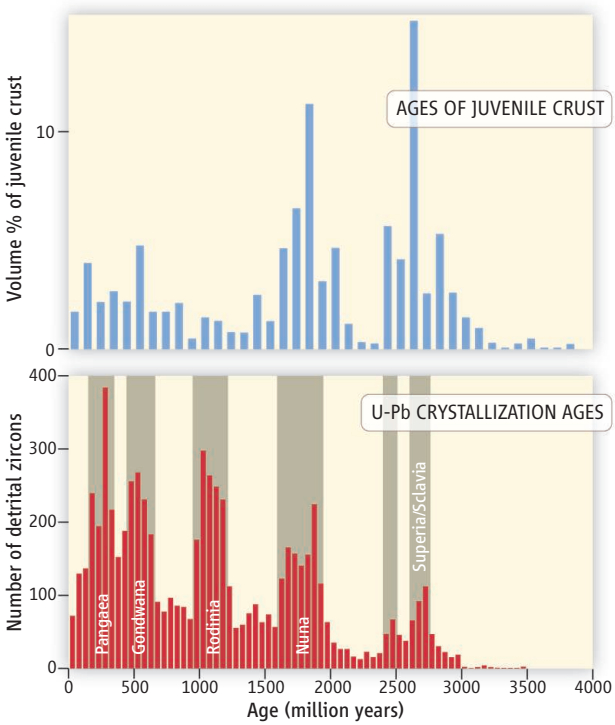
Individual magmatic events involve generation of new crust, reworking of preexisting crust, or a combination of the two processes. Thus, peaks of crystallization ages are not necessarily associated with periods of enhanced crustal growth (see the first figure). Nonetheless, several authors have linked peaks of magmatic activity to the development of supercontinents (*2*). As a result of plate tectonics, continental fragments periodically amalgamate to form large supercontinents. The igneous record associated with the development and breakup of supercontinents may be divided into three consecutive stages: subduction-related magmatism, collisional mountain building and magmas generated by crustal melting, and extensional magmatism (see the second figure). At issue is the extent to which the geological record of each stage will be preserved and represented in today's rock record.

Despite their similarity to the bulk composition of the continental crust (*3*), subduction-related rocks appear to have poor preservation potential. The global rates of removal of continental and island-arc crust through subduction into the mantle are similar to the crust generation rates at modern magmatic arcs (*8*). Instead, there is increasing evidence that subduction-related magmatic rocks are better preserved in extensional basins that lie inboard from the subduction zone (*9*).

Magmas associated with collisional mountain building are dominated by partial melting of preexisting crust. They are granitic in composition and, although the volumes generated may be small relative to other tectonic settings, it will tend to be protected within the enveloping supercontinent. It thus has good preservation potential in the geological record.

The extensional phase is dominated by basaltic magmatism (exemplified by the flood basalts and subordinate rhyolite associated with the breakup of Gondwana). The rocks may also be relatively sensitive to erosion into the oceans. Rocks from this phase do not contain large amounts of zircon and have relatively poor preservation potential.

These considerations show that preserved rocks tend to be from the end of the subduction-related and the collisional mountain building stages (see the second figure), because they are insulated in the cratonic interior and thus preserved from convergent plate margin erosion. Thus, the reason the continental evolution record is likely to be dominated by periods of supercontinent assembly is that these periods provide a setting for the selective preservation of crust. Age peaks reflect the interplay between zircon crystallization and preservation bias related to tectonic setting and do not require accelerated pulses of crust generation or magmatic activity. The peak between 2.9 and 2.5 billion years ago may be different, in that it coincides with the time of the stabilization of Archean crust as presently preserved and may thus preserve a more representative record of the rocks of those ages.



Age is not everything. The U-Pb age of the mineral zircon (calculated from its present-day U and Pb isotope ratios) corresponds to the age of crystallization of the parent igneous rock. Analysis of 7000 detrital zircons shows several peaks in their U-Pb crystallization ages over the course of Earth history (*2*) (**bottom**). Similar peaks are observed in the relative volumes of rocks of different ages that reflect juvenile crust (normalized to 100% for the total volume of crust) (*4*) (**top**). The peaks in both graphs often but not always coincide with the periods of supercontinent formation (gray bars in the bottom panel).

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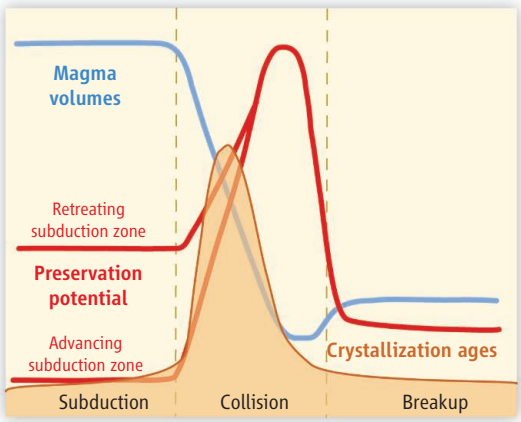

Preservation potential. The volumes of magma generated (blue line), and their likely preservation potential (red lines), may vary in the three stages associated with the convergence, assembly, and breakup of a supercontinent. The preservation potential in the first stage is greater at margins where the subduction zone retreats oceanward to form extensional basins than at margins where the subduction zone advances toward the continent. Thus, peaks in the crystallization ages that are preserved (brown area) reflect the balance between the magma volumes generated in the three stages and their preservation potential.

Rocks formed before 2.9 billion years ago have an extremely poor preservation potential (see the first figure). Thus, the late Archean marks the transition from a period of uniformly poor preservation potential to one in which the geological record is biased by the tectonic setting in which the rocks were formed. The peaks of ages illustrated in the first figure have been interpreted in terms of episodic, rather than continuous, generation of continental crust (*6*, *7*). Such models are largely based on the records of igneous and sedimentary rocks selectively preserved in "stable" areas and are therefore biased by the formation of supercontinents. The challenge now is to explore the geological records from stages with poor preservation potential.

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EDUCATION

Farewell, Lecture?

A physics professor describes his evolution from lecturing to dynamically engaging students during class and improving how they learn.

Eric Mazur

Discussions of education are generally predicated on the assumption that we know what education is. I hope to convince you otherwise by recounting some of my own experiences. When I started teaching introductory physics to undergraduates at Harvard University, I never asked myself how I would educate my students. I did what my teachers had done—I lectured. I thought that was how one learns. Look around anywhere in the world and you'll find lecture halls filled with students and, at the front, an instructor. This approach to education has not changed since before the Renaissance and the birth of scientific inquiry. Early in my career I received the first hints that something was wrong with teaching in this manner, but I had ignored it. Sometimes it's hard to face reality.

When I started teaching, I prepared lecture notes and then taught from them. Because my lectures deviated from the textbook, I provided students with copies of these lecture notes. The infuriating result was that on my end-of-semester evaluations—which were quite good otherwise—a number of students complained that I was "lecturing straight from (his) lecture notes." What was I supposed to do? Develop a set of lecture notes different from the ones I handed out? I decided to ignore the students' complaints.

Click here. Students continually discuss concepts among themselves and with the instructor during class. Discussions are spurred by multiple-choice conceptual questions that students answer using a clicker device. See supporting online text for examples of such "clicker questions."

A few years later, I discovered that the students were right. My lecturing was ineffective, despite the high evaluations. Early on in the physics curriculum—in week 2 of a typical introductory physics course—the Laws of Newton are presented. Every student in such a course can recite Newton's third law of motion, which states that the force of object A on object B in an interaction between two objects is equal in magnitude to the force of B on A—it sometimes is known as "action is reaction." One day, when the course had progressed to more complicated material, I decided to test my students' understanding of this concept not by doing traditional problems, but by asking them a set of basic conceptual questions (*1*, *2*). One of the questions, for example, requires students to compare the forces that a heavy truck and a light car exert on one another when they collide. I expected that the students would have no trouble tackling such questions, but much to my surprise, hardly a minute after the test began, one student asked, "How should I answer these questions? According to what you taught me or according to the way I usually think about these things?" To my dismay, students had great difficulty with the conceptual questions. That was when it began to dawn on me that something was amiss.

In hindsight, the reason for my students' poor performance is simple. The traditional approach to teaching reduces education to a transfer of information. Before the industrial revolution, when books were not yet mass commodities, the lecture method was the only way to transfer information from one generation to the next. However, education is so

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much more than just information transfer, especially in science. New information needs to be connected to preexisting knowledge in the student's mind. Students need to develop models to see how science works. Instead, my students were relying on rote memorization. Reflecting on my own education, I believe that I also often relied on rote memorization. Information transmitted in lectures stayed in my brain until I had to draw upon it for an exam. I once heard somebody describe the lecture method as a process whereby the lecture notes of the instructor get transferred to the notebooks of the students without passing through the brains of either (*3*). That is essentially what is happening in classrooms around the globe.

Since this agonizing discovery, I have begun to turn this traditional information-transfer model of education upside down. The responsibility for gathering information now rests squarely on the shoulders of the students. They must read material before coming to class, so that class time can be devoted to discussions, peer interactions, and time to assimilate and think (*4*). Instead of teaching by telling, I am teaching by questioning.

I now structure my time during class around short, conceptual multiple-choice questions. I alternate brief presentations with these questions, shifting the focus between instructor and students. The questions address student difficulties in grasping a particular topic and promote thinking about challenging concepts. After posing the question, I give the students 1 to 2 minutes to think, after which each must commit to an individual answer. They do this by submitting their answers using handheld devices called "clickers" (see the figure). Because of the popularity of these devices, questions posed this way are now often referred to as "clicker questions." The devices transmit the answers to my computer, which displays the distribution of answers. If between 35% and 70% of the students answer the question correctly, I ask them to discuss their answers and encourage them to find someone in the class with a different answer. Together with teaching assistants, I circulate among the students to promote productive discussions and guide their thinking. After several minutes of peer discussion, I ask them to answer the same question again. I then explain the correct answer and, depending on the student answers, may pose another related question or move on to a different topic. This approach has two benefits: It continuously actively engages the minds of the students, and it provides frequent and continuous feedback (to both the students and the instructor) about the level of understanding of the subject being discussed.

I often meet people who tell me they have implemented this "clicker method" in their classes, viewing my approach as simply a technological innovation. However, it is not the technology but the pedagogy that matters (*5*). Unfortunately, the majority of uses of technology in education consist of nothing more than a new implementation of old approaches, and therefore technology is not the magic bullet it is often presumed to be. Although clickers offer convenience and (at least for now) an amount of trendiness that appeals to students, the method can be implemented with flash cards, which are inexpensive and never prone to technological glitches (*6*).

Data obtained in my class and in classes of colleagues worldwide, in a wide range of academic settings and a wide range of disciplines, show that learning gains nearly triple with an approach that focuses on the student and on interactive learning (*7*, *8*). Students are given the opportunity to resolve misunderstandings about concepts and work together to learn new ideas and skills in a discipline. Most important, students not only perform better on a variety of conceptual assessments, but also improve their traditional problem-solving skills (*9*). Also, data show that such interactive engagement helps to reduce the gender gap that exists in introductory physics classrooms (*10*).

So, evidence is mounting that readjusting the focus of education from information transfer to helping students assimilate material is paying off. My only regret is that I love to lecture.

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MICROBIOLOGY

Mosquitoes Cut Short

Andrew F. Read[1,2] and Matthew B. Thomas[2]

Can the life-shortening effect of a bacterium on mosquitoes control the transmission of dengue?

Forty years ago, as the first drug- and insecticide-based global malaria eradication plan was being abandoned, the concept was raised of using evolutionary genetics to fight vector-borne diseases like malaria, dengue, and river blindness (*1*). The idea was to exploit selfish genetic elements, entities that can spread through host populations by distorting normal Mendelian inheritance, thereby enhancing their own transmission. Theoretically, such elements could be used to drive antipathogen effector genes through mosquito populations. On page 141 of this issue, McMeniman *et al*. (*2*) report a major step in a lateral development of this approach. They have infected the mosquito species that transmit dengue viruses to humans with an inheritance-distorting bacterium that kills mosquitoes likely to be infectious.

Wolbachia are maternally inherited bacteria found in a diverse range of arthropods. Because only female hosts can keep a lineage of *Wolbachia* alive, the bacteria have acquired mechanisms to ensure the overrepresentation of infected female offspring. One of these strategies is called cytoplasmic incompatibility, in which uninfected females that mate with *Wolbachia*-infected males fail to produce offspring. This reproductive asymmetry can allow the bacteria to spread through a population even if they reduce host fecundity (see the figure). Rapid invasion of fruit fly (*Drosophila*) populations by *Wolbachia* has been seen in real time in nature, raising the prospect of using these bacteria to spread disease-controlling genes through mosquito populations.

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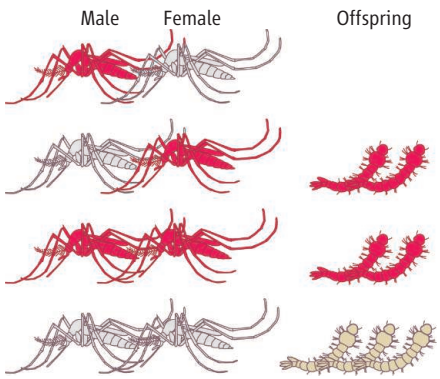
Yet in a cruel twist of fate, the mosquito that transmits dengue (*Aedes aegypti*) and the mosquito that transmits human malaria (*Anopheles* spp.) are not naturally infected by *Wolbachia*, even though many other mosquitoes are. But 3 years ago, microinjection of *Ae. aegypti* embryos with *Wolbachia* that were derived from *Ae. albopictus* was reported (*3*). The resulting infections had strong cytoplasmic incompatibility, highly efficient maternal inheritance, and in the laboratory increased from 20 to 100% of mosquitoes in just eight generations. Since then, the question has been how to translate this advance into dengue control.

One way would be to genetically engineer *Wolbachia* to carry a foreign gene (transgene) whose product attacks the flavivirus that causes dengue. A less intuitive alternative is to either provide *Wolbachia* with a gene whose product kills its host mosquito, or to find a *Wolbachia* strain that kills its host naturally. One strain, *w*MelPop, halves the life span of its natural host, *D. melanogaster*. McMeniman *et al*. now report the successful infection of the mosquito *Ae. aegypti* with *w*MelPop, and show that it also halves the life span of the new host. There are no data yet showing that *w*MelPop will spread in populations of *Ae. aegypti*, but the very strong cytoplasmic incompatibility is sufficient to prevent the loss of *w*MelPop from laboratory populations, despite its lethality.

Can the life-shortening effect of *Wolbachia* on mosquitoes achieve dengue control? Most vector-borne pathogens require many days to develop within the vector before becoming infectious to humans. For the virus and protozoan parasites that cause dengue and malaria, respectively, this extrinsic incubation period is about 2 weeks, although it is very temperature sensitive and for dengue, can be as short as 1 week (*4*). Because mosquitoes generally die quickly, the extrinsic incubation period of many infectious agents is longer than the average life span of the mosquitoes that transmit them. This means that only old mosquitoes are potentially dangerous to humans.

By killing old mosquitoes, *w*MelPop could thus impact on dengue transmission. But determining whether it can remove enough infectious mosquitoes to be useful will be a challenge. McMeniman *et al*. found that the mean longevity of *w*MelPop-infected mosquitoes is at least 3 weeks. Mosquitoes acquiring dengue from their first human meal would therefore be infectious for about a week. However, if *w*MelPop also halves life spans in the field, where background mortality rates are higher, substantial reductions in transmission could occur. For the right set of parameters, dengue control is possible in theory (*5*).



Ready for combat? A bacterial strain of *Wolbachia* has been adapted to infect the mosquito vector that transmits dengue virus to humans. The bacterial infections (red) are only transmitted through females to offspring, and cut mosquito life span by up to 50%. This may block disease transmission to humans.

A critical factor is *Wolbachia* virulence. All else being equal, the greater the impact on mosquito life span, the greater the disease control. But there are important downsides. Excessively virulent bacteria strains will spread very slowly, if at all; there are limits to what even cytoplasmic incompatibility can drive. Moreover, the reproductive advantage of cytoplasmic incompatibility largely accrues from *Wolbachia*-infected individuals mating with each other (see the figure). To ensure that such matings are sufficiently frequent, control programs will need to initially release large numbers of *Wolbachia*-infected, mating-viable individuals. The required numbers increase markedly with virulence (*5*). High virulence will also start its own evolutionary games: selection for *Wolbachia*-resistant mosquitoes and for more benign *Wolbachia*. Moreover, *Wolbachia* virulence is environmentally sensitive (*6*), as are pathogen extrinsic incubation period (*4*) and other determinants of disease transmission. Epidemiological models with explicit evolution are needed to determine the virulence required to give maximal and evolutionarily stable disease control in diverse ecological settings. Appropriate phenotypes could be selected from what is available naturally, or engineered by inserting appropriate virulence transgenes into the *Wolbachia* genome. It seems unlikely that one phenotype will fit all circumstances. There may even be geographic locations where no phenotype provides adequate disease control. Evolutionary epidemiological models would also help determine whether life-shortening *Wolbachia* could contribute to controlling other vector-borne diseases, not least malaria.

Other strategies for targeting old, potentially infectious adult mosquitoes are being developed. These include engineering densoviruses (natural viruses of mosquitoes) (*7*) and biopesticides that are based on entomopathogenic fungi (*8*). Such biopesticides effectively reduce malaria transmission in the laboratory by killing older insects (*9*), and theoretical models demonstrate good malaria-control potential in endemic areas (*10*, *11*). But interventions aimed only at older mosquitoes control disease transmission, not mosquito densities. This is in stark contrast to chemical insecticides which, as currently used, suppress mosquito densities by killing individuals of any age. Large-scale removal of mosquitoes is popular with local people but opens up niches for new vector strains or species and comes at the very high price of massive selection for insecticide resistance.

Would the release of life-shortening *Wolbachia* select for dengue viruses that develop more rapidly in *Ae. aegypti*? This possibility is highly relevant for any interventions that alter mosquito age structure (*12*). The high rates of mortality typical of mosquitoes must already be imposing intense natural selection for shorter extrinsic incubation periods. The apparent lack of response to this selection implies that prolonged development substantially enhances pathogen fitness (*13*). Even if life-shortening *Wolbachia* impose sufficiently strong selection to offset these fitness gains, the resulting evolution of more rapidly developing dengue viruses would presumably generate otherwise less fit pathogens. The impact of such evolution on public health will be important to understand. It may be less problematic than the evolution of insecticide resistance, the main evolutionary consequence of last century's vector-control strategies.

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INTRODUCTION

Adding a T to the Three R's

A STUDENT IN SCOTLAND INVESTIGATES THE EFFECTS OF POLLUTION ON THE Yangtze River in China. Students in Colorado learning from classroom discussions register their progress using clickers, or individual response systems. And when they're not in school, an astounding number of students spend their off hours playing technology-based games, either in immersive environments that millions of users share or individually on a cell phone. Opportunities abound for squeezing in a bit of learning along with the game-playing.

Although technology can be grand fun, the gee-whiz effect is only part of the story. Its real value lies in the underlying learning effects. Technologies that emphasize peer interactions can aid a collaborative approach to learning, as when soldiers build team function across distances using interactive training simulations. And technologies that place knowledge in the context of what a student already knows can aid learning. But technology is not a magic bullet for education: A fancy bit of electronics distributed without context and support may leave the laptop functioning as a doorstop.

Nor is it easy to know what works. A peer-reviewed national science education digital library initiative, supported for nearly a decade by the U.S. National Science Foundation, has made scant progress in getting the technology into the classroom and training teachers to use it. And when today's students are immersed in technology, how does one tease out the impact of such interventions? Even a proven success can be undermined by the ever-changing nature of the ways in which we communicate: Today's best practices may soon be embedded in antiquated technology.

In this special issue, we have collected a range of articles in the research, opinion, news, and book review sections that examine how education is changing in the face of technology. Related videos and podcasts feature interviews with some of the authors. As examples, we explore what one can learn from video games, how large-scale testing might be improved by technology, where cognitive science meets education, and what resources are being developed to facilitate a more effective use of technology.

There are both exciting opportunities and challenges in this fast-moving field, and we view this special issue as only the beginning of our exploration of the frontiers of learning for the next generation of scientists—and citizens.

– PAMELA J. HINES, BARBARA R. JASNY, AND JEFFREY MERVIS

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NSF Rethinks Its Digital Library

A $175 million investment has fostered collaboration and created vast amounts of material. But the digital world is changing

THE WEB IS A DOUBLE-EDGED SWORD FOR TEACHERS. Linda Lai has seen it deliver wonderful answers to the toughest questions posed by her third- and fourth-grade students at Edith Bowen Laboratory School in Logan, Utah. But separating the wheat from the vast amount of chaff on the Web takes time. Lai also worries that her students may be exposed to inappropriate material as they search for knowledge.

Mimi Recker, a professor of instructional technology at Utah State University in Logan, which runs the kindergarten through grade-5 lab school, knows that the Web poses many challenges for teachers. That's why she asked the U.S. National Science Foundation (NSF) to fund development of a Web-based tool to help teachers find, manage, and manipulate high-quality educational materials for use in the classroom. The software, called Instructional Architect (IA), is one of hundreds of research projects funded by NSF's National Science, Mathematics, Engineering, and Technology Education Digital Library (NSDL) program.

NSDL was launched in 2000 to help scientists and science educators tap into the rapidly expanding online world. Since then, the foundation has spent about $175 million "to provide organized access to high quality resources and tools that support innovations in teaching and learning at all levels." In practice, that has meant three things: creating and maintaining a Web site (nsdl.org) with a vast assortment of peer-reviewed materials, including lesson plans, videos, lectures, examples, and teacher guides; providing support for more than a dozen disciplinary

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and sector-based portals, called Pathways, that offer suitable content to NSDL; and funding individual research projects, such as IA, that are aimed at helping researchers and educators make better use of online learning. [AAAS, which publishes *Science*, has received $5.3 million since 2000 to oversee the BiosciEdNet (BEN) Pathway.] The money also goes toward administration, outreach, and the support of a global community of users.

Although NSDL has always focused on education, precollege classroom teachers are only one of many audiences. In fact, the thrust of NSDL in its early years was to help the research community make available something—high-quality educational materials—that by and large didn't exist.

Because NSDL serves several different purposes, the payoff from NSF's investment, which has averaged almost $18 million a year (see graph, p. 57), has been hard to quantify. Its biggest advocates admit that relatively few educators and researchers have even heard of NSDL, much less visited the Web site or contributed material. It's proven to be no match for Google as a search engine for finding good sites. And there's no evidence to date that NSDL has improved student learning.

Although NSF officials insist that NSDL has been a success, the agency is in the process of reducing its support for digital libraries. Last year, the initialism NSDL was redefined as the National Science Distributed Learning program and subsumed under a new, broader cyberlearning initiative for which digital libraries are only a small component. In September, NSF cut its support to the organizations that manage NSDL by more than half and described the new round of funding as a "rampdown … toward self-sufficiency." The consortia operating the various Pathway portals say they don't expect to get another bite of the apple. In 2007, NSF ended its funding of DLESE, a digital library for earth system education that is separate from NSDL but serves as an informal pathway for the earth sciences community (see sidebar).

Those in the trenches remain committed to making digital libraries an integral part of science education. But they face serious obstacles. Lai, who began teaching in 1971 and who admits that she's "technology challenged" by the profusion of communications devices now available, says she's found IA very user-friendly. "The site is easy for my kids to access, and it's very safe," she says. Lai prefers IA (ia.usu.edu) to the school's Web site, for which she must give an instructional aide a list of URLs and which allow no supplemental materials. "And I can do IA from home," she adds.

So how often do Lai and the other teachers at Edith Bowen, all of whom were trained on IA several years ago, use this powerful new online tool? Recker has found that only 20% of teachers in one study were still using IA 1 year later, and Lai and her colleagues are no exception.

"I haven't used it this year," Lai confesses. "My third graders are just learning how to use the laptops" that are kept in the school's media lab and carted around to classrooms as needed. "And I don't know how many [of the other teachers] still use it. There's so much else that we're expected to be doing, we don't really talk about IA."

Promise and reality

NSDL was launched in the waning days of the Clinton Administration, at the height of the dot-com boom, when expectations about the Web's potential were sky-high. One leading online policy guru, Thomas Kalil, then of the National Economic Council (and now a member of the transition team for President-elect Barack Obama), wrote in 1996 about the need to "leverage cyberspace" for economic growth. NSDL was seen as a way to do that in the realm of education, and Kalil and other White House officials promised that it would improve student performance,

New Landscape for the Earth Sciences

A digital library for the earth sciences community and by the community is now also *of* the community.

Four years before the U.S. National Science Foundation (NSF) launched the National Science Digital Library (NSDL) (see main text), leaders in the earth sciences community began planning what would become DLESE (Digital Library for Earth System Education). In 2002, NSF took the plunge, making a 4-year award to the University Corporation for Atmospheric Research (UCAR) to operate the distributed, community-based library of materials for undergraduate education and research. But in 2005, after getting a scathing report from a visiting committee on how NSF's $21 million investment had been managed, NSF decided to phase out its support. After much soul-searching, a slimmed-down DLESE found a home in 2007 within UCAR's National Center for Atmospheric Research in Boulder, Colorado, where it is now part of NCAR's library.

From the start, DLESE had twin goals: to unite earth and environmental scientists and to improve how those disciplines were taught, including the research component. In addition to vetting material for the library to assure high quality, researchers hoped DLESE would be their online watering hole, a place to foster collaborations and share their knowledge. But the NSF visiting committee found that DLESE's horizontal management structure actually inhibited good communications and that the sum of its various parts added up to less than the whole. "DLESE cannot be all things to all groups. … More is not always better," its report concluded. The program's fiscal stewardship was another problem. "Even with the documentation provided [us], we still have almost no idea how $21 million have been spent over the past 5 years," the committee noted.

Christopher Maples, a paleontologist and former NSF program officer who chaired the panel, acknowledges that "we were pretty tough on them." But the report also lauded DLESE for taking "a terrific, creative idea" and proving that it is "both promising and deserving." Maples, who this fall became president of the Oregon Institute of Technology in Klamath Falls, says "I haven't followed them since our report. But I'm glad to hear that they've landed on their feet."

Jill Karsten, program director for education and diversity within NSF's geosciences directorate, certainly agrees. She says that DLESE's "soft landing" is exactly what the foundation hopes will happen to worthy projects after their NSF funding runs out. (DLESE was supported largely by the geosciences directorate, which also funds NCAR, rather than the education directorate, which has funded NSDL.)

But Mary Marlino, DLESE's longtime director and now head of the NCAR library, sees the transition a bit differently. In addition to laying off staff, she had to jettison several community-based activities and outreach efforts. "We're at maintenance level," she says. "It's no longer DLESE on steroids. But we've survived, and we're moving forward."

DLESE initially targeted undergraduates and those who teach them, Karsten says, but it has gradually moved into elementary and secondary schools. In one such project, DLESE worked with middle and high school earth science teachers to prepare electronic "teaching boxes" for the classroom. "In the early days, we thought that access would be the ticket," says Marlino. "But now we realize teachers need to understand the resources in context and how to use them."

Earth science teachers will have to do it on their own, however. The instructional units, on topics from weather to plate tectonics, are online and ready to use (teachingboxes.org/). But after the loss of NSF funding, Marlino says there are no plans to develop any more boxes. **–J.D.M.**

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A Nobelist's Passionate Pursuit of Excellence

In its role as a collection of collections, the National Science Digital Library (NSDL) offers more than 1.5 million resources to science educators. But even that impressive figure doesn't capture the work of everyone who is generating serious online content. One academic who operates outside NSDL's network is Harry Kroto, 1996 chemistry Nobelist for his co-discovery of fullerenes.

Kroto, who retired from the University of Sussex in the U.K. and moved in 2004 to Florida State University in Tallahassee to focus on science education, believes that it's possible to produce high-quality materials without following NSDL's protocol of first putting everything under a disciplinary microscope. Instead, he argues that the best materials often come from "people who are passionate about what they are doing and want to share it. I'm committed to the ideals of the Dead Poets Society—you know, the charismatic teacher being the vehicle to excite students." That principle, he adds, is why Wikipedia has become so much more popular than *Encyclopedia Britannica*.

Toward that end, he's built a studio on campus that films presentations from fellow scientists. The materials are then posted on a site called GEOSET (Global Education Outreach for Science Engineering and Technology). The process is idiosyncratic—"if I hear about a good presentation on a particular topic, I ask the person to come by," he explains—and runs on a tiny budget drawn mostly from university start-up funds. "I generally like to show people what I can do before I ask them for money," Kroto says. "It was the same for my research on C_{60}."

With 75 modules available, the bulk aimed at students from high school through graduate school, Kroto says it's time to start thinking of scaling up his digital library (geoset.group.shef.ac.uk). "I'd love to see this happening on hundreds of campuses," he says, ticking off collaborators in the United Kingdom, Portugal, Croatia, and Japan. "All it needs is a room with the right equipment and someone who's really committed to the task." **–J.D.M.**

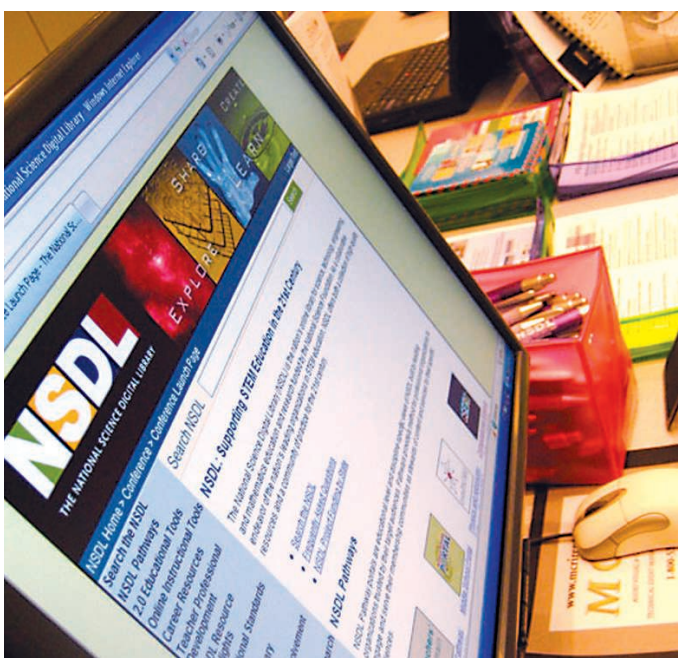
heighten student interest in science, and make high-quality material readily available to parents, teachers, and students.

Lee Zia, NSDL's longtime program director, says that those statements, in hindsight, were "idealistic. The rhetoric at the time—greater connectivity would usher in this new era of educational achievement—was compelling." He insists that NSF never tried to hype NSDL. "We'd rather be in a position of underpromising and overdelivering," he explains.

NSDL was not NSF's first exposure to digital libraries. In 1994, it joined with other federal agencies to fund a team at Stanford University trying "to develop the enabling technologies for a single, integrated and universal digital library." A few years later, two graduate students on that project, Sergey Brin and Larry Page, used the search engine being created to found Google.

NSF officials weren't trying to replicate Google's commercial success when they launched NSDL. But as the Internet grew in importance, they began to ask how it could help teachers and students. There was already a lot of potentially useful content on the Web, some of it funded by other NSF programs and considered to be of high quality. But it was neither easy to find nor readily tailored for the classroom. As Zia puts it, "If you wanted to turn great piles of stuff into piles of great stuff, the first thing you needed was an infrastructure."

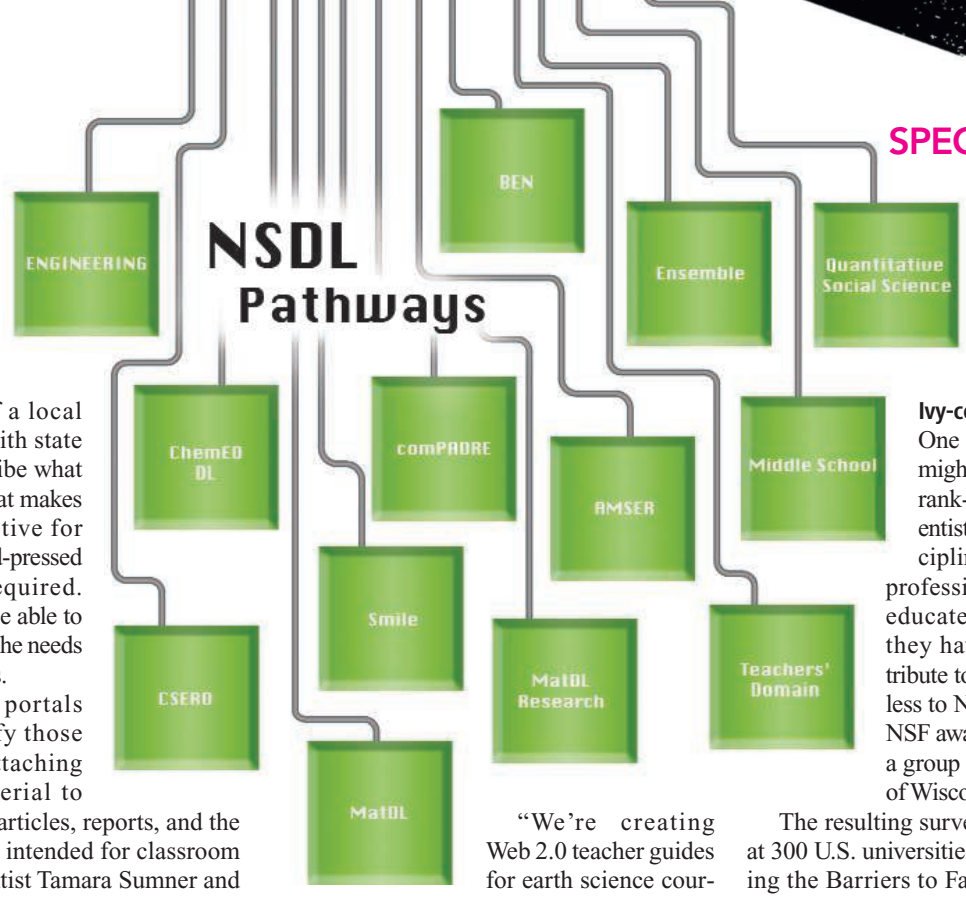
To provide that infrastructure, NSF funded "core integration" groups at the University Corporation for Atmospheric Research in Boulder, Colorado; Cornell University; and Columbia University to operate the main portal. They set out to develop technical standards, define the scope of the library, troubleshoot problems, and anticipate users' future needs. The service component was a new wrinkle on NSF's traditional approach of supporting cutting-edge research. "It was an unusual activity for NSF," explains Zia. "NSDL was both a traditional program to fund research on an emerging technology [the Web] and an evolving entity that needed to be supported and sustained."



Once the administrative structure was in place, NSF put out a call for the community to compile collections that would link with NSDL. Over the years, NSF has funded 13 so-called Pathways serving both individual scientific disciplines and various user communities, including new ones this fall for the computing sciences and the quantitative social sciences.

One major Pathway activity is to post material that other NSF programs had funded over the years, much of it created by those same organizations managing individual Pathways. "NSF has invested in a lot of educational resources, and this is a way to archive everything that the societies had done, including things that may have gone out of print," says Yolanda George, deputy director of education and human resources at AAAS in Washington, D.C., which operates BEN (biosciednet.org). Each Pathway is also supposed to be the hub for an interactive community of users.

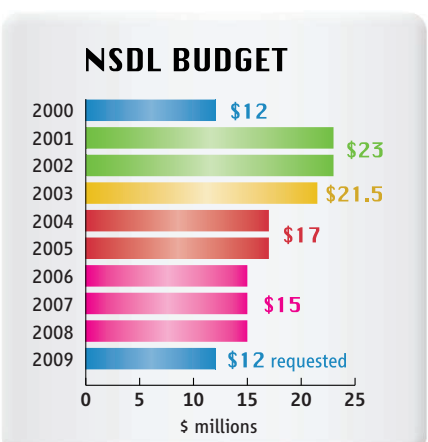
Run by a consortium of 26 professional societies, BEN has put up 11,000 peer-reviewed resources—everything from scientific papers and reports to lectures and lab experiments—toward a goal of 25,000 by 2010. But to George, quality is more important than quantity. "Other people think that more is better," she says. "I don't agree. I'd rather have a nice-sized catalog of peer-reviewed material that promotes active learning than a vast amount of stuff that hasn't been vetted."



To teach a child. NSF has funded 13 Pathways projects to gather and disseminate material by discipline and sector. See below for URLs.

Low quality isn't the only reason teachers might avoid Web-based material. Most online resources aren't aligned with the existing curricula of a local school district or with state standards that describe what should be taught. That makes it much less attractive for teachers already hard-pressed to cover what's required. Teachers must also be able to tailor the material to the needs of individual students.

Many Pathway portals have tried to satisfy those requirements by attaching supplemental material to resources—journal articles, reports, and the like—not originally intended for classroom use. Cognitive scientist Tamara Sumner and her colleagues at the University of Colorado, Boulder, are tackling the issue head on. In 2007, she began working with secondary school earth science teachers in the Denver Public Schools to use NSDL resources to customize the district's curriculum in an interactive fashion. The curriculum was created by the American Geological Institute and is published by It's About Time, the Armonk, New York–based education division of Herff Jones that works mainly with NSF-funded curricula. Sumner, who has several NSDL-related research grants and is also co–principal investigator with Cornell on one of the two remaining infrastructure grants that NSDL supports, contributes both her expertise in teacher training and her knowledge of NSDL.



CREDITS (TOP TO BOTTOM): N. KEVITIYAGALA/*SCIENCE*; (CHART) SOURCE: NSDL PROGRAM

A digital rise and fall. The budget of NSF's National Science Digital Library has fallen from its early days.

"We're creating Web 2.0 teacher guides for earth science courses," she explains about a pilot study now under way to give Denver teachers an interactive platform to develop individualized lesson plans. It allows them to integrate information from the district's own IT system, which teachers now use to maintain student records and track their performance on ongoing formative assessments as well as year-end standardized tests, with material tailored to address the needs of students across a range of abilities, from gifted and talented to English language learners.

Jim Short, then science coordinator for the Denver schools, says he was attracted by Sumner's previous work in organizing NSDL material to give teachers immediate access to exactly what they might need, when they need it. "I wasn't interested in more curriculum," says Short. "But imagine how useful it would be for a teacher to link the concepts from an activity to an embedded assessment, then ask, 'What key question would help me know if the student got the concept?' and then not have to search for the answer because the appropriate resources are already tagged."

A former biology teacher who now directs the Gottesman Center for Science Teaching and Learning at the American Museum of Natural History in New York City, Short believes that the guides should help even the most experienced teachers. "I don't think teachers have the time or expertise to put it all together themselves," he says.

Ivy-covered indifference

One group that presumably might have such expertise is rank-and-file academic scientists. Despite their vast disciplinary knowledge and a professional responsibility to educate the next generation, they have been slow to contribute to digital libraries, much less to NSDL. To find out why, NSF awarded a grant in 2005 to a group based at the University of Wisconsin, Madison.

The resulting survey of faculty members at 300 U.S. universities (its title was "Lowering the Barriers to Faculty Participation in NSDL") doesn't provide a direct answer. But it adds to the previously meager pool of information on how academics use online resources. The survey found, among other things, that they are prone to do their own searches and value speed as highly as they do quality, and that the material they download is most likely to be used in lectures. An overwhelming number say they are self-taught.

"The biggest surprise to me is their reliance on Google," says Alan Wolf, a co-author of the study, which he says was one of the first to ask such questions. Their methods are likely crude, he says—"they tend to spend a great deal of

Links...

A Pathway to the Pathways

To access these NSDL portals, go to:

Biology: biosciednet.org
Chemistry: jchemed.chem.wisc.edu
Computational science: shodor.org/refdesk
Computing science: computingportal.org
Engineering: engineeringpathway.com/ep/
Materials science: matdl.org
Mathematics: mathdl.maa.org
Physics and astronomy: compadre. org
Social sciences: qssdl.org
Community colleges: amser.org
Informal science: howtosmile.org
Middle school: msteacher.org
Teachers: teachersdomain.org

A Vision in Search of Funding

Last summer, the U.S. government created an awkwardly named entity to fund research on how the Internet can improve U.S. education. But so far, the National Center for Research in Advanced Information and Digital Technologies exists only on paper.

Anecdotal evidence abounds of students getting excited about science through video games and other electronic educational resources. But the research to back up that premise is thin. Advocates of the new center persuaded Congress to take the first step as part of legislation reauthorizing U.S. policies toward higher education that became law last summer. But none of the $50 million that supporters are seeking in start-up funding has been appropriated.

Its backers have been deliberately vague about what type of research the nonprofit center would fund so that experts can set the agenda—and to attract as much support as possible. Michelle Lucey-Roper of the Federation of American Scientists (FAS) in Washington, D.C., which has led the campaign, sees it funding "precompetitive research on innovative learning tools serving all levels of society," from preschoolers to retirees, and in settings as different as the battlefield and the factory floor. The center would be an odd creature in the federal research zoo: Although housed within the Department of Education, it would nevertheless have an independent policymaking board and be free to solicit money from other agencies and from the private sector.

Supporters hope President-elect Barack Obama will ask Congress to fund the center in his 2010 budget submission. They note that his campaign promises to use technology to improve education and to double funding for education research point to his support for the concept. But FAS isn't waiting for the new Administration. It's already raised a bit of money to come up with an operating plan and management structure so that the center can hit the ground running if Congress ever funds it. **–J.D.M.**

time looking for something that they assume exists"—and they often do it with a sense of urgency, "say, for a lecture the next day."

Wolf, a biologist who directs the New Media Center program at Wisconsin, doesn't know how elementary and secondary schoolteachers would answer a similar survey. But he guesses that "their use of Google as a search engine is likely to be similar." He also speculates that the quality of what's on the Web is less of an obstacle to faculty members than to K–12 teachers. "They are experts and probably feel capable of judging the accuracy and suitability of the material" for their students, he says.

An uncertain future

In fact, Zia expects expert knowledge to play a bigger role in NSDL as its progress tracks the evolution of the Web. Begun as an information commons, the Web then became a mechanism to foster social networking and interactivity. But NSF is already planning for Web 3.0.

"That's a return to editorialization," explains Zia, on leave this year as a policy fellow in the office of U.S. Senator Jay Rockefeller (D–WV). "It's something to help the user get the content in the right context. It adds an interpretative component that's now missing."

Whether NSDL will be part of that next iteration of the Web remains an open question, however. The program was always an odd fit at NSF. The agency's deep roots in the academic research community don't necessarily help it nourish science teachers in thousands of local school districts. Those teachers need help solving problems that are often unfamiliar to academic researchers. "When we began 8 years ago," says Utah State's Recker, "we assumed that we could build a resource bank of high-quality interactive material and that change would follow. That was naïve. Once we started to go into the classrooms, we realized the complexity of the environment."

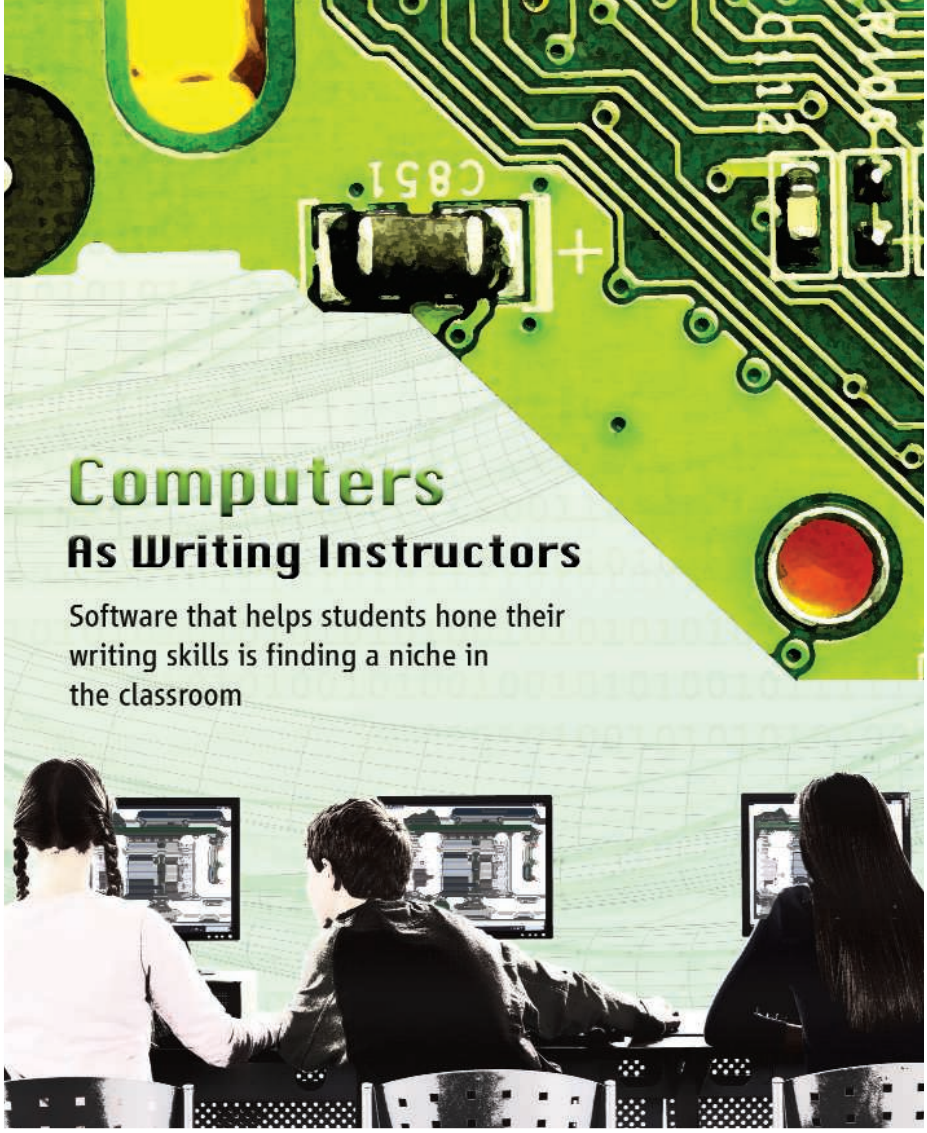
NSDL must also operate within the rules that have made NSF a model agency for federal support of academic research: Applicants should address cutting-edge research questions, funding is for a finite period, and successful projects must figure out other ways to scale up or be sustained indefinitely. "A colleague of mine is fond of saying that there's only one thing that's certain about an NSF grant: It always ends," says Sumner, who is quick to add that she's very grateful for NSF's continued support of her research at the University of Colorado, Boulder.

Still, Sumner and others are disappointed that NSDL isn't better known. "Could NSF have done a better job of marketing NSDL? Absolutely, but NSF doesn't fund marketing efforts," she says. "They funded the research and the service components. There was never a product-development group with a marketing department, as would be the case for any commercial business."

In addition to a marketing department, companies also have an advantage over NSDL because their products are obvious. Identifying exactly what NSDL has to offer is much trickier. Although a contractor is laying the groundwork for outside experts to do a thorough program evaluation, Zia admits that "it's going to be much harder to figure out the impact of NSDL on a particular student or school." Given the privacy issues involved in trying to trace a user's Web behavior, he adds, "I don't even know if it could be done." **–JEFFREY MERVIS**

Pulling it together. An NSF-funded project is giving these Denver high school teachers a chance to customize their district's curriculum with online resources.

CREDITS (BOTTOM): LYNNE DAVIS



Computers
As Writing Instructors

Software that helps students hone their writing skills is finding a niche in the classroom

CREDITS: (PHOTO MONTAGE): N. KEVITIYAGALA/*SCIENCE*; (PHOTOS TOP TO BOTTOM) JUPITER IMAGES; JOHN CUMMING/GETTY IMAGES

CAN COMPUTERS TEACH CHILDREN TO write better? Michael Jenkins, who teaches language arts at Estancia Middle School in central New Mexico, tells the story of Maria (a pseudonym), who so struggled to put her ideas on paper that she used to cry whenever he gave the class a writing assignment. That was before Jenkins began using writing-instruction software that provides feedback on students' essays and offers suggestions on how to improve them, all within seconds. By the end of the school year, Maria had more confidence in her writing abilities—and passed the writing portion of the state assessment test. "It's not a cure-all, but what a difference it's made in what the kids have shown they can do," says Jenkins, who began using the software last year.

He is not alone. Pearson Education, which makes the WriteToLearn software Jenkins uses, declined to provide an estimate of how many students use it, but its two main competitors, Vantage Learning and Educational Testing Service (ETS), each say that more than a million students use their respective software. Although teacher testimonials abound, few studies have directly investigated whether computer writing instruction works better than the traditional kind. Moreover, some skeptics question how well the programs capture the essence of good writing.

Writing-instruction software has emerged in the past 5 to 10 years, but it traces its origins to the 1960s, when Ellis Page, a computer scientist who once taught high school English, realized that the time it takes for teachers to grade and return writing assignments limits the number of writing assignments they can dole out—and, therefore, the amount of writing practice students get. Hoping to speed things up, he identified several features that could be extracted from a writing sample by a computer—such as average word length and the number of prepositions—that correlated well with the scores human graders assigned. His automated grader gave scores that consistently agreed with human readers, but the punch-card data entry required by computers of the day limited its use.

By the late 1990s, computing power had finally caught up, and several companies introduced software that expanded on Page's ideas and incorporated newer methods from machine learning, natural language processing, and statistics. (Page died in 2005.) These programs, for example, can analyze the vocabulary used in a passage to determine whether a writer strayed from the assigned topic and evaluate sentence variety—generally considered a mark of good writing.

The first application of the software was to grade essays. Millions of essays are scored by computer each year as part of test-preparation software for standardized tests such as the Graduate Record Examination taken by prospective graduate students. The programs are also used in some high-stakes situations: the Graduate Management Assessment Test, taken each year by about 200,000 students aspiring to MBA programs, uses software in scoring its analytical writing section. (A human reader also scores each essay, and a second person weighs in if the human and computer scores differ widely.)

Today, Vantage, Pearson, and ETS dominate the field. "All three use slightly different mathematical models but a similar basic approach," says Scott Elliot, a private consultant who previously worked at Vantage. The programs typically have to be "trained" on 100 or more human-graded essays before they can score new material.

So how well do these programs evaluate writing? "The system has to agree with a teacher as often as two teachers would agree," says Peter Foltz, vice president for research at Pearson. Studies have found that the programs consistently hit this mark for the type of everyday writing that's found in standardized tests and high school essay assignments, says Mark Shermis, an educational psychologist at the University of Florida, Gainesville: "They handle 95% of the writing that's out there, but I don't think they will ever do poetry … or identify the next great novelist."

Over the past decade, several companies have adapted their essay-grading software to identify the strengths and weaknesses of an essay instead of simply issuing a grade. Rigorous studies on the effectiveness of this writing-instruction software are lacking, however. Those done by vendors typically examine the progress of a group of students using the software rather than comparing them with students who receive more traditional instruction, says Shermis. He has asked the Department of

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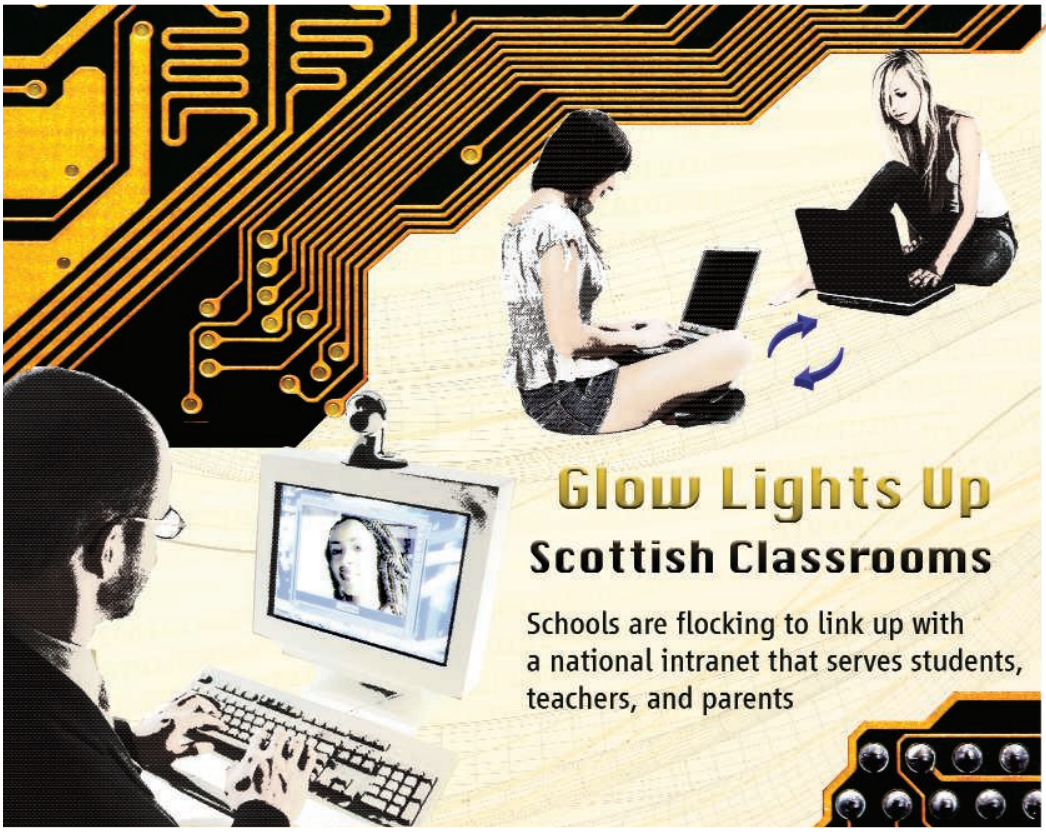
Education's Institute of Education Sciences to fund a study that would analyze the performance of two such groups on the Florida state assessment test. But Jenkins, who has taught for 14 years, says he is already convinced that the programs work.

The software that Jenkins uses, sold by Pearson at a cost of $30 per student, generates colored bars that show students, among other things, how well their essay states and supports a main idea and whether their sentences flow smoothly and logically. Jenkins says his students can now write and refine an essay several times in a single class period; in the past, Jenkins needed a couple of days to return their first drafts. He welcomes the electronic assistance. "It builds their confidence because they keep trying till they get it," Jenkins says. The software lets him spend more time with struggling students and also identifies topics that the entire class needs to review.

Jenkins suspects that English language learners (ELL)—educationese for children who speak another language at home—may be among those who can benefit the most from using writing-instruction software. Last year, 92% of his ELL students passed the writing portion of the state assessment test, he says, compared with 31% of his ELL students before he started using the software. That percentage is also well above the statewide ELL rate of 58%.

Richard Haswell, a professor emeritus of English at Texas A&M University, Corpus Christi, sees benefits and drawbacks to the writing software. Haswell says he's sympathetic to the plight of time-strapped teachers who want to incorporate more writing assignments into their classes, but he worries that "expediency is trumping validity." One peril, says Haswell, who has studied both traditional and electronic measures of writing, is that the programs pick up quantifiable indicators of good writing—average sentence length, for instance—yet ignore qualities such as whether an essay is factually accurate, clear, or concise, or whether it includes an element of wit or cleverness. "Those are all qualities that can't be measured by computer," he says. **–GREG MILLER**



Glow Lights Up Scottish Classrooms

Schools are flocking to link up with a national intranet that serves students, teachers, and parents

JAYE RICHARDS IS A BIOLOGY teacher at Cathkin High School in the Glasgow suburb of Cambuslang. When she teaches the effects of pollution on rivers and seas, she asks her 14- and 15-year-old students to look far beyond Scotland to the River Don in Sheffield, England, the Yangtze River in China, and the Gulf of Mexico. She doesn't just turn them loose on the Internet, however. Instead, she taps into Glow, Scotland's national intranet for schools.

By accessing video clips, podcasts, and newspaper articles—which Richards has identified in advance—her students can see how the theories they learned in class apply to the real world. "They can go to the Gulf of Mexico, find out the level of oxygen in the water, see the dead zones," Richards says. The students collaborate on the tasks, communicate via instant messaging, and post their work on an online discussion board. Such a lesson is possible in any classroom with networked computers, but the teacher would need to be pretty tech savvy. Glow hopes to make such online learning routine. "For a standard classroom teacher, this is a very good system," Richards says.

Glow is essentially a Web site that aims to be a one-stop shop for Scotland's teachers, students, and their parents. A teacher who logs in can access e-mail within Glow. Discussion groups provide a forum for lively debates over curriculum, discipline, and other topics with colleagues in their school or across the country. Glow offers instant messaging as well as Web conferencing, using audio, video, or a shared whiteboard. And there are teaching materials, too. Students can use the system in class, in a moderated chatroom, or at home. "The pupils are talking about it," says Richards. "I have to drag them out of the classroom at the end of the lesson."

The driving force behind Glow is Learning and Teaching Scotland (LTS), an agency funded by the Scottish government to develop the school curriculum. LTS began thinking about such a system in 2001 when the nascent Scottish parliament, created in 1999, was flexing its new policy muscles. Like many countries, Scotland had made large invest-

CREDITS (TOP TO BOTTOM) (PHOTO MONTAGE) N. KEVITIYAGALA/*SCIENCE*; (PHOTOS: LEFT TO RIGHT) JUPITER IMAGES; ISTOCK PHOTOS

ments in providing computers for schools and access to the Internet. But their use varied widely among the country's 700,000 primary and secondary school students and their teachers, explains Laurie O'Donnell, director of learning and technology for the agency. LTS saw a national intranet as a way to help those among Scotland's 32 education authorities that were struggling, while encouraging all its teachers to collaborate.

The Scottish government allocated $64 million to develop Glow and operate it for 3 years. LTS set about designing the system in collaboration with local authorities and the Oxford-based company Research Machines (RM). When it was ready, authorities were invited to come on board and provide Glow to their schools via their existing networks. LTS and RM provide training for local authority staff, who then train their own teachers. "It's driven by improving learning and teaching, not by forcing technology down teachers' throats," O'Donnell says.

Glow debuted in September 2007 and was immediately a hit: Half of Scotland's 32 education authorities are already using it, and all but two of the rest have signed up. (One decided that it couldn't afford the additional staff required to implement Glow, and a second has not signed up because of the bandwidth required.) "Glow looks to be groundbreaking," says Seb Schmoller, head of Britain's Association for Learning Technology, a professional society for the field.

It's even attracted notice far from Scotland's shores. Last summer, filmmaker George Lucas appeared before the U.S. Congress in his role as benefactor of the George Lucas Educational Foundation and held up Glow as a model of how schools could use information technology. "This kind of common platform makes perfect sense," he told a panel of legislators looking to expand a program to wire up U.S. schools. O'Donnell says 20 countries have sent people to Scotland to see Glow in action.

The biggest benefit of Glow, according to O'Donnell, is increased communication. Although Scottish schools already had access to the Internet and enough computers to tap into its vast potential, teachers were still developing their own teaching plans and materials and were generally unaware of similar efforts by other teachers, even those within the same school. "Glow creates an environment where teachers can collaborate and share," says O'Donnell.

Teachers have found they have much in common. Glow has helped a class in Dundee hold a videoconference with a museum curator who runs a recreated Victorian classroom. A school studying global citizenship linked up with a class in Malawi, and a violin teacher who routinely travels to remote schools in the region of Argyll and Bute is now able to hold a violin class simultaneously with six pupils in different places.

A new Glow. Students in East Dunbartonshire, Scotland, work on Glow with Laurie O'Donnell, whose agency developed the project.

Glow has also made it easier for schools to meet the needs of their top students. Although Scotland offers extra final exams called advanced highers, small high schools often don't have suitable teachers. But Glow allows students to learn from a larger pool of teachers. "They can link to the best teachers in Scotland and the world," O'Donnell says. LTS also expects parents to take advantage of Glow to keep up with their children's homework assignments, announcements, and school events.

The system hasn't been around long enough for officials to assess its impact. But Richards devised her own study to see if Glow was worth the time and effort. With funding from the General Teaching Council for Scotland, Richards studied Glow's impact on four biology classes in her school, all of which followed the same syllabus. Three classes used the traditional syllabus for a module on water pollution, while Richards used Glow in one of three weekly lessons to reinforce her normal teaching.

An end-of-year exam and other assessments found that her class performed 14% better than the other classes in the pollution module, and 32% better in that module than they achieved in other modules taught without Glow. Stephen Draper, a researcher into technology and learning at the University of Glasgow, will help Richards with follow-up studies on how the teacher's level of experience and other factors affect student learning with Glow, and how much time and effort is required to use it.

Draper says he was impressed by Richards's results but doesn't think technology is an educational cure-all. Instead, he believes that the Glow modules helped by connecting concepts to concrete examples. "It's not technology that causes learning but lesson design," he says. The Glow lessons are self-paced and allow pupils to learn from one another. Meanwhile, Richards moves around the classroom dealing individually with problems, which Draper believes is "a much more sensible way to use your teachers."

Richards says Glow doesn't replace traditional teaching. But for students accustomed to using electronics outside the classroom, she says, Glow is an "enhancement. They are true digital natives. You have to tailor your teaching to the pupils in front of you, and Glow materials are suited to this." **–DANIEL CLERY**

CREDITS (TOP TO BOTTOM): LEARNING AND TEACHING SCOTLAND

Published by AAAS

Korea Tries To Level the Field

A cyber home learning system seeks to reduce the huge cost of private tutors, a practice not available to every Korean family

NAMYANGJU, SOUTH KOREA—When Kwangdong Middle School lets out, many of the students in this hard-scrabble town northeast of Seoul head for pricey cram schools or private tutors for help with their studies. It's part of an educational rat race throughout primary and secondary education, culminating in entrance exams that determine which students get into Korea's elite universities.

It's also a big business: The Hyundai Research Institute estimates that the average family spent 19% of its household income on extracurricular primary and secondary education in 2006. That investment totaled about $23 billion and represented 4% of the country's gross domestic product. In the same year, the Korean government spent only $21 billion on education. A recent government survey found that 77% of primary and secondary students were enrolled in various academic classes to supplement regular classroom instruction.

On a recent afternoon, however, a handful of Kwangdong students have headed to the school's computer room instead of going to private tutors. Like their classmates, they are getting individualized help to improve their school performance. But they are getting it for free thanks to Korea's online Cyber Home Learning System (CHLS).

"In class, the pace has to be the same for all," says Sang Mi Kim, a math teacher who has tweaked the CHLS system to suit the needs of students in her cybergeometry class. "[In the computer room], students study at their own pace." Initially skeptical of e-learning, Kim is now a vocal supporter. "It really is effective," she says. One of her students, Yun Sik Lee, says the video lectures and animations help him keep up with his regular class. "I log on every day after school."

Korean education officials hope that enthusiasm is contagious. Their goal is to wean many students and their parents away from private educational services and to create a more equitable and affordable system. They hope the 3-year-old CHLS can help reduce the imbalance between urban areas, where private tutors are plentiful, and rural areas, where they are rare, and also relieve the middle class of what is now a crushing financial burden.

Paul Resta, director of the Learning Technology Center at the University of Texas, Austin, calls CHLS "a very coherent and comprehensive approach to enhancing teaching and learning by integrating the school experience with resources available in the home. It really is a model [for] other countries." Resta chaired a jury that in 2006 awarded CHLS the UNESCO King Hamad Bin Isa Al-Khalifa Prize for the Use of Information and Communication Technologies in Education. The next year, the cybersystem won the IMS Global Learning Consortium's Learning Impact Platinum Award for the use of technology to enhance learning. Toru Iiyoshi, director of the Knowledge Media Laboratory at the Carnegie Foundation for the Advancement of Teaching in Stanford, California, and a member of the IMS award panel, believes the CHLS model could be extended to adult and vocational education. "We need more individualized learning. The future possibilities are just huge," he says.

Time to learn

CHLS relies on the nation's well-established broadband Internet infrastructure. Korea has one of the world's highest rates of home personal computer ownership. Every public classroom and practically every household has broadband Internet access, and all public school teachers have computers and must take information technology training courses. So in 2004, when the Ministry of Education turned to e-learning to provide an

Cyber convert. Sang Mi Kim, a middle school math teacher, has switched from being a doubter to a booster of the system.

CREDITS: (PHOTO MONTAGE) N. KEVITIYAGALA/*SCIENCE*; (PHOTOS IN MONTAGE TOP TO BOTTOM) JUPITER IMAGES, GOGO IMAGES CORPORATION / ALAMY, CORBIS; (BOTTOM PHOTO) DENNIS NORMILE/*SCIENCE*

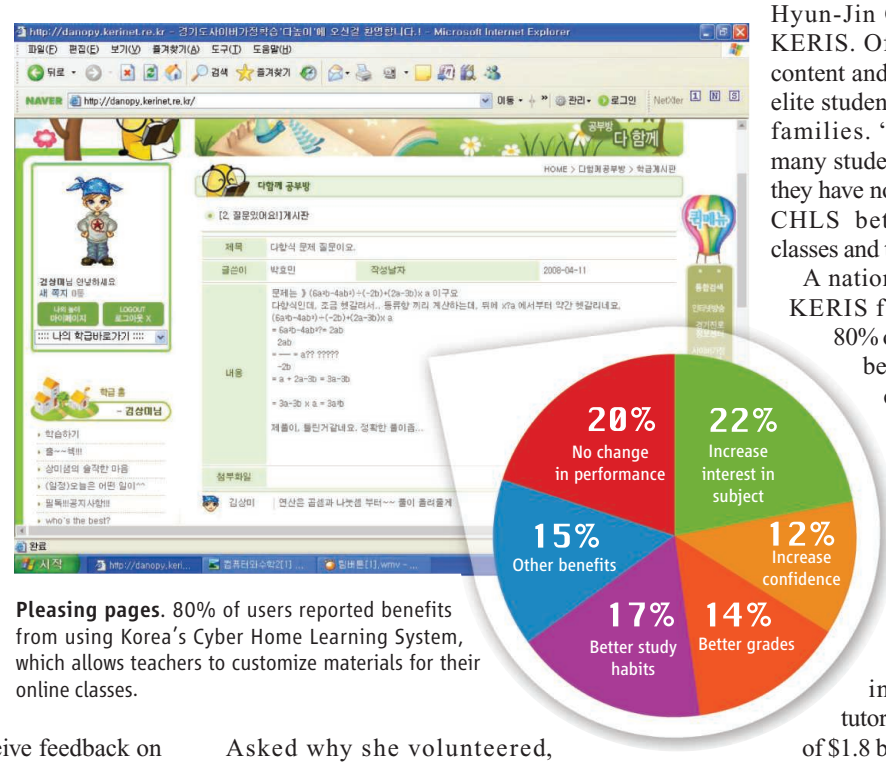
alternative to private tutoring, the infrastructure already existed.

From the outset, the objective was to supplement classroom instruction with materials students could use on their own either at school or at home with the support of cyberteachers, cybertutors (usually parents), and online counselors. "In a classroom, you have a limited amount of time," says Jae-Myung Yang, director of the cyberlearning team at the government-affiliated Korea Education & Research Information Service (KERIS), which has led development of CHLS. "But with e-learning, you can have an extended amount of time with more individualized content and more personal interaction with teachers."

CHLS provides materials for grades four through 10. It covers major subjects—Korean, social studies, mathematics, science, and English—and elective subjects such as composition, Chinese, and drawing. For each subject, there are supplementary, basic, and advanced contents. Demonstrations and explanations feature animations as well as video clips involving real teachers. Students can replay the appropriate part of a lecture and request practice problems. Wrong answers are corrected immediately, and students receive feedback on areas they have mastered as well as those in which they need improvement.

With access to a pool of exercises and problems, cyberteachers can work one-on-one with students or give online lectures to an entire class in real time. Students can also interact among themselves. There are even materials to help students prepare for midterm and final examinations, and they include room for the students' own notes. In some areas, CHLS also offers contemporary touches such as avatars and "cybermoney."

National and local governments invested at least $11 million to develop CHLS contents, and the system went live in April 2005. Although a national initiative, CHLS permits the country's 16 metropolitan and provincial offices of education to modify content to suit local priorities and recruit cyberteachers and regulate cyberclasses. Some local education offices have assigned those with special needs—such as disabled or gifted students—to limited-enrollment cyberclasses with specially trained teachers. Some teachers use CHLS to tailor homework for their regular students, whereas others have gathered cyberstudents from throughout their district. For example, Kim created a cyberclass with a customized home page and problem sets selected for her students, adding her own explanations for some concepts.



Pleasing pages. 80% of users reported benefits from using Korea's Cyber Home Learning System, which allows teachers to customize materials for their online classes.

Asked why she volunteered, Kim flashes her glacier-melting smile and jokes that "there are predictions schools will disappear, and I felt my job was at risk." In fact, she felt CHLS gave her the chance to stay at the forefront of trends in education. Watching a boy who was last in his class become one of her best students after a semester with CHLS erased her initial skepticism. "It's really effective because students can always log on and access material," she says.

Hak Song Lee, Kwangdong Middle School principal, says CHLS also gives shy students an opportunity to get help without embarrassing themselves. "Korean students hesitate to show [their classmates] they don't understand something. With the CHLS, they feel free to ask the teacher questions," he says.

Help for teachers

By 2008, in its 4th year of operation, about 65% of the country's nearly 5 million students in grades 4 through 10 were enrolled in CHLS, although officials acknowledge that some students enroll but don't really rely on the system. It's serving half of those from small rural schools and low-income families—priority targets for Korean officials.

Despite international praise and high marks from users, Korean education officials consider CHLS a work in progress. "We are trying to improve its effectiveness," says Hyun-Jin Cha, a researcher at KERIS. Officials are tweaking content and trying to attract more elite students from affluent urban families. "What we hear from many students," she says, "is that they have no time to work with the CHLS between their regular classes and their private tutoring."

A national student survey by KERIS found that more than 80% of respondents reported better grades, increased confidence in a subject, or other benefits (see illustration). It also seems to be having the desired effect on parents' pocketbooks: Nearly one in six CHLS users said they had dropped or were considering dropping private tutors, an estimated savings of $1.8 billion.

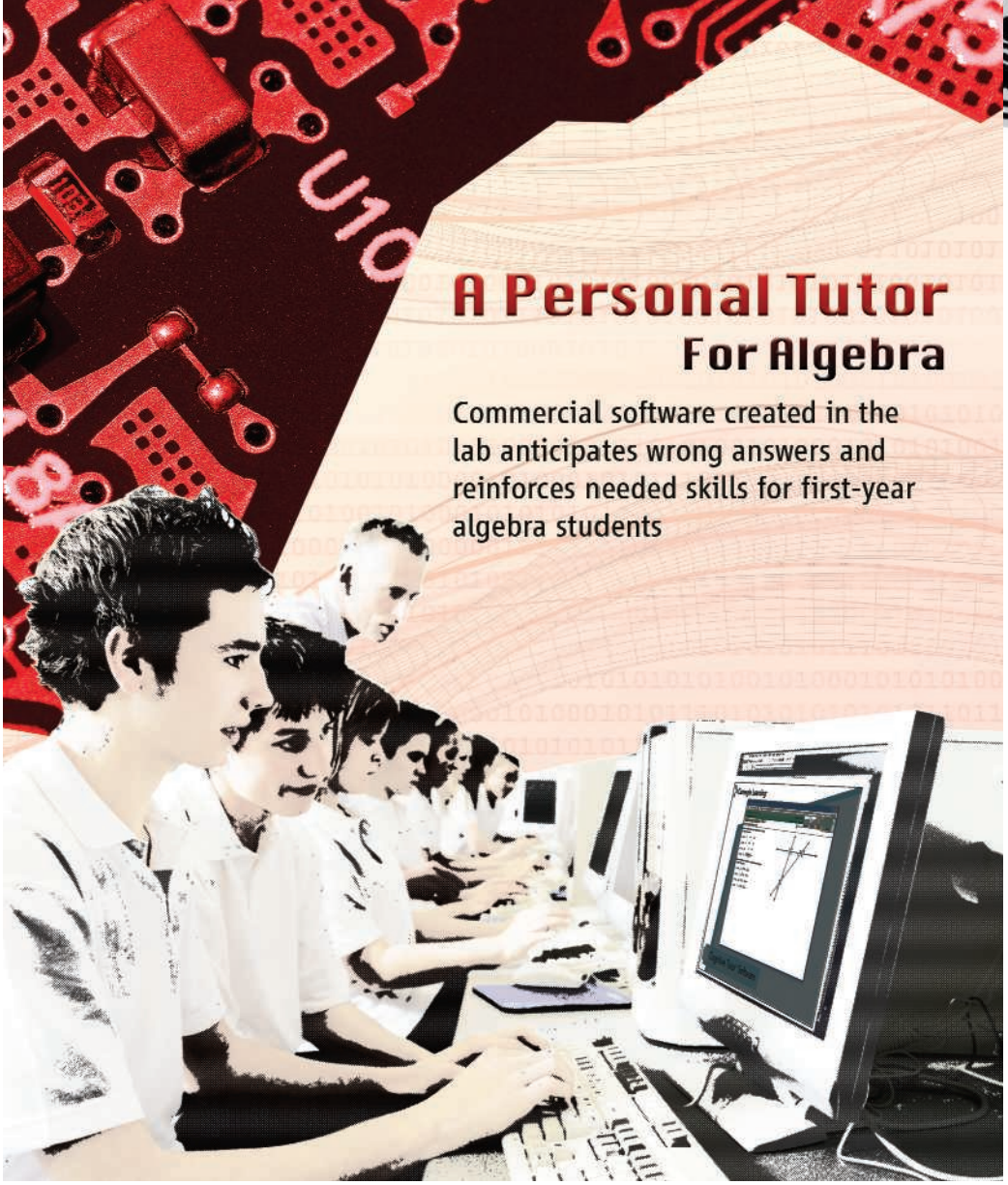
The biggest issue, according to some observers, is attracting and retaining the interest of talented teachers. Cyberteachers get at most nominal additional compensation, and a study by Hakjin Bae and colleagues at the Korea National University of Education in Chungbuk found that they received no systematic training on the system. And the teacher is key, says Kim. CHLS "is just a tool; how successful it is depends on the teacher," she says.

KERIS is looking at ways to keep teachers motivated and provides guidelines on training them. But those policies ultimately rest in the hands of the local educational offices. If better training and compensation for cyberteachers can be figured into the equation, more Korean students are likely to be turning to CHLS when school lets out rather than making a beeline to private tutors. **–DENNIS NORMILE**

CREDITS (TOP TO BOTTOM): (SCREEN GRAB) KERIS; (CHART) KOREA EDUCATION & RESEARCH INFORMATION SERVICE (KERIS)

A Personal Tutor For Algebra

Commercial software created in the lab anticipates wrong answers and reinforces needed skills for first-year algebra students



IN 1983, PSYCHOLOGIST JOHN Anderson wrote a computer program to test his theory of how people solve math problems. The program was designed to compare the steps subjects take in solving a problem with those predicted by a theoretical model. It also gave students—typically in high school—feedback to get back on track if they were having trouble. Anderson, a professor at Carnegie Mellon University (CMU) in Pittsburgh, Pennsylvania, expected the studies to uncover flaws in his theory. After being pleasantly surprised at "how well it seemed to work," Anderson had another revelation: "Here was something that could be used to improve student learning."

His program became the foundation for Cognitive Tutor (CT), a software product being used to teach algebra in more than 2600 middle and high schools across the United States. Developed at CMU and commercialized by a spinoff company called Carnegie Learning, CT has been shown to be effective in a small, randomized field study, the gold standard for evaluating curricular interventions.

Yet there's no guarantee that using CT will improve learning: In at least two other studies, the product led to no significant rise in student test scores. And even though it has been around for 15 years, only now is the software going through a large-scale trial. Experts say the cloudy picture shows how difficult it is to rigorously evaluate educational technologies.

Nonetheless, researchers say CT has benefited from two factors rarely found together in instructional products—a product with deep roots in academia that the school decided to market. Most commercial tutors are developed by textbook publishers, with little or no research behind them, says Alan Lesgold, an education professor at the University of Pittsburgh. And interventions developed at university departments rarely get the financial backing to advance beyond a small scale, he says.

CT's creators say that ongoing improvements to the software would not have been possible without the steady revenue from sales or its enduring relationship to the university. CT "embeds the principles of good, cognitively based instruction into a well-designed software environment," says Mitchell Nathan, an education professor at the University of Wisconsin, Madison, who has no connection to CT. "The tutor has continually grown."

Here's a hint

What distinguishes CT from the majority of tutors on the market is the precision with which it is able to assess a student's skills and provide instruction tailored to that assessment at different steps in the problem-solving process. Many tutors assume that a student has learned a concept if he or she gets the right answer, says Lesgold. Conversely, he says, CT attempts to "figure out what's going on inside the kid's head."

CT presents students with a series of progressively more difficult problems. It provides hints tailored to the particular difficulty that an individual student may be facing. These hints are the tutor's fundamental instructional tool, says CMU cognitive psychologist Kenneth Koedinger, one of the creators of CT and a co-founder of Carnegie Learning.

Take the equation $3(x-2)=21$. If a student makes an error trying to simplify the left side of the expression and writes "3x minus 2," the tutor might provide a hint saying "you forgot to multiply correctly." The system would simultaneously note on a "skillometer" that the student is weak at "distribution," that is, applying the multiplication function to all the quantities within the brackets. If the student repeats the error, the system might provide a second, more obvious hint, such as "multiply 3 by 2." The system would provide a different set of hints for students pursuing another approach, say, dividing both sides by 3. If the student repeatedly requests a hint, the system offers many examples of the same type of problem to build mastery of the skill.

The software's ability to present relevant hints is based on a technique developed by Anderson and his colleagues in the 1980s called model tracing. The tutor essentially

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Top tutors. From left, Ken Koedinger, John Anderson, and Steve Ritter created Cognitive Tutor and have watched it grow.

CREDIT: TODD KELLY

maps the student's error to one of many possible violations of mathematical rules. The tutor's ability to track the student's expertise in acquiring the needed skills to solve a task is based on a related technique called knowledge tracing.

Anderson was working on these techniques when Koedinger joined his lab in 1986 as a graduate student. For his thesis, Koedinger developed software to teach geometry proofs and tried it out at a local high school. That's where Koedinger discovered the importance of professional development. "The teacher I was working most closely with got the best results," he says. He also decided to integrate the software with the rest of the curriculum. Armed with these insights and a grant from the U.S. National Science Foundation (NSF), Koedinger and fellow CMU researchers decided to create a complete algebra course, including a textbook and software. Students typically use CT twice a week and receive regular instruction the remaining 3 days.

After funding from NSF ended in 1993, the researchers kept CT alive with grants from local charities. "These organizations were very concerned about strengthening Pittsburgh's technology base," Anderson says. Campus efforts to develop educational technology usually don't receive that kind of support, says Lesgold. He says CT benefited from the desire of local foundations such as the Heinz Foundation to see "social return on their investment."

Eager to commercialize the software, CMU founded Carnegie Learning Inc. in 1998, combining the university's own capital with outside investments. Anderson and Koedinger were given a stake in the company, and Steve Ritter, another member of the founding team who earned his Ph.D. under Anderson in 1992, was hired as its chief scientist.

The university first considered licensing the software to textbook publishers, Ritter says, but without any requirement that they improve it. He says a second option, making it open source, would have helped fix bugs but would not have provided the necessary resources for further development.

Ritter says independent studies have shown that the software helps students learn algebra. In a 2001 study that met the U.S. Department of Education's standards of evidence, 224 middle school students in Moore, Oklahoma, scored higher on average on a standardized test after using CT than did 220 fellow students taught by the same teacher using a different textbook. Nearly one-third of the students who used CT in this study were rated proficient in an Educational Testing Service assessment compared with 17% taught using traditional materials.

Other studies have failed to show any significant rise in student achievement, however. The first part of a $10 million government study on the effectiveness of reading and mathematics software reported in April 2007 that CT was one of five algebra products that, in a collective evaluation, produced no improvement in student performance compared with a control group. Another randomized study in the Maui School District in Hawaii, which only looked at CT, came to the same conclusion.

Ritter says the different results illustrate the difficulties in evaluating the effectiveness of educational technology: The outcome depends heavily on the way they are tested and used. Both studies measured the impact of CT after only 1 year, which he says is not long enough for teachers to become familiar with it. "People think technology somehow minimizes the effects of the teacher, but if the teacher isn't trained and committed to making the curriculum work, then it can be hard to show results." A $6 million randomized field trial launched by the RAND Corp. in 2007 will follow students in 130 schools across six states for 5 years in an attempt to measure long-term impacts.

Instructional synergy

Steve Spence, a math teacher at Old Mill High School in Millersville, Maryland, likes that CT keeps track of an individual student's minute-by-minute performance. "I can go into a student's account and know how long they have been working on a section, how many times they clicked on the hint button, how many errors they made," says Spence. "I can then try to focus on whatever skills the student is missing."

CT has something to offer both stronger and weaker students. Tenth grader Aneisha Vester used CT last year to leapfrog ahead of most of her classmates and complete all 40 chapters in the tutor. (The rest of the class did less than half that number.) "It gave me something to do," says Vester, who adds that proceeding on her own from one chapter to the next gave her a great sense of accomplishment. At the same time, Old Mill math teacher Janet Liimatta says the flexibility of being able to login from anywhere, anytime provided another student with the extra time needed to acquire the necessary skills.

Henry Kepner, president of the National Council of Teachers of Mathematics in Reston, Virginia, says there's always a danger that instructional software will become less effective once the novelty wears off. "After a while, it becomes routine and they learn to play the system," he says. Spence agrees that teachers must intervene if a disengaged student tries to breeze through entire sections by repeatedly clicking the hint button. "The instructor and the tutor [must] work together," he says.

–YUDHIJIT BHATTACHARJEE

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PERSPECTIVE

Immersive Interfaces for Engagement and Learning

Chris Dede

Immersion is the subjective impression that one is participating in a comprehensive, realistic experience. Interactive media now enable various degrees of digital immersion. The more a virtual immersive experience is based on design strategies that combine actional, symbolic, and sensory factors, the greater the participant's suspension of disbelief that she or he is "inside" a digitally enhanced setting. Studies have shown that immersion in a digital environment can enhance education in at least three ways: by allowing multiple perspectives, situated learning, and transfer. Further studies are needed on the capabilities of immersive media for learning, on the instructional designs best suited to each type of immersive medium, and on the learning strengths and preferences these media develop in users.

As another article in this special issue discusses, the information technologies used by children during their formative years influence their learning strengths and preferences (*1*). An increasingly prevalent type of media, immersive interfaces, can aid in designing educational experiences that build on students' digital fluency to promote engagement, learning, and transfer from classroom to real-world settings.

Immersive Presence

Immersion is the subjective impression that one is participating in a comprehensive, realistic experience (*2*, *3*). Immersion in a digital experience involves the willing suspension of disbelief, and the design of immersive learning experiences that induce this disbelief draws on sensory, actional, and symbolic factors (*4*). Sensory immersion replicates digitally the experience of location inside a three-dimensional space; total sensory interfaces utilize either head-mounted displays or immersive virtual reality rooms, stereoscopic sound, and—through haptic technologies that apply forces, vibrations, and motions to the user—the ability to touch virtual objects. As described below, interactive media now enable various degrees of sensory immersion.

Actional immersion involves empowering the participant in an experience to initiate actions impossible in the real world that have novel, intriguing consequences. For example, when a person playing an Internet game can make new discoveries by becoming a bird and flying around, the degree of concentration this activity creates is intense.

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Inducing a participant's symbolic immersion involves triggering powerful semantic, psychological associations by means of the content of an experience. As an illustration, digitally fighting a terrifying, horrible virtual monster can build a mounting sense of fear, even though one's physical context is unchanging and rationally safe. Invoking digital versions of archetypical situations from one's culture deepens the immersive experience by drawing on the participant's beliefs, emotions, and values about the real world. The more a virtual immersive experience is based on design strategies that combine actional, symbolic, and sensory factors, the greater the participant's suspension of disbelief that she or he is "inside" a digitally enhanced setting.

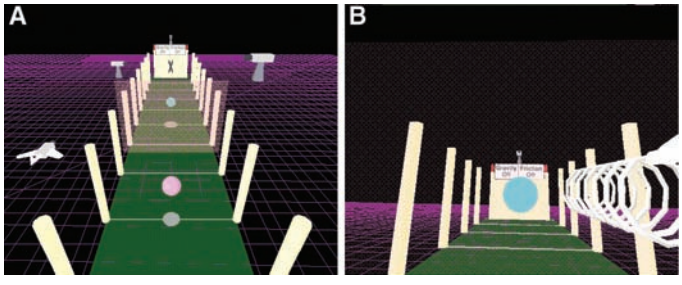

Fig. 1. (**A**) Exocentric view of NewtonWorld. (**B**) Egocentric view inside a ball.

Immersion Enhances Learning Through Multiple Perspectives

According to studies, immersion in a digital environment can enhance education in at least three ways: by enabling multiple perspectives, situated learning, and transfer. First, the ability to change one's perspective or frame of reference is a powerful means of understanding a complex phenomenon. Typically, this is done by shifting between an exocentric and an egocentric frame of reference. The exocentric frame of reference (Fig. 1A) provides a view of an object, space, or phenomenon from the outside; the egocentric frame of reference (Fig. 1B) provides a view from within the object, space, or phenomenon. With funding from the National Science Foundation (NSF), in the 1990s, our Project ScienceSpace research team conducted studies on sensory immersion in frames of reference and found that the exocentric and the egocentric perspectives have different strengths for learning (*5*) (SOM Text 1).

A major advantage of egocentric perspectives is that they enable participants' actional immersion and motivation through embodied, concrete learning, whereas exocentric perspectives foster more abstract, symbolic insights gained from distancing oneself from the context (seeing the forest rather than the trees). Bicentric experiences that alternate these views combine these strengths.

Immersion Enhances Learning Through Situated Experience

Immersive interfaces can foster educational experiences that draw on a powerful pedagogy: situated learning. Situated learning requires authentic contexts, activities, and assessment coupled with guidance from expert modeling, mentoring, and "legitimate peripheral participation" (*6*, *7*). As an example of legitimate peripheral participation, physical science graduate students work within the laboratories of expert researchers, who model the practices of scholarship in field work and laboratory work. These students tacitly learn through watching experts in research, as well as by interacting with other team members who understand sophisticated scholarship to varying degrees. While in these settings, students gradually move from novice researchers to more advanced roles, with other team members' expectations for them evolving as their skills develop.

Potentially quite powerful, situated learning is seldom used in classroom instruction because arranging complementary, tacit, relatively unstructured learning in complex real-world settings is difficult. However, immersive interfaces can draw on the power of situated learning by enabling digital simulations of authentic problem-solving communities in which learners interact with other virtual entities (both participants and computer-based agents) who have varied levels of skills.

As discussed in another article in this special issue, scholars are studying the extent to which Internet games and virtual environments such as Second Life provide situated learning that leads to knowledge useful in the real world; their findings thus far are promising (*8*, *9*). The research my colleagues and I are conducting on

gamelike virtual simulations for educating young people about higher-order inquiry skills illustrates how immersion can aid engagement and educational achievement through situated learning.

The NSF-funded River City multiuser virtual environment is centered on skills of hypothesis formation and experimental design, as well as on content related to national standards and assessments in biology and epidemiology (*10*). Students learn to behave as scientists as they collaboratively identify problems through observation and inference, form and test hypotheses, and deduce evidence-based conclusions about underlying causes. Learners immerse themselves inside a simulated, historically accurate 19th-century city (Fig. 2A). Collaborating in teams of three or four participants, they try to figure out why people are getting sick and what actions can remove sources of illness. They talk to various residents in this simulated setting, such as children and adults who have fallen ill, hospital employees, merchants, and university scientists (Fig. 2B and SOM Text 2).

Our research results from River City show that a broader range of students gain substantial knowledge and skills in scientific inquiry through immersive simulation than through conventional instruction or equivalent learning experiences delivered via a board game. Our findings indicate that students are deeply engaged by this curriculum through actional and symbolic immersion and are developing sophisticated problem-finding skills (in a complex setting with many phenomena, problems must be identified and formulated before they can be solved). Compared with a similar, paper-based curriculum that included laboratory experiences, students overall (regardless of factors such as gender, ethnicity, or English language proficiency) were more engaged in the immersive interface and learned as much or more (*11*, *12*).

Many academically low-performing students do as well as their high-performing peers in River City, especially on performance-based measures (such as a letter to River City's mayor describing an intervention to help reduce illness and providing evidence to support this claim). Digital immersion allows these students to build confidence in their academic abilities by stepping out of their real-world identity of poor performer academically, which shifts their frame of self-reference to successful scientist in the virtual context. This suggests that immersive media may have the potential to release trapped intelligence and engagement in many learners, if we can understand how best to design instruction using this type of immersive, simulated experience.

Other researchers who study educational multiuser virtual environments designed for young people, such as Quest Atlantis or Whyville (*13*, *14*), also are finding that immersive digital settings enhance their participants' engagement and learning. Research indicates that active learning based on immersive situated experiences that include frequent opportunities for reflection via combining egocentric and exocentric perspectives (e.g., participant inside River City versus external observer of the town's overall dynamics) is both motivating and powerful for a broad spectrum of students. The success of immersive simulations in corporate and military training (*15*, *16*) suggests that these positive findings also apply to learners considerably older than those we study.

Immersion may enhance transfer through simulation of the real world. Situated learning through immersive interfaces is important in part because of the crucial issue of transfer. Transfer is defined as the application of knowledge learned in one situation to another situation and is demonstrated if instruction on a learning task leads to improved performance on a transfer task, ideally a skilled performance in a real-world setting (*17*).

Researchers differentiate between two ways of measuring transfer: sequestered problem-solving and preparations for future learning (*18*). Sequestered problem-solving tends to focus on direct applications that do not provide an opportunity for students to utilize resources in their environment (as they would in the real world); standardized tests are an example of this. Giving students presentational instruction that demonstrates solving standard problems, then testing their ability to solve similar problems involves near-transfer: applying the knowledge learned in a situation to a similar context with somewhat different surface features.

When evaluation is based on the success of learning as a preparation for future learning, researchers measure transfer by focusing on extended performances where students "learn how to learn" in a rich environment and then solve related problems in real-world contexts. With conventional instruction and problem-solving, attaining preparation for future learning requires far-transfer: applying knowledge learned in a situation to a quite different context whose underlying semantics are associated, but distinct.

One of the major criticisms of instruction today is the low rate of far-transfer generated by presentational instruction. Even students who excel in educational settings often are unable to apply what they have learned to similar real-world contexts. The potential advantage of immersive interfaces for situated learning is that their simulation of real-world problems and contexts means that students must attain only near-transfer to achieve preparation for future learning. Flight and surgical simulators demonstrate near-transfer of psychomotor skills from digital simulations to real-world settings; a variety of studies are currently under way to assess whether other types of immersive learning show transfer to the real world to some degree.

Lesser Degrees of Immersion Can Still Provide Situated Learning

Our research team is currently studying augmented reality, in which users are immersed in a mixture of real and virtual settings. Participants in these immersive simulations use location-aware handheld computers [generally with global positioning system (GPS) technology], which allow

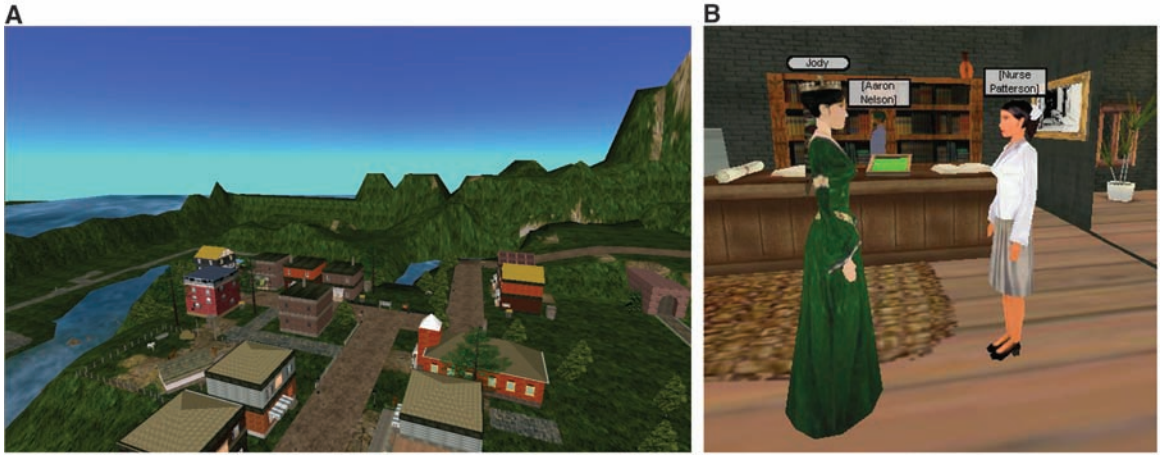

Fig. 2. (**A**) River City. (**B**) Avatar talking to agent.

users to physically move throughout a real-world location while collecting place-dependent simulated field data, interviewing virtual characters, and collaboratively investigating simulated scenarios (*19*). Today, augmented reality relies on coupling a handheld computing device to a GPS receiver; however, in the near future, sophisticated cellular phones will provide a ubiquitous infrastructure for this type of immersive learning.

The Handheld Augmented Reality Project, funded by U.S. Department of Education, is part of a collaborative effort by Harvard University, the University of Wisconsin, and the Massachusetts Institute of Technology to study the efficacy of augmented-reality technology for instruction in math and language arts at the middle-school level. Alien Contact! is a curriculum my research team designed to teach math and literacy skills to middle- and high-school students. (*20*) This narrative-driven, inquiry-based augmented-reality simulation is played on a Dell Axim X51 handheld computer and uses GPS technology to correlate the students' real world location to their virtual location in the simulation's digital world (Fig. 3A).

As the students move around a physical location, such as their school playground or sports fields (Fig. 3B), a map on their handheld displays digital objects and virtual people who exist in a simulated world superimposed on real space (Fig. 4). When students come close to these digital artifacts, the augmented reality and GPS software triggers video-, audio-, and text files, which provide narrative, navigation and collaboration cues, as well as academic challenges to build math and literacy skills (SOM Text 3).

Early research on the Alien Contact! curriculum found high levels of student engagement, as well as educational outcomes in literacy and math equivalent to students playing a similar, engaging board game as a control condition (*21*). Further design-based research is needed to determine the extent to which more powerful learning outcomes emerge from augmented-reality experiences. Such studies will aid in determining what degree of digital immersion is necessary for achieving various types of engagement, learning, and transfer.

Next steps in research on immersive interfaces for learning. Due to the growing ubiquity of sophisticated mobile phones and multiplayer Internet games, people of all ages increasingly will have life-style choices involving engaging forms of immersion in both virtual and augmented realities. Understanding the strengths and limits of these immersive media for education is important, particularly because situated learning seems a promising method for learning sophisticated cognitive skills, such as using inquiry to find and solve problems in complicated situations.

Further studies are needed on the affordances immersive media offer for learning, on the instructional designs best suited to each type of immersive medium, and on the learning strengths and preferences use of these media develops in users. Illustrative research questions include:

- To what extent does good instructional design for immersive environments vary depending on the subject matter taught or on the characteristics of the learner? For what types of curricular material is full sensory immersion important?
- To what extent can the successes of one's virtual identity in immersive environments induce greater self-efficacy and educational progress in the real world?
- To attain transfer, what is the optimal blend of situated learning in real, augmented, and virtual settings?
- What insights about bicentric frames of reference can generalize from immersive environments to pedagogical strategies in face-to-face settings?

Results from studies of immersive environments for learning seem sufficiently promising that further investment in this type of research is indicated.

Fig. 3. (**A**) Dell Axim and GPS receiver. (**B**) Students exploring school grounds.

Fig. 4. Handheld display of digital resources on school grounds.

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Figs. S1 to S7

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PERSPECTIVE

Technology and Informal Education: What Is Taught, What Is Learned

Patricia M. Greenfield

The informal learning environments of television, video games, and the Internet are producing learners with a new profile of cognitive skills. This profile features widespread and sophisticated development of visual-spatial skills, such as iconic representation and spatial visualization. A pressing social problem is the prevalence of violent video games, leading to desensitization, aggressive behavior, and gender inequity in opportunities to develop visual-spatial skills. Formal education must adapt to these changes, taking advantage of new strengths in visual-spatial intelligence and compensating for new weaknesses in higher-order cognitive processes: abstract vocabulary, mindfulness, reflection, inductive problem solving, critical thinking, and imagination. These develop through the use of an older technology, reading, which, along with audio media such as radio, also stimulates imagination. Informal education therefore requires a balanced media diet using each technology's specific strengths in order to develop a complete profile of cognitive skills.

Informal education—what goes on outside of the classroom—shapes our thought processes as they develop from early childhood. Media technologies are an extremely important part of informal learning environments. Media are also part of formal learning environments, the subject of other papers in this special issue on educational technology. The technologies composing the informal learning environment are generally intended for entertainment rather than education. However, they are important sources of cognitive socialization, often laying the foundation for knowledge acquisition in school.

In the midst of much press about the decreasing use of the print medium and failing schools, a countervailing trend may come as a surprise: the continuing global rise in IQ performance over more than 100 years. This rise, known as the Flynn effect, is concentrated in nonverbal IQ performance (mainly tested through visual tests) but has also occurred, albeit to a lesser extent, in verbal IQ (*1*–*5*). Rising IQ performance is attributable to multiple factors: increased levels of formal education, urbanization, societal complexity, improved nutrition, smaller family size, and technological development (*5*–*7*). These are interrelated rather than independent factors; they are part and parcel of the worldwide movement from smaller-scale, low-tech communities with subsistence economies toward larger-scale, high-tech societies with commercial economies (*8*). Which specific factor is most important in raising IQ performance at a given time and place depends on the locus of social change occurring then and there (*6*, *8*). Increasing levels of formal education and urbanization were particularly important in the United States and Europe in the first half of the 20th century (*9*, *10*). More recently, technological change may have taken the dominant role.

The changing balance of media technologies has led to losses as well as gains. For example, as verbal IQ has risen, verbal SATs have fallen. Paradoxically, omnipresent television may be responsible for the spread of the basic vocabulary (*11*) that drives verbal IQ scores, while simultaneously the decline in recreational reading may have led to the loss of the more abstract vocabulary driving verbal SAT scores (*6*, *12*, *13*).

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Evidence for the Flynn Effect

Among several kinds of test data from 20 industrialized countries, Flynn compared records of British people tested in 1942 and 1992 on Raven Progressive Matrices (Fig. 1 shows a sample item). Between 1942 and 1992, average performance increased for all age groups (Fig. 2) (*4*). Note that the oldest members of the first cohort tested grew up in the last two decades of the 19th century, extending the baseline back that far.

The new organization of Flynn's data in Fig. 2 reveals another important point: Not only is performance on the matrices better in the later cohort but cognitive aging is also reduced—witness an almost flat slope of performance across the age groups tested in 1992. This slope contrasts with the age-related decline seen in the groups tested in 1942.

Male military recruits supplied most of Flynn's data, skewing samples toward a relatively low socioeconomic population and excluding women. A University of California, Los Angeles, team (*5*) later demonstrated the Flynn effect in rural

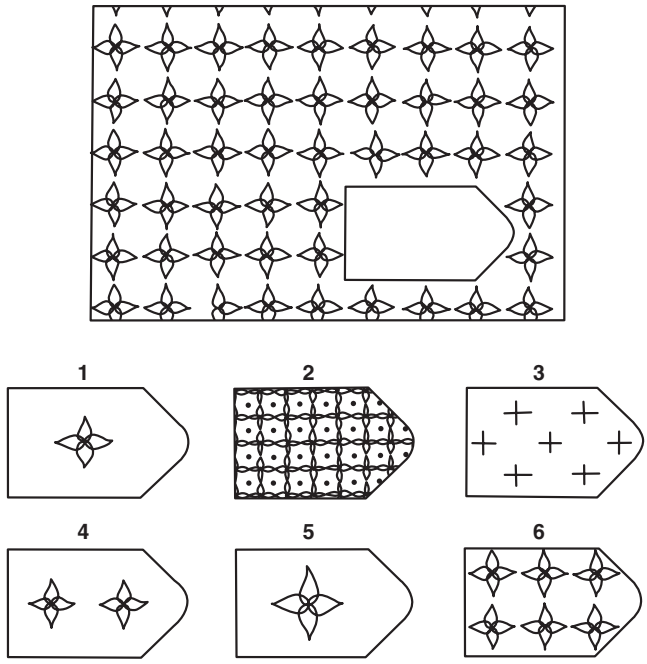

Fig. 1. A simple item from Raven Standard Progressive Matrices. From the six inserts at the bottom of the figure, the participant selects the one that logically fits in the matrix above. [Figure A5 of the Raven Standard Progressive Matrices, by J. C. Raven. Copyright 1938, 1976 by J. C. Raven Ltd. Reprinted with permission]

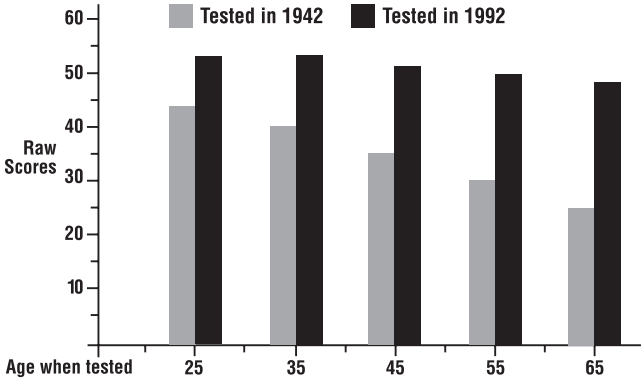

Fig. 2. Comparing performance on Raven Progressive Matrices in British people of different ages tested in 1942 and 1992 (*4*). Each bar represents 50th-percentile performance for a particular age group tested in a particular year. It is necessary to equate for age because of the influence of cognitive aging, seen in the decline of raw scores for pairs of bars with increasing age. However, decline was less pronounced in 1992 than in 1942.

Kenya, testing younger people, both boys and girls.

Technology and Visual Intelligence

Raven Progressive Matrices, like most so-called nonverbal IQ tests, provide a measure of visual intelligence, a concept that includes but is broader than Gardner's "spatial intelligence" (*14*). From the second half of the 20th century to the present time in the United States, highly popular forms of technology, such as television (currently with 99% penetration) (*15*) and, more recently, video games (currently with 97% penetration) (*16*), have taken a major role in developing visual intelligence on a mass scale, producing learners with capabilities that match the visual demands of modern science and technology.

The understanding of pictures or icons develops at an earlier age than the ability to read words (*17*). Building on this ontogenetic primacy, television, film, and video games augment basic visual literacy skills such as iconic representation (*18*), spatial orientation (*19*, *20*), spatial visualization (*20*–*26*), and other visual skills (*27*) that are important in the virtual world of computers (*28*, *29*). This cognitive socialization produces learners who are particularly well suited to take advantage of media-rich environments for formal education (*30*, *31*) and possess the visual literacy skills used in many modern professions.

Iterative and reciprocal processes are undoubtedly involved: Designers raised on visual media themselves create ever-more-sophisticated visual environments, in turn augmenting the visual skills of the next generation of young consumers. Take divided attention: keeping track of multiple events at different locations on a screen. Correlational and experimental data collected a decade apart show that divided attention is enhanced by playing action video games (*32*, *33*). However, the game *Medal of Honor*, used as an experimental treatment in the more recent study, was much more visually sophisticated and had broader effects on visual attention than did *Robotron*, used as an experimental treatment a decade earlier [see also (*34*–*36*)].

Technology and Multitasking: Benefits and Costs

Divided attention is the precursor and prerequisite for multitasking, defined as carrying out more than one task simultaneously. So do video games promote skill in multitasking? Research provides an affirmative answer. Kearney measured multitasking with *SynWork*, which simulates elements of work-based activities and measures composite performance on four tasks carried out simultaneously. Playing 2 hours of a shooting game called *Counter-Strike* improved multitasking scores significantly over those of a no-play control group (*37*). What we do not know from this study is whether each of the four tasks could have been performed better or processed more deeply if done alone, rather than in a multitasking environment. This is an important question, given the all-pervasiveness of multitasking in today's technological environment, especially for youth (*38*, *39*).

An experimental study by Foerde and colleagues answers this question (*40*). They developed a weather prediction task in which one condition used a distractor task (multitasking condition), whereas the other did not (single-task condition). In both conditions, participants learned to use cues equally well to predict the weather; however, they often were unaware of what cues they had used when they were in the dual-task distractor condition. Under multitasking conditions, cognitive processing was less mindful and more automatic.

Another study of the cognitive effects of multitasking used CNN Headline News to simulate a socially realistic and important cognitive task; understanding the news. While news anchors present their stories as talking heads on Headline News, weather forecast icons, sports scores, stock quotes, and textually delivered news crawls all appear at the bottom of the screen. To process these simultaneous stimuli requires multitasking. Such formats are very popular with younger viewers (ages 18 to 34), whereas older viewers (over 55) dislike them most (*41*, *42*). Nonetheless, the distracting information exacts a cognitive cost, even from the younger generation who have had more experience with multitasking. A controlled experiment showed that college students recalled significantly fewer facts from four main news stories in CNN's visually complex environment than from the same stories presented in a visually simple format, with the news anchor alone on the screen and the news crawls etc. edited out (*41*).

Implications for Education and Training

Internet multitasking also has costs for classroom learning. What is the effect on learning if college students use their laptops to access the Internet during a classroom lecture? This was tested in a communication studies class where students were generally encouraged to use their laptops during lectures, in order to explore lecture topics in greater detail on the Internet and in library databases (*43*). Half of the students were allowed to keep their laptops open, while the other half (randomly assigned) had to close their laptops. Students in the closed laptop condition recalled significantly more material in a surprise quiz after class than did students in the open laptop condition. Although these results may be obvious, many universities appear to be unaware of the learning decrement produced by multitasking when they wire classrooms with the intention of improving learning.

Laparoscopic surgery provides an example in which visual skills developed by video games have implications for training. Surgeons recognize that laparoscopy has changed the required skill profile of surgeons and their training needs (*44*). In laparoscopic surgery, a small incision is made, and a viewing tube with a small camera on the eyepiece is inserted through it. The surgeon examines internal organs on a video monitor connected to the tube and can also use the viewing tube to guide actual surgical procedures. Navigating through and operating in a three-dimensional space represented on a two-dimensional screen with minimal tactile feedback constitute basic parallels between laparoscopy and action video games. A study of the relation between video game skill and success in training for laparoscopic surgery yielded positive results (*44*): Action video game skill (as demonstrated in the laboratory) and past video game experience (assessed through self-report) predicted laparoscopic skills; in contrast, neither laparoscopic experience in the operating room nor years of training significantly predicted laparoscopic skill. The best game players (the top third) made 47% fewer errors and performed 39% faster in the laparoscopy tasks than the worst players (the bottom third). These results indicate the value of video game play as informal educational background for specific training in laparoscopic surgery, a finding that is applicable to other lines of work (such as piloting a plane) whose skill profiles overlap with those required by action video games.

Violent Games: Are the Costs Worth the Benefits?

Up to now, the discussion has ignored content and centered on the cognitive effects of video

game forms, forms that can be used to present any content. However, game content is crucial to psychosocial effects, such as the effects of violent screen activity. Indeed, more than 85% of games contain violence (*45*). Research shows that playing violent video games produces aggressive behavior, aggressive affect, aggressive cognition, physiological arousal, desensitization to real-life violence, and a decrease in prosocial behavior (*45*, *46*). The cost/benefit tradeoff of violent games is epitomized by the finding that Chinese children who play video games extensively not only have higher nonverbal IQs but also are more aggressive (*47*).

Fostering Scientific Thinking Through Informal Learning

Although visual literacy is a tool in scientific thinking and can lead to discoveries (such as Hack's discovery of a new jaw muscle when he altered the normal visual perspective used by dentists for dissection) (*6*), scientific thinking goes beyond the techniques provided by visual literacy, highlighting the importance of a number of other qualities: reflection, inductive analysis, critical thinking, mindful thought, and imagination. We start with reflection and inductive analysis.

By their very nature as a real-time medium, action video games penalize the player who stops to reflect. Indeed, no real-time medium—including film, television, and radio—permits time to reflect (*28*). The one communication technology that does provide time to reflect is the written word. Indeed, we have known for more than 40 years that there is an association between reading skill and reflection: Starting in first grade, better readers are also more reflective than less skilled readers (*48*). And reflection (contrasted with impulsivity) is associated with inductive problem-solving competence in children as young as first grade (*49*). Whereas reading is associated with reflection, television is associated with impulsivity. Over a 6-week period, an experimental reduction in television watching in a group of 6-year-olds decreased intellectual impulsivity, increased reflection, and increased time spent reading (*50*).

Reading is also key to the development of critical thinking. The amount of out-of-class reading done during the college years is a statistically significant predictor of critical thinking skills (*51*). One reason for this may be that books are perceived as a "hard" medium, requiring mental effort (*52*).

Imagination is important in scientific discovery as well as in the creation of literature and art. Here there is evidence that visual technology inhibits imaginative response. In controlled experimental studies, the audiovisual (television) presentation of stories, as compared with audio or print presentation of the same stories, led to better story recall and inferences (*53*, *54*). However, as compared with radio or print, the visual element in television also led to weaker imaginative responses, defined as the creation of original elements not found in the preceding stimuli (*53*–*55*).

Conclusions

Schools often rely on older media such as print and lectures to communicate with learners who increasingly lack the cognitive socialization—the informal education—that would enable them to process these media with maximum efficiency. Not only that, but schools rely almost entirely on the print medium to test that knowledge. Indeed, as science and technology have become increasingly visual in their intrinsic nature, there may be a mismatch between the structure of the knowledge and the structure of the print and oral language media traditionally used to both impart and test that knowledge.

However, the preceding makes it clear that no one medium can do everything. Every medium has its strengths and weaknesses; every medium develops some cognitive skills at the expense of others (*28*). Although the visual capabilities of television, video games, and the Internet may develop impressive visual intelligence, the cost seems to be deep processing: mindful knowledge acquisition, inductive analysis, critical thinking, imagination, and reflection. It is difficult for schools to teach reflective habits of mind to children whose informal education and cognitive socialization have not prepared them for this kind of learning and thinking. Yet society needs reflection, analysis, critical thinking, mindfulness, and imagination more than ever. The developing human mind still needs a balanced media diet (*28*), one that is not only virtual, but also allows ample time for the reading and auditory media experiences that lead to these important qualities of mind.

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PERSPECTIVE

Education and Training Technology in the Military

J. D. Fletcher

The United States Department of Defense (DOD) has contributed to the development of various education and training technologies, two of which are computer-assisted instruction and simulation-based instruction. DOD investment in computer-assisted instruction has continued from the 1950s to the present. Its contributions have ranged from drill and practice to computers capable of generating instructional interactions on demand and in real time. DOD investment in instructional simulation began with pilot trainers but evolved to include computer-controlled simulators serving a wide range of purposes, including simulators that are networked for collective education and the training of crews, teams, and units. Past and continuing contributions of the DOD in areas such as effectiveness, cost-effectiveness, instructional efficiency, and collaborative collective activity are worthy of attention.

Military organizations rely on education and training to prepare individuals and groups of individuals to perform extremely difficult tasks at high levels of proficiency under stressful conditions. Both education and training are needed: training to provide the knowledge and skills needed to perform military tasks and jobs, and education to help military personnel at all levels decide when and how to apply the knowledge and skills that they acquire through training (*1*).

Accordingly, the U.S. Department of Defense (DOD) provides training and education for the 2.1 million members of its active and reserve armed forces, 700,000 civilian employees, and 85,000 dependent children. This enterprise has been accompanied since the 1960s by an investment of $150 to 250 million each year on research and development in education, training, training devices, and training simulators.

For military organizations, both education and training are means to an end. Efficiency (time and resources expended) and effectiveness (production of human competence) are critical. Military organizations have historically turned to technology to maximize the efficiency and effectiveness of all their activities, training and education included. Two examples of instructional technologies whose development have been substantially stimulated by the DOD are computer-assisted instruction (CAI) and simulation-based instruction.

Computer-Assisted Instruction

The contributions of the DOD to the development of computers have been well noted. They range from the first vacuum tube–driven calculators to the Internet. Perhaps less well known are its contributions to the development and use of computers for instruction, which began in the 1950s (*2*–*4*) and have continued to the present (Fig. 1). Many DOD techniques and technologies developed in these areas are open and nonclassified and have found their way into both private and other public sectors. After reviewing contributions to CAI from all sectors, the Congressional Office of Technology Assessment concluded that "The military has been a major, and occasionally, the major player in advancing the state-of-the-art … without [military research and development] … it is unlikely that the electronic revolution in education would have progressed as far and as fast as it has" (*5*).

One early example was PLATO (Programmed Logic for Automated Teaching Operations), which was specifically designed for the development and presentation of instruction (*6*). PLATO was one of several projects at the University of Illinois Coordinated Science Laboratory that were supported by all three military departments in the 1950s. A prominent feature of the PLATO system was its plasma panel, which allowed digitized graphics (with some primitive animation) to be displayed along with text during its "teaching operations." Hardware aside, the major impact of PLATO was in encouraging individuals to develop and use CAI through its authoring language, TUTOR.

The other major CAI development efforts of the time took different approaches. The Institute for Mathematical Studies in the Social Sciences at Stanford University concentrated on CAI curriculum structure and strategies, and the MITRE Corporation working with the University of Texas (and later Brigham Young University), developed Time-Shared Interactive Computer-Controlled Television (TICCIT), an entire computer system designed to implement formal principles of instructional design. All three of these efforts received support from the DOD throughout the 1960s. Many techniques and capabilities that they developed found their way into K–16 education (*4*, *7*, *8*).

The effectiveness of CAI was recognized by the 1970s (*9*, *10*). However, costs posed major impediments to its widespread adoption. Researchers found the costs of computer technology itself and the costs of anticipating responses to all possible learner states and interactions to be problems.

Moore's Law, that which posits, roughly, a doubling of computer capabilities every 18 months, appears to be solving the first problem. The second problem led the DOD to support the development of intelligent CAI (ICAI) (*11*). This support was partially motivated by developments in artificial intelligence but especially by the promise of reducing production costs by enabling computers themselves to generate instructional interactions on demand and in a near-conversational manner. Development of the mixed-initiative instructional dialogue capabilities envisioned by Uttal (*12*) and Carbonell (*13*) was key to this approach. Work on ICAI—today generally called intelligent tutoring systems—has continued in both military and private sectors (*11*, *14*, *15*).

With time, a growing body of data permitted the application of meta-analytic techniques to assess CAI effectiveness. Among other findings from comparisons of CAI with standard classroom learning in military, academic, and industry sectors were reductions of 24 to 54% in the time taken to learn (*11*). Technology costs aside, a 30% reduction in the time needed to learn would save the DOD 15 to 25% of the $4 to 5 billion it spends annually for specialized skill training (from novice to journeyman).

Today, much CAI developed for the military emphasizes portability, which reduces costs by allowing digital learning objects (anything from entire courses to course modules to raw media, such as video and audio clips) to operate across a variety of computer systems. It enables instructional materials developed, for example, by the active forces to be reused without reprogramming by reserve and specialized training commands. It also allows both instruction and performance aiding to be distributed on demand, to locations from classrooms to the field, on computer platforms ranging from desktop to handheld devices.

Efforts to increase both the portability and reusability of learning objects and capabilities for on-demand instruction integrated with performance aiding have cumulated in the DOD Advanced Distributed Learning (ADL) initiative (*16*). ADL is intended to take advantage of the distribution capabilities of the global information infrastructure (today's World Wide Web). It has joined with industry and academia to develop the Sharable Content Object Reference Model (SCORM). This model has been adopted globally by academic, industrial, and military organizations to satisfy criteria for the portability, durability, and reusability of digital learning objects. In cooperation with the Corporation for National Research Initiatives, the ADL initiative also developed the Content Object Resolution, Discovery, Registry/Repository

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Architecture (CORDRA), which permits digital objects to be located globally while allowing their developers to retain control over access to them.

Simulation

The military has been developing simulation for many years as an instructional technique to represent the visual, auditory, haptic, and occasionally olfactory sensations of the operational world. CAI concentrates on teaching, whereas simulation concentrates on learning through interactions with authentic "real world" experiences. Military research on simulation effectiveness, techniques, and costs may supply much of the empirical assessment that educational researchers have suggested is absent from these experience-based "situated" approaches (*17*).

Simulation reduces costs while increasing safety, visibility of events, and reproducibility of actions. It is often included in CAI to train individuals in operating, maintaining, and deploying equipment. Other applications, such as training for complex tasks and collective operations, use simulation as the primary instructional approach to enhance application, analysis, and evaluation of facts, concepts, and procedures already acquired by individuals.

Simulation for complex tasks. Today's technology-infused military operations have spawned what Wulfeck and Wetzel-Smith (*18*, *19*) have called "incredibly complex tasks." Such tasks are cognitively demanding and increasingly common in today's military operations. CAI is very good at producing journeymen from novices, but these tasks require higher levels of mastery, involving analysis, evaluation, and creativity defined by common hierarchies of learning [for example, (*20*)]. Training for these tasks must compress years of experience into intense instructional interactions with a comprehensive range of realistic situations. Simulation is viewed as essential for preparing large numbers of individuals, many with limited success in traditional academic settings, to perform these tasks. Examples include learning how to apply sophisticated knowledge of oceanography in using advanced sonar to detect submarines (*18*, *19*), how to apply electronics in maintaining complex avionics equipment (*21*), and how to apply operational procedures and tactics in making collaborative decisions in confused time-pressured environments (*22*).

The best early example of an incredibly complex task may be the operation of aircraft. Military pilots must process numerous multimodal stimuli arriving from equally numerous sources, interpret and prioritize attention among them, pursue an integrated plan of action while performing difficult and interdependent manual control movements, navigate and direct the aircraft, adjust for the transport and launch of weapons, and avoid lethal attacks arriving anytime from anywhere in three-dimensional space (*23*). Preparing individuals to perform these tasks has, since the beginning of manned flight, relied on simulation (*24*).

Early versions used human instructors to control simulators and simulations. Increasingly, instructor-generated stimuli and responses were replaced with those produced by mechanical, electrical, and finally electronic devices (Fig. 2).

Fig. 1. U.S. Marine using a tactical training simulation embedded in CAI. [Image made by Amela Sadagic, MOVES Institute, Naval Postgraduate School (NPS)]

Most of these military simulation capabilities have been adopted elsewhere (*25*).

Simulation training for collectives. Today, military use of simulation has incorporated computer-networking techniques to link simulators and simulations together to enable members of collectives—crews, teams, and units—to train together. This approach remains important for training aircrews (*26*) but has expanded well beyond aviation applications.

Most operations-oriented training focuses on collectives. In their review of collectives in industry and business, Cannon-Bowers, Oser, and Flanagan reported a clear "consensus … that work groups are the cornerstone of modern American industry" (*27*). A review by the National Research Council (*28*) suggested that what the military is learning about training teams is widely applicable elsewhere. Until recently, however, the use of technology-based simulation for collective training was rarely found outside of military organizations.

Fig. 2. An Apache attack helicopter pilot engaged in a simulated mission using a Longbow Crew Trainer located in a combat zone. [U.S. Army photo/Sgt. Brandon Little. Image provided courtesy of the U.S. Department of Defense Office of Public Affairs through the Digital Video and Imagery Distribution System]

Fig. 3. U.S. Air Force A-10 pilots training with British ground troops 3700 miles away on the tactics, techniques, and procedures of close air support. [U.S. Air Force photo/Staff Sgt. Joe Laws. Image provided courtesy of the U.S. Department of Defense Office of Public Affairs through the Digital Video and Imagery Distribution System.]

Since the 1980s, the U.S. military has categorized collective simulation training into three categories: live, constructive, and virtual (*29*). These three approaches complement one another; each provides unique capabilities for developing human performance beyond the basic knowledge and skills provided by individual training.

Live-simulation participants employ real-world materiel on exercise ranges instrumented to record all relevant events. Technologies such as eye-safe laser transmitters and receivers provide opportunities for free play, tactical creativity, and participant motivation. Events are recorded in extensive detail for later analyses, which can be facilitated by experts but is primarily expected to come from the participants themselves (*30*).

Constructive simulation is more academic. It is best exemplified by computerized war games. Participants establish scenarios, parameters, and command decisions. They then use computers to play out missions and use the consequences of their decisions to support the development of tactics, techniques, and procedures.

Virtual simulation occupies the middle ground between live and constructive simulation (Fig. 3). It employs human-controlled simulators networked together to collaborate or otherwise engage each other on common electronically generated terrain (*31*, *32*). The simulators may be physically located anywhere because they are modular and share a common model of the situation and its virtual terrain; a tank crew in a tank simulator in Germany can receive air support from aircraft simulators in Nevada when they are being attacked on the electronic terrain by helicopter simulators located in Alabama. Virtual simulation provides more realistic experience and feedback than constructive simulation but with less cost and time than live simulation.

Civilian applications of virtual networked simulation for education may include globally dispersed groups engaging collaboratively in scientific experiments, problem solving, or decisionmaking using otherwise unaffordable equipment, visiting otherwise inaccessible locations for field research, or guided by otherwise unavailable experts.

Simulation fidelity. One perennial issue for military and civilian simulation-based instruction alike is the amount of realism, or "fidelity," that is needed. Optimal choices key on the careful explication of instructional objectives and their subsequent use in selecting levels of simulation fidelity (*33*). Development of techniques to trade off the costs of fidelity against instructional effectiveness is a particular concern and contribution of military research. Simulation effectiveness may be

judged by the transfer of what is learned in instruction to what is done in practical operations. Quantitative attempts to deal with this issue have used transfer effectiveness ratios to balance the cost of simulator time to the cost of using real equipment (*34*). "Isoperformance" curves have also been developed to help instruction designers identify points at which different combinations of training inputs produce equivalent performance output with minimal costs (*35*).

Conclusion

Military organizations have their own perspectives and emphases, but the techniques and technologies that they have developed in the following areas, among others, continue to be of interest and value beyond the military.

Training technology. After reviewing the issue of tailoring instruction to the needs of each learner, Scriven (*36*) concluded that it was both an educational imperative and an economic impossibility. Continued DOD interest in developing CAI arises from an expectation that computer technology will make this imperative affordable (*11*). The results from the 1960s on have been instructional technologies that adjust the pace, sequence, and difficulty of tasks so that learning is accelerated, allowing learners to focus on what they need to learn rather than what they already know.

Instructional efficiency. Military organizations, which assume responsibility for individuals from enlistment through retirement, have concentrated on the development of techniques and principles that increase instructional efficiency and assess the cost-effectiveness of alternate approaches.

Collective performance. Instructional technology for crews, teams, and units is a particular concern of military organizations. Techniques for developing shared mental models, conducting group assessments, encouraging collaboration, and measuring the competence, productivity, and readiness of collectives should be of value to all sectors.

Research and development. The military continues to invest substantially in research and development for instructional technology. Some of its instructional technology programs, particularly those in skill-training areas, have been transferred to specific civilian applications. However, its open nonproprietary development of techniques, technologies, and capabilities in nonclassified areas, particularly those of CAI and simulation, has influenced instructional practice in all sectors.

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PERSPECTIVE

Technology and Testing

Edys S. Quellmalz[1]* and James W. Pellegrino[2]

Large-scale testing of educational outcomes benefits already from technological applications that address logistics such as development, administration, and scoring of tests, as well as reporting of results. Innovative applications of technology also provide rich, authentic tasks that challenge the sorts of integrated knowledge, critical thinking, and problem solving seldom well addressed in paper-based tests. Such tasks can be used on both large-scale and classroom-based assessments. Balanced assessment systems can be developed that integrate curriculum-embedded, benchmark, and summative assessments across classroom, district, state, national, and international levels. We discuss here the potential of technology to launch a new era of integrated, learning-centered assessment systems.

A new generation of technology-enabled assessments offers the potential for transforming what, how, when, where, and why testing occurs. Powered by the ever-increasing capabilities of technology, these 21st-century approaches to assessment expand the potential for tests to both probe and promote a broad spectrum of human learning, including the types of knowledge and competence advocated in various recent policy reports on education and the

economy [e.g., (*1*, *2*)]. The use of assessment to support the attainment of such goals will require interdisciplinary partnerships and considerable additional research and development. It will also demand major shifts in educational policies regarding the use of assessment data for various purposes, including student, teacher, and system-level accountability. Here, we look at both the current state of technology use in testing and some of the emergent cases that have used technology to push the envelope with regard to educational assessment.

Technology Applications in Current Large-Scale Assessment Programs

In large-scale assessment programs such as those run by states or nations and by major testing companies, technology currently supports a myriad of assessment functions, including test development, delivery, adaptation, scoring, and reporting [e.g., (*3*, *4*)]. Authoring shells that guide the process of item writing and item banks aligned to content standards enable efficient development and assembly of items into comparable test forms. Online administration eliminates costs for shipping, tracking, and collecting print booklets while simultaneously introducing other logistical complexities related to equipment and security. Computer scoring provides rapid return of results and generation of reports tailored to multiple audiences. Flexible administration times and locales shift annual, on-demand testing to interim and just-in-time challenges.

Online testing now occurs in numerous international, national, and state assessment programs. The 2009 Programme for International Student Assessment (PISA) will include electronic texts to test reading, and in 2006 PISA conducted a pilot of computer-based assessment in science (*5*). The National Assessment of Educational Progress (NAEP) studied online versions of mathematics and writing tests in preparation for transitioning NAEP to electronic administrations in the near future (*6*). Currently, more than 27 states have operational or pilot versions of online tests for their statewide or end-of-course exams. This includes Oregon, which pioneered online statewide assessment, North Carolina, Utah, Idaho, Kansas, Wyoming, and Maryland. The landscape is changing rapidly, as is the growth of computer-administered tests. For example, the Educational Testing Service (ETS) estimates that more than 4 million people will take ETS-developed tests on computer in 2008. Those tests range from the Graduate Record Exam (GRE), to state and national teacher competency tests, to selected areas of the Advanced Placement Program. This is representative of what is happening industry-wide and on a much larger scale.

Computerized adaptive testing (CAT) procedures, in which items are selected based on the examinee's prior response history and an underlying measurement model of proficiency, have been developed to reduce testing time and examinee burden. However, their use in high-stakes testing contexts has been confined largely to admission, professional certification, and credentialing exams. Despite requests to use CAT in state testing programs, regulations of the No Child Left Behind (NCLB) legislation have prohibited their implementation for assessment of student academic achievement in reading, mathematics, and science.

A transformative advance in large-scale testing programs is the machine scoring of essays and constructed responses, including testing programs for the military, industry training, higher education admissions, and statewide kindergarten through grade 12 achievement testing. Computerized scoring of free responses uses complex statistical methods and techniques such as latent semantic analysis (LSA) (*7*). Test publisher Pearson is in its second year of using Knowledge Analysis Technologies, based on LSA techniques, to pilot the automated scoring of 46,000 brief constructed responses for the Maryland School Assessment science test. ETS has developed E-rater for scoring essays and C-rater for scoring constructed responses and has deployed them in a variety of high-stakes testing programs such as the GRE.

Klein (*8*) recently reviewed the literature on automated scoring methods and presented results from a study comparing hand and machine scoring of college-level, open-ended items of the type found on the Collegiate Learning Assessment. Findings across studies using a variety of machine scoring methods consistently show comparability of human and machine scoring (~0.85 score correspondence) at levels approximating the agreement between two human scorers (~0.86) and sufficient to warrant using computerized scoring alone, or as an augmentation to human scoring.

In summary, the current genre of online testing applications remains focused on (i) electronic delivery of conventional selected-response and constructed-response item formats, (ii) automation of existing processes, and (iii) comparability of scores and interpretation of results across computer-based and paper forms. A next generation of assessments, however, is attempting to move beyond this limited framing of assessment issues and overcome many of the limitations of conventional testing practices. A goal is to harness technology to enable assessment of those aspects of cognition and performance that are complex and dynamic and that were previously impossible to assess directly. Such work involves reconceptualizing assessment design and use and tying assessment more directly to the processes and contexts of learning and instruction.

Toward the Next Generation of Technology-Enabled Assessment

Across the disciplines, technologies have expanded the phenomena that can be investigated,

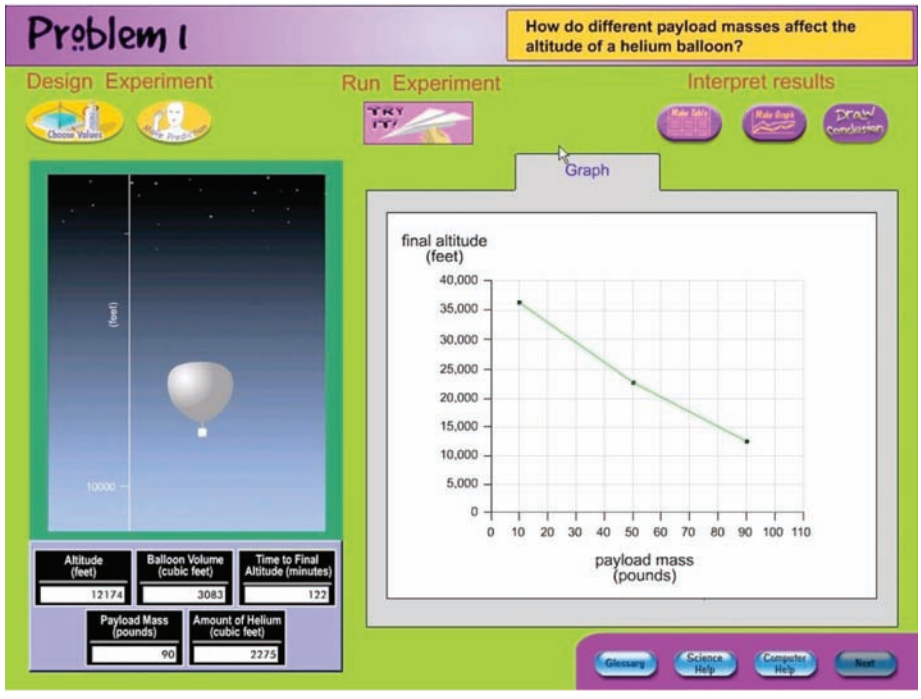

Fig. 1. The ETS simulation model (*10*).

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the nature of argumentation, and the use of evidence. They allow representation of domains, systems, models, and data, and their manipulation, in ways that previously were not possible. Dynamic models of ecosystems or molecular structures help scientists visualize and communicate complex interactions. Models of population density permit investigations of economic and social issues. This move from static to dynamic models has changed the nature of inquiry among professionals and the way that academic disciplines can be taught and tested. Moreover, the computer's ability to capture student inputs permits collecting evidence of processes such as problem-solving sequences and strategy use as reflected by information selected, numbers of attempts, and time allocation. Such data can be combined with statistical and measurement algorithms for the extraction of patterns associated with varying levels of expertise [e.g., (*9*)]. In addition, technology can be used to design new forms of adaptive testing that integrate diagnosis of errors with student and teacher feedback.

Large-scale assessment programs. Information and communications technologies such as web browsers, word processors, editing, drawing, and multimedia programs support research, design, composition, and communication processes. These same tools can expand the cognitive skills that can be assessed, including planning, drafting, composing, and revision. For example, the NAEP writing assessment in 2011 will require the use of word processing and editing tools to compose essays. In professional testing, architecture examinees use computer-assisted design programs as part of their licensure assessment. The challenge offered by such technology-based presentation and data-capture contexts now lies in the analysis of complex forms of data and their meaningful interpretation relative to models of the underlying components of competence and expertise.

Science assessment is perhaps leading the way in exploring the presentation and interpretation of complex, multifaceted problem types and assessment approaches. In 2006, PISA pilot-tested a Computer-Based Assessment of Science specifically to test knowledge and inquiry processes not assessed in the paper-based booklets. Their assessment included student exploration of the genetic breeding of plants. At the state level, Minnesota has an online science test with tasks engaging students in simulated laboratory experiments or investigations of phenomena such as weather or the solar system. ETS pioneered the design of technology-based assessments for complex learning and performance (*10*). An example of the type of item that Bennett *et al.* evaluated in (*10*) is shown in Fig. 1.

Students were presented with a scenario involving a helium balloon and asked to determine how different payload masses affect the altitude of the balloon. They could design an experiment, manipulate parameters, run their experiment, record their data, and graph the results. Figure 1 also shows the types of data that might be obtained by a student and plotted before reaching a conclusion and writing a final response. The 2009 NAEP Science Framework and specifications drew upon ETS work and other research in developing their rationale for the design and pilot testing of Interactive Computer Tasks to test students' ability to engage in inquiry practices. Such innovative items will be included in the upcoming 2009 NAEP science administration.

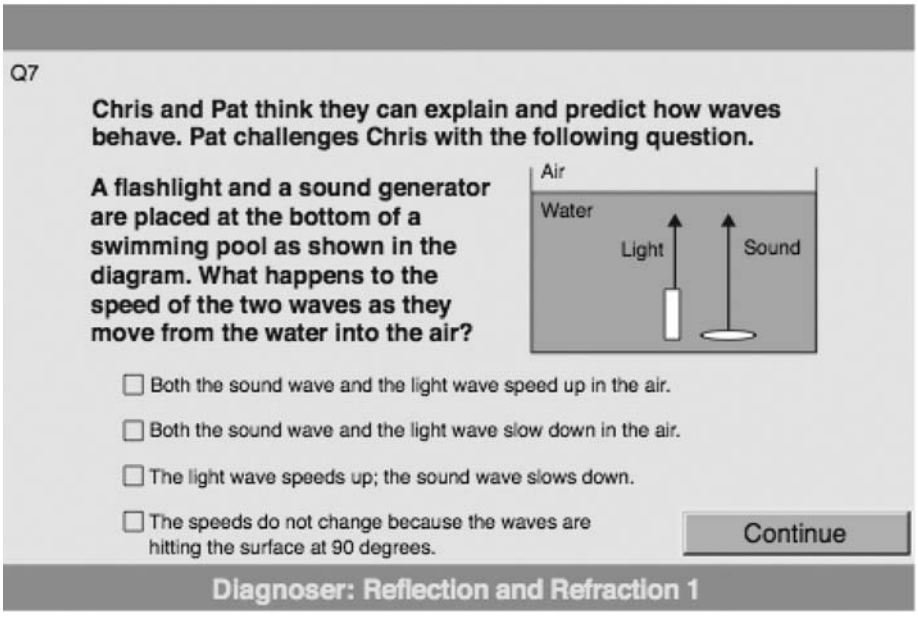

Fig. 2. The DIAGNOSER assessment (*15*).

Large-scale testing programs such as those mentioned above are just beginning to explore the possibilities of using dynamic, interactive tasks for obtaining evidence of student content knowledge and reasoning. However, in the realm of high-stakes assessment for NCLB accountability, a number of regulatory, economic, and logistical issues still constrain the breadth and depth of the content and performance standards that are assessed in annual on-demand tests. Standard, multiple-choice item formats continue to dominate large-scale computer-based high-stakes testing, resulting in an overreliance on simple, well-structured problems that tap fact retrieval and the use of algorithmic solution procedures.

Classroom instructional uses of assessments. A distinction has been made between assessments of the outcomes of learning, typically used for grading and accountability purposes (summative assessment), and assessments for learning, used to diagnose and modify the conditions of learning and instruction (formative assessment). The formative use of assessment has been repeatedly shown to significantly benefit student achievement (*11*, *12*). Such effects depend on several classroom practice factors, including alignment of assessments with state standards, quality of the feedback provided to students, involvement of students in self-reflection and action, and teachers actually making adjustments to their instruction based on the assessment results (*13*).

Technologies are well suited to supporting many of the data collection, complex analysis, and individualized feedback and scaffolding features needed for the formative use of assessment (*14*). Two illustrative projects, one drawn from science and the other from mathematics, rely on detailed analyses of subject domains and student thinking to provide in-depth assessment and feedback during instruction.

DIAGNOSER is an Internet-based tool that delivers continuous formative assessment and feedback to students and teachers (*15*, *16*). The online assessment identifies problematic "facets" of students' thinking, then provides counterexamples and additional lessons. An example of an item in the DIAGNOSER system is provided in Fig. 2.

Based on a student's response to this item, as well as others in a set related to this topic area, a diagnosis is done of the student's level of understanding, with feedback provided to both the student and the teacher. In a validation study in a Washington state district, students using DIAGNOSER outperformed their peers on items from the state science test.

ASSISTments is a comprehensive intelligent tutoring system to provide student instruction and support teachers in collection and analysis of their students' data for topics in mathematics (*17*). Like DIAGNOSER, the system has shown promising initial results and is now being extended to areas of science.

Technology can also support the design of complex, interactive tasks that extend the range of knowledge, skills, and cognitive processes that can be assessed (*18*). For example, simulations can superimpose multiple representations and permit manipulation of structures and patterns that otherwise might not be visible or even conceivable. Simulation-based assessments can probe basic foundational knowledge such as the functions of organisms in an ecosystem, but, more important, can probe students' knowledge of how components of a system interact as well as abilities to investigate the impacts of multiple variables changing at the same time (*19*). Moreover, because simulations use multiple modalities and representations, students with diverse learning styles and language backgrounds may have better opportunities to demonstrate their knowledge than are possible in text-laden print tests.

In an ongoing program of research and development, WestEd's SimScientists projects are studying the suitability of simulation-based science assessments as summative assessments with the technical quality required for components of an accountability system (*19*). New SimScientists projects are also studying the use of simulations for curriculum-embedded formative uses of assessment.

Figures 3 and 4 present screen shots of tasks in a SimScientists assessment designed to provide evidence of middle school students' understanding of ecosystems and inquiry practices. Students are presented with the overall problem of preparing a report to describe the ecology of a lake for an interpretive center. They investigate the roles and relationships of the fish and algae in the lake, answering conventional items and also, as shown in Fig. 3, constructing responses such as drawing a food web.

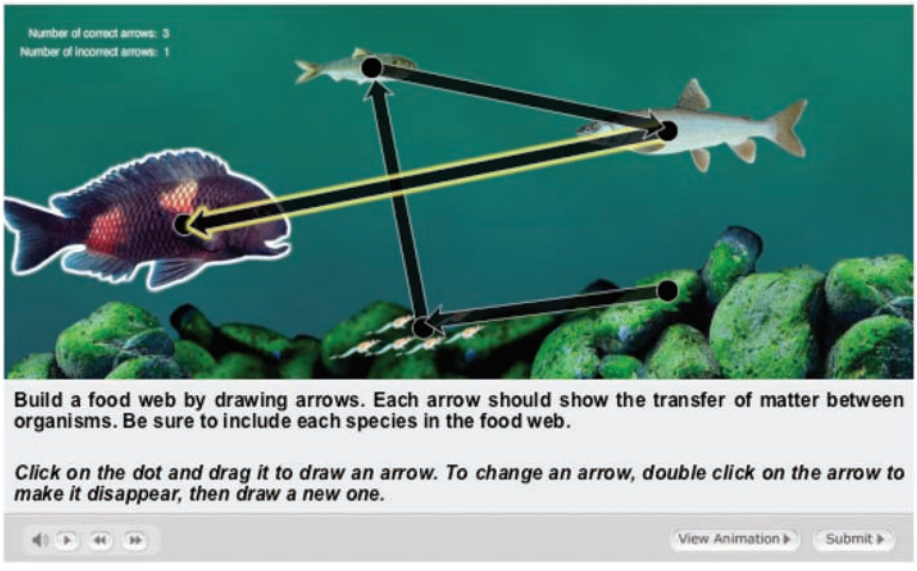

Fig. 3. SimScientists Food Web Construction (*19*).

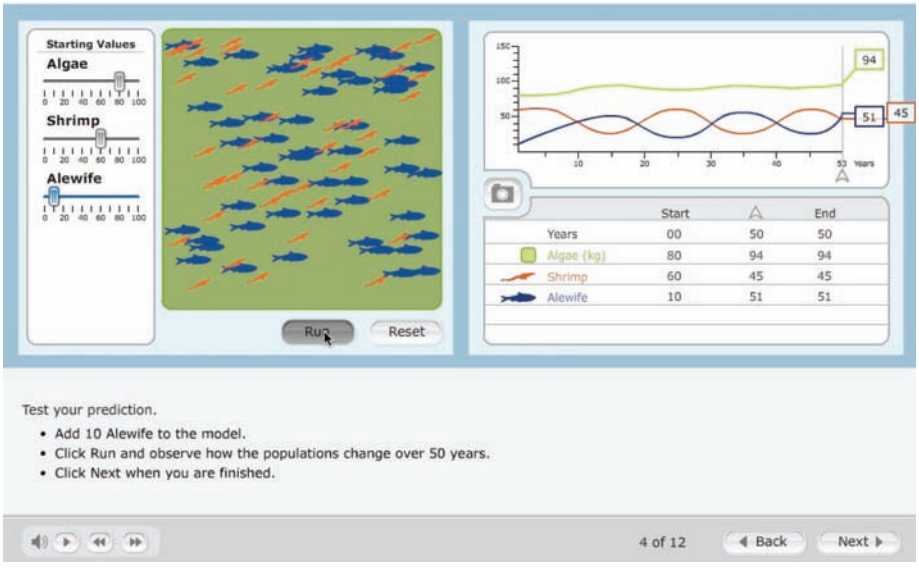

Fig. 4. SimScientists Lake Population Simulation (*19*).

To assess inquiry skills, Fig. 4 shows one of several tasks in which students conduct investigations by manipulating the numbers of fish or plants in a model of the lake and predicting and explaining outcomes. A graph and table provide multiple representations of the population levels. A graph inspector arrow allows students to reexamine the numbers of organisms at different points in time. A camera permits saving each run to a folder for later comparison and analysis.

In a culminating task, students write a report of their findings about the lake. No feedback is presented in the end-of-unit assessment. In a set of embedded assessments, the system identifies types of errors and follows up with increasing levels of feedback and coaching. In the assessment screen shown, after observing animations of the organisms interacting, students draw a food web to depict the flow of matter. An incorrect arrow the student has drawn is highlighted. Levels of feedback and coaching progress from identifying that an error has occurred and asking the student to try again, to explaining the concept (flow of matter), to demonstrating and explaining correct drawing of the arrows.

Research in the SimScientists projects is studying the technical quality of the assessments, the potential of the end-of-unit assessments as components of an accountability system, and the impact of the curriculum-embedded assessments and feedback on student learning. Project designs such as these can document the validity and utility of technology-based assessments for instructional and accountability purposes.

Multilevel State Assessment Systems

It is widely recognized that states must aim for balanced state assessment systems in which classroom, district, and state tests are aligned and mutually reinforcing. The National Research Council (NRC) report *Knowing What Students Know* argued that a balanced assessment system relies on a nested system of assessments that exhibit multiple features (*20*). One feature is the use of multiple measures covering the full range of standards. Another involves alignment of standards, assessments, curriculum, and instruction within and across different levels of the system (school, district, and state). A third important feature is going beyond annual, on-demand tests to multiple assessments distributed over time combined with timely reporting that offers teachers the opportunity to tailor instruction.

Because of concerns about the adequacy and coherence of state-level assessment systems, the National Science Foundation funded the NRC to offer recommendations to states on the design and

implementation of their science assessment systems (*21*). In a report commissioned by that project, Quellmalz and Moody (*22*) proposed strategies for states to form collaboratives and use technology to create multilevel science assessment systems. With the goal of helping schools and students meet the NCLB goals, states are seeing classroom-based, instructional uses of assessment as a powerful tool for driving student achievement. Such assessment is distinguished from interim assessments administered periodically on a larger scale that are intended to describe the status of student performance after instruction (*23*).

A key feature in creating a balanced multilevel system is the use of common design specifications that can operate across classroom, district, state, and national levels (*22*). To enable implementation, online authoring systems are being developed that can assist in creating such common specifications, in streamlining test design, and in reducing development costs (*24*). Online design systems can also support adaptations of assessments to offer accommodations for special populations while preserving the linkages between targeted standards and designs of the tasks for eliciting evidence of achievement.

Conclusion

Technology helps us do many conventional things in the world of testing and assessment better and faster, and it holds the key to transforming current assessment practice for multiple purposes and at multiple levels ranging from the classroom to state, national, and international levels. We are not there yet, and although many obstacles remain to their widespread use, the next generation of technology-enabled assessments is under development with several promising cases of design, implementation, and use. Such demonstrations provide a vision of the possible and can help move education toward the design and adoption of more integrated and effective learning-centered assessment tools and systems.

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PERSPECTIVE

Video Games: A Route to Large-Scale STEM Education?

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Video games have enormous mass appeal, reaching audiences in the hundreds of thousands to millions. They also embed many pedagogical practices known to be effective in other environments. This article reviews the sparse but encouraging data on learning outcomes for video games in science, technology, engineering, and math (STEM) disciplines, then reviews the infrastructural obstacles to wider adoption of this new medium.

In the 2000-to-2005 time frame, ~450,000 students graduated annually in the United States with a bachelor's degree in STEM (*1*). These numbers pale in comparison to the reach of a single computer video game (Figs. 1 and 2). *World of Warcraft* (*2*), a fantasy game, has over 10 million current subscribers, with ~2.5 million in North America (*3*). *Food Force* (*4*), the U.N.-produced game on the mechanics of food aid distribution, saw 1 million players in its first 6 weeks and 4 million players in its first year (*5*). Additionally, in the realm of K-to-12 science and math education, the virtual world *Whyville* (*6*), with its game-based activities, now sports 4 million subscribers (90% North American), with the dominant demographic being 8- to 14-year-old girls (*7*, *8*). Although traditional education institutions pride themselves on educating citizens, they do so at a relatively small scale compared with the media now available. Is it possible to greatly expand the reach of STEM education with the use of video games as the medium? And to what level of effectiveness?

At first, the idea of using video games to teach science and engineering seems laughable. However, sophisticated video game content already exists in topics ranging from immunology (*9*) (Fig. 3) to numerical methods (*10*, *11*). The examples in Table 1 suggest that video games can yield a 7 to 40% positive learning increase over a lecture program. What's more, there may be additional benefits to poor learners: One variant of the *River City* ecology game (*12*) diminished the learning gap between D and B students to the point where nearly all students were performing at the B-student level (*13*).

Learning outcomes are by no means uniformly positive. Results from review studies (*14*, *15*) make

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it clear that there are both well-designed games and poorly designed ones. Where learning benefits appear, they are attributed to effective pedagogical practices embedded in the game design (*14*–*17*). Of course, many of these same practices can also be applied to classroom, Web, or other forms of instruction with similar benefits, an approach known as game-informed learning (*18*).

Unlike lectures, games can be adapted to the pace of the user. Games also simultaneously present information in multiple visual and auditory modes, which capitalizes on different learning styles. J. P. Gee (*16*) identifies the former as the "just-in-time principle" and the latter as the "multimodal principle" in his book on video game–based learning (*16*), reviewed in (*19*). Games are also particularly adept at dosing information delivery. Complex tasks are presented first as a small core experience that is practiced multiple times before being progressively extended into a longer, more complex sequence. The superior efficiency of this approach (known as concurrent chaining) has been compared with whole-task learning in (*20*). Gee (*16*) describes this kind of task structuring through his "incremental principle," "concentrated sample principle," and "bottom-up basic-skills principle."

Games are also useful for reinforcing information acquisition. The rich environment of objects and activities within games gives information "situated meaning": the other contextual elements support the information being conveyed. Social surroundings can also reinforce content. Well-constructed social interactions around societal goals within the game will drive learner engagement and achievement, as has been studied in depth by S. Barab *et al.* in their *Quest Atlantis* project (*21*, *22*). Content is further reinforced through continuous, immediate feedback: Almost every keystroke yields a response from the game. In contrast, students in a typical classroom get to ask 0.11 questions per hour (*23*). And, finally, a steady stream of positive rewards accompanies a game's rapid feedback. Players accumulate points, levels, titles, or magic swords with some visible progress for even the tiniest successes. These rewards contribute to greater self-confidence/self-efficacy. Greater self-efficacy, in turn, translates to greater persistence and thus a higher level of accomplishment (*24*).

Learner control over navigation through tasks and activities is a surprisingly important feature of effective learning games. The metastudy by J. J. Vogel *et al.* (*15*) found learner control/autonomy to be one of the few easily identified predictors of enhanced learning outcomes, whereas the study by R. M. Ryan *et al.* (*25*) found that it was critical to enjoyment and motivation as well. Goals in games can often be reached by multiple routes [Gee's "multiple routes principle" (*16*)]. But, in these branching decision structures, the learner must navigate between choices based on a considered estimation of relative consequences. In other words, the learner must operate at the highest level in B. S. Bloom's *Taxonomy of Educational Objectives* (*26*), "Evaluation."

The active, participatory style of learning in games also departs from the traditionally passive lecture [Gee's "active, critical learning principle" (*16*)]. Game-based tasks often require the formation of hypotheses, experimentation, and discovering the consequences of actions taken; in other words, they are very similar to the inquiry-based learning lauded by science educators (*27*). Increasingly, game activities are multiplayer in design, meaning problems are set up to be solved in teams. Anywhere from a handful up to 40 players interact at a time via text or voice, sharing strategies in the pursuit of game goals and learning from each other as they engage in the activity. In this context, the teacher becomes a "wise guide" who participates alongside the students. Although no game-based data are available, classroom studies show that collaborative learning yields, on average, a 50% improvement over solo learning (*28*).

Finally, with all else being equal, games invite more time on task. Teenagers commonly spend 5 to 8 hours per week playing games, and this equals or surpasses the time spent on homework each week (*29*). B. D. Coller's racing car game, designed to teach numerical methods, resulted in twice the time spent by students on homework as a traditional class, with greater depth of understanding of the relations between concepts, and an overwhelming demand for the follow-up course (*10*, *11*).

In contrast to the pedagogical and motivational elements found in games, some studies suggest that the lecture format is severely wanting. E. Seymour and N. Hewitt (*30*) chronicle near-universal antipathy to the undergraduate lecture experience, showing that 98% who leave science and engineering majors cite "poor teaching by faculty" as a major concern and that even 86% of those who stay say the same. R. R. Hake's metastudy (*31*) of 6542 students in 62 introductory physics classes demonstrated only a 17% SD in learning outcomes across lecture-based classes. In contrast, the same study showed that switching to any interactive mode of instruction (e.g., group projects, Socratic lectures, participatory demonstrations) easily improved learning outcomes in

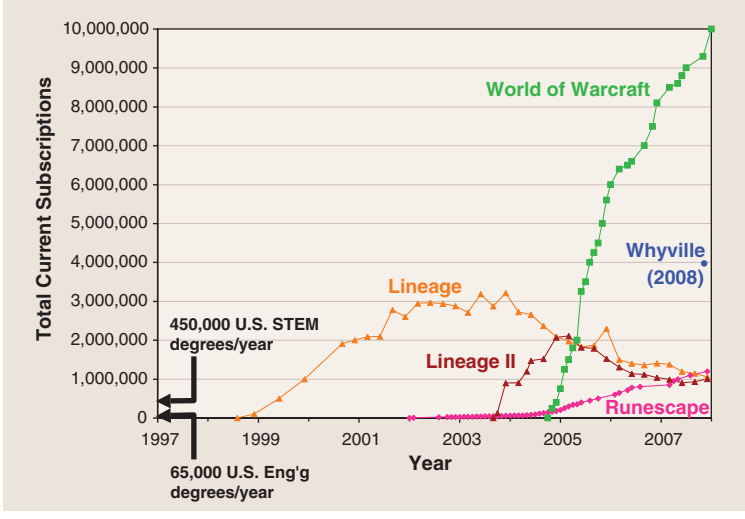

Fig. 1. Comparison of online game subscriptions (*3*, *7*) to U.S. bachelor's degrees awarded across all STEM disciplines (*1*) as well as in just the engineering disciplines (*1*). Games having more than 1 million subscribers are shown.

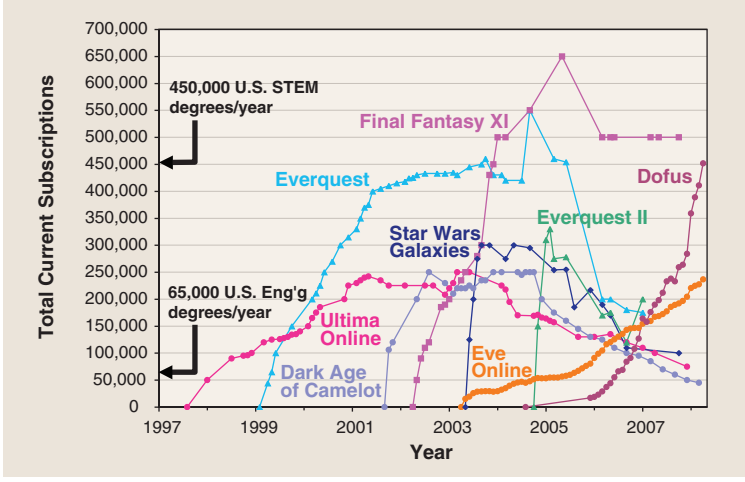

Fig. 2. Comparison of online game subscriptions (*3*) to U.S. bachelor's degrees awarded across all STEM disciplines (*1*) as well as in just the engineering disciplines (*1*). Games having less than 1 million subscribers are shown.

introductory physics by 108%. One could certainly argue that games are about the most interactive type of content that exists today. If video games are valid pedagogical delivery vehicles and they reach many more people than lectures, why do we not see video games adopted as the learning vehicle of choice? Cultural adoption lag exists, but we also face challenges of quantity, quality, and sustainability.

Quantity

It is often assumed that games with academic content are inherently uninteresting. Yet, 4 million children voluntarily play math-and-science–based exploration games on Whyville.net (*7*). In my opinion, most academically developed games suffer from infrastructural challenges rather than content challenges, with respect to mass adoption. Examples include the lack of any distribution mechanism for the product, the lack of product discoverability, the prohibitive expense of content creation, the dearth of meaningful assessment (and therefore of consumer confidence in the product), and the lack of sustainable business models.

The first infrastructural challenge is the lack of any mechanism for distribution, sales, or marketing. Grants will not pay for these essential business functions that are required to reach audiences in the millions. Instead, academic games are often relegated to the office shelf or personal Web site of their creator as soon as the grant is over. One way around this dilemma is for a third entity—for example, a not-for-profit organization—to take on the business activities in exchange for intellectual property rights from the content creator.

Fig. 3. Protein-sized drone flying over macrophage surface in *Immune Attack* (*9*). The player is required to call neutrophils by using the drone's ray gun to activate CXCL8 release.

Table 1. Learning outcomes of several games compared to lecture on same material.

Game	Topic	Audience	N (study size)	Learning outcome over lecture	Reference
Dimenxian/ Evolver	Algebra	High school	193	7.2%	(*37*–*39*)
Geography Explorer	Geography	College	273	15 to 40%	(*40*)
NIU Torcs	Numerical methods	College	86	2× more time spent on homework, much more detailed concept maps	(*10*–*11*)
River City	Ecology/ biology	Middle/high school	≈2000	15 to 18%, on average	(*13*)
Supercharged!	Electrostatics	Middle school	90	+8%	(*41*)
Virtual Cell	Cell biology	College	238	40%, on average	(*40*)

Regarding the challenge of discoverability, academic game producers often use the Web as their distribution mechanism. However, three-dimensional (3D) content is not discoverable by search engines, which read text and text-based tags. For someone interested in capacitors, for example, Google cannot discover a virtual 3D capacitor in the middle of a game about electronics. Therefore, a key need in the area of 3D immersive games is the institution of a standardized metadata tagging system that allows users to locate appropriate 3D content with the use of common search engines. For the visually impaired who "see" 3D content only via voiced expression of tags, this tagging system is crucial. At present, there are multiple inconsistent tagging systems in use by specialized communities, but most games embed none of these.

Expense is also an important factor. User-created 2D content floods the Web. We can imagine a future in which the same is true of 3D content, and this richness of content could spur a concurrent, expanding user base of 3D games, large and small. However, the reason that 2D content is so cheap and easy to generate is the fact that almost all of it can be easily repurposed: copied, pasted, and moved from one application, document, clip-art bank, or Web site to another. In contrast, 3D content has no standard file format and thus has a limited ability to repurpose content between applications. Moving to a common file format for 3D objects—Collada and/or X3D (*32*, *33*)—would greatly reduce graphics development costs, moving high-quality video game creation into the academic/home-user price range.

Quality

The ability to distinguish between a high- and low-quality product will be essential to the growth and credibility of game-based learning as a field. However, the first step in delivering quality is to be able to measure it. Assessment data are notoriously expensive to obtain, typically costing as much to develop as the original game. Few funders are willing to bear this double cost. To address this issue, the Ewing Marion Kauffman Foundation (*34*) has begun investigating the possibility of creating a software infrastructure to automate certain assessment tasks, thereby standardizing assessment across different games, lowering the cost of assessment per game, and making it more likely that researchers and funders will engage in assessment activities. Automated assessment is surprisingly advanced in certain areas: For example, automated essay grading is now nearly identical to human essay grading (*35*, *36*).

Games may also extend assessment into new areas. Whereas we say that we value 21st-century skills such as problem solving, teamwork, communication, and leadership, these essential traits are nowhere to be found on a modern transcript. An attractive dimension of game-based assessment is the potential to track sequences of user actions and communications, then map these onto higher-order

skills and abilities. For example, in the case of problem solving, one can easily measure how often a user attempts a given problem. Attempt frequency (especially if each attempt is different) correlates highly to improved problem solving. Similarly, by monitoring users' keystrokes while they navigate search engine results, we can distinguish between hypothesis-driven searches and random searches, another key indicator of advanced problem-solving skills.

Sustainability

The last major hurdle in expanding the use of game-based learning is arriving at sustainable business models. Academic game development, which depends on living from one grant to the next, is inherently unsustainable. However, if funders could lay the foundations in an initial grant, the same learning materials could transition to profit-generating models that could be used to expand the material's reach after small-scale academic development is completed. These models could include corporate sponsorship, dual pay (free to some, but a fee for others) or sliding-scale fee models, subscriptions, site licensing, and the sale of virtual goods (e.g., virtual clothing to be worn by the player's in-game character, downloadable wallpapers, electronic books that give game hints). Other business models could include leader sales to countries with nationalized education systems and hence centralized buying power, partnerships with commercial game distributors, and microcredits for microknowledge (a far-future economic concept wherein a user would pay, say, $0.99 to learn the Pythagorean theorem via a small educational module, in exchange for a math mini-credit that could aggregate with other mini-credits toward a degree). To my knowledge, none of these methods has yet been used to sustainably support academically developed games, with the possible exception of corporate sponsorship, which has supported the growth of academically developed but for-profit–operated Whyville.

Summary

Although the field is still in its embryonic stages, game-based learning has the potential to deliver science and math education to millions of users simultaneously. Unlike other mass-media experiments in education (e.g., TV, Webinars), games are a highly interactive medium with many key attributes shared with sophisticated pedagogical approaches. Large-scale adoption, however, still awaits key infrastructural developments to improve quantity (of users), quality (of product), and sustainability (of business models).

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PERSPECTIVE

Laptop Programs for Students

Andrew A. Zucker[1] and Daniel Light[2]

With the continuing decline in costs of technology, programs are proliferating worldwide to put networked laptop computers into the hands of millions of students on a routine basis. The reasons policy-makers support these programs are based on economic arguments, equity concerns, and widespread interest in education reform. Studies of laptop programs in schools report that they increase students' engagement in school, improve technology skills, and have positive effects on students' writing. However, evidence of the effectiveness of large-scale laptop programs in other learning domains is scarce. Research in many nations suggests that laptop programs will be most successful as part of balanced, comprehensive initiatives that address changes in education goals, curricula, teacher training, and assessment.

Interest in providing laptops to schoolchildren has been growing for more than a decade, with a school in Australia beginning what may have been the first such program in 1990 (*1*). Traditional manufacturers now offer many laptop models costing under US$900. In addition, less-expensive laptops especially designed for children and schools have become available, including the XO computer designed and distributed by One Laptop Per Child [a spinoff of the Massachusetts Institute of Technology (MIT) Media Lab] and the Intel Classmate personal computer (PC). Ultra-

low-cost computers such as these typically include flash memory instead of a spinning hard drive, smaller screens, and fewer external ports. At the same time, they and others like them may offer features of particular interest to schools in developing nations, such as low power consumption, a free or low-cost operating system, and the capability to establish a mesh network with other computers.

With declining unit costs, policy-makers around the world are investing large sums of money in laptop computers for students and teachers in elementary and secondary schools. In the United States, the state of Maine provided every middle-grade student (ages 13 to 14 years old) with a laptop beginning in 2002, on loan from schools like a textbook. Pennsylvania's Classrooms for the Future program is providing classroom sets of laptops to more than 500,000 high school students. Uruguay has recently distributed 120,000 laptops and plans to buy 300,000 more. Portugal announced it will provide 500,000 computers to students, and Venezuela has ordered 1 million laptops for children, which will be assembled in Portugal based on the Intel Classmate PC design. Australia, Chile, Columbia, Libya, Mongolia, Nigeria, and South Africa are among the many other nations supporting at least pilot programs with laptops.

The reasons given by policy-makers for investing in these programs vary. There are economic arguments, based on improving students' technology skills, creating a better educated work force, and attracting new jobs; equity concerns, to support students from low-income families whose access to technology and information is otherwise restricted; and education reform issues, as policy-makers try to make schools more effective and provide students an education that prepares them for life in the 21st century.

The growth of laptop programs globally has been fueled by widespread discontent with the status quo in elementary and secondary education. Computers and globalization have changed skill requirements. Schools are being asked to increase the quality of education, notably by providing more students than in the past with advanced skills and the ability to be flexible thinkers and problem-solvers. At the same time, in many developing countries there is a demand for deep reforms in education to help create a more democratic, participatory, and responsible society, which calls for substantial changes in the schools (*2*, *3*). Those countries, which are only now beginning to move away from traditional education systems, often see technology as one of the keys to transforming their education systems.

Programs to provide students with laptops and related technologies use various devices and differing usage or ownership models. In almost all cases, the laptops are wireless and provide students with access to the Internet and a local school network. Programs providing students with personal laptops to use during the school year are often called one-to-one (1:1) computing. Some 1:1 programs allow students to take their computer home; others do not. A few 1:1 programs (such as in Henrico County, Virginia, involving more than 25,000 laptops) subsidize home access to the Internet for low-income families. Australia is aiming for 1:1 but has begun by offering schools grants for one computer per two students (www.digitaleducationrevolution.gov.au). In contrast to 1:1 computing, tens of thousands of schools in many nations have invested in classroom sets of computers, generally with wireless networking capability, which are either shared by many classrooms or assigned to only one. Although students in those cases are not provided with a personal laptop, some personalization can be provided if students' documents are stored on a server and are thus available from any machine. A few school systems have adopted "thin client" solutions, meaning that the machines students use are cheap and easy to maintain, whereas the major computer power and the software are provided by a small number of computers or servers. The republic of Macedonia (formerly part of Yugoslavia), for example, uses inexpensive computer terminals linked to regular PCs, at a seven-to-one ratio, for its 360,000 students (*4*).

What these varied approaches have in common is putting powerful, networked computer capability into the hands of more students on a routine basis. And as interest has grown in laptop programs, especially since 2001, schools have also adopted related digital tools and services, including online courses, interactive electronic whiteboards, handheld devices (sometimes called clickers) that beam students' answers to a receiver and a display, graphing calculators, and "probes" for collecting science laboratory data in digital form (*5*).

Evidence of Effectiveness

To measure the extent to which a laptop or other technology program is effective, one must know the goals against which success is measured as well as the outcomes. One would also like to have information about the nature and quality of the program design and about details of implementation so that reasons why programs do or do not work can be better understood (*6*). Gathering and analyzing all of those data is expensive and a challenge.

A widely reported outcome in both the developed and the developing world is that programs providing computers to schools increase the technology skills of teachers and students (*7*, *8*). Sometimes, as in the case of the World Links program supported by the World Bank in 26 developing countries, this is an explicit program goal (*8*).

Research and evaluation studies also report that laptop programs increase students' engagement with academic work, which is an important finding given the large dropout rates in many secondary schools (*7*, *9*). Participants are often enthusiastic about laptop programs, including teachers, students, parents, and administrators (*10*, *11*). As a result, many programs have been supported for years.

Not surprisingly, research also shows that at school students use laptops frequently to search for information of various kinds. Students report that they benefit from Internet search tools and digital resources that allow them to access information more quickly and efficiently (*12*).

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Table 1. The estimated 5-year total cost of ownership (TCO) for a smart classroom in an urban secondary school in a developing nation, based on using ultra-low-cost computers and the Linux operating system. [Adapted from (*31*)]

Cost item	5-year TCO	% of TCO
Initial costs per classroom		
Hardware	$11,397	17.5%
Retrofitting, networks, cabling, and deployment	$1,779	2.7%
Total initial costs	$13,176	20.2%
Recurrent costs		
Training	$16,000	24.5%
Internet	$2,100	3.2%
Service and support	$20,801	31.9%
Electricity and consumables	$636	1.0%
Total recurrent costs	$39,537	60.6%
Hidden costs		
Replacement computers, repurchase, and deployment	$10,043	15.4%
Other	$2,533	3.9%
Total hidden costs	$12,576	19.3%
Total 5-year TCO, 32 seats	$65,290	100.0%
TCO per seat	$2,040	

There is strong evidence—but usually drawn from studies besides those focused on laptop programs—that a variety of specific educational applications of computers are effective. For example, using a word processor has been shown to help students learn to write (*13*); certain drill-and-practice applications assist students in learning facts and skills (*14*); some science laboratory simulations can be more effective learning tools than actual laboratory equipment (*15*). (Other articles in this issue supply additional information about particular computer applications.)

In some cases, there is evidence that laptop programs are important contributors to students' academic achievement. A public charter high school in Colorado that serves many students from low-income families provides an example (*16*). Physics classes there make use of an interactive electronic textbook stored on students' laptops, computer-based physics simulations developed by a Nobel Prize winner, probes for collecting laboratory data, and other digital tools. In 2007–2008, 30% of the seniors at the school took an Advanced Placement physics test, compared to about 3% nationally, yet students at the school scored at or slightly above the national average on the test, making the yield of capable physics students exceptionally high. The school does not claim that laptops alone cause this outcome—the teachers are excellent and many other factors play a role, including the digital resources used to teach physics—but data show the laptops are heavily used for academic work, and the administrators, teachers, and students believe that the 1:1 laptops contribute to the school's success. Animated physics simulations are part of the electronic textbook, so students interact and experiment with this textbook, not just read it. Teachers routinely make use of computer software called ExamView that provides instant feedback about how students have responded to assessment questions, and teachers may then assign students to groups on the basis of that information. Several times each year the teachers study assessment data, gathered using ExamView, to find out how well students are performing on education standards. The school's alignment of educational goals, instructional materials, student assignments, teacher practices, and assessment techniques illustrates that computers will be most effective when used as part of a thoughtfully coordinated, systemic approach.

A review of 30 studies of 1:1 programs found few with rigorous designs, but the studies measuring learning outcomes showed consistent, positive effects on students' writing skills (*7*). However, studies finding evidence of other academic achievement gains in laptop programs involving large numbers of schools, particularly studies using quantitative methods, are scarce in wealthy countries (*7*) and rarer in developing countries. In what is probably the first study of its kind, the Canadian government recently funded an evaluation of 1:1 pilot programs in Argentina, Costa Rica, Uruguay, and Columbia (www.idrc.ca/en/ev-129437-201_104122-1-IDRC_ADM_INFO.html). A study underway in Texas is unusual because it is a 4-year longitudinal study, costs millions of dollars, and includes not only more than 20 experimental schools that use laptops but also a matched comparison group of schools that do not (*12*). After 3 years, the researchers found positive impacts of laptops on technology use and proficiency, increased interest among teachers in student-centered instruction, reduced student disciplinary actions, and greater teacher collaboration. However, there was generally no significant impact on students' test scores in reading and writing and only a weak impact in mathematics.

Policy research from many countries finds that a key difficulty is that instruction often focuses mostly on basic skills and memorizing facts and less on complex ideas or teaching students to be flexible thinkers (*17*–*20*). Some policy-makers hoped that the introduction of computers would lead directly to better instruction. However, the Texas study found that the availability of computer technology by itself had little or no impact on the intellectual challenge of teachers' lessons, concluding that across classrooms lessons generally failed to intellectually challenge students.

It is clear is that simply providing computers to schools is not enough to increase student achievement or change the nature of instruction. At a minimum, learning goals, curricula, teaching strategies, and assessments must change as well. Also, computers are often underutilized (*21*–*23*). Leaders must provide teachers and administrators with a clear vision of how computers are to be used; appropriate digital resources must be made available; effective, ongoing professional development needs to be provided to teachers; technical support must be available for computers, networks, printers, software, and other components; local leaders, including school principals and teacher leaders, need to be trained and supported; and so on. For example, one crossnational study found that teachers' competence in using technology, as well as the amount of their training in uses of technology for instruction, was associated with greater use of technology for instruction (*24*).

Costs/Affordability

Despite these challenges, interest in laptop programs is likely to continue growing, in part because a new generation of low-cost laptops has been developed, including the OLPC XO machine, the Intel Classmate, the Asus Eee PC, and others. Someday there may be a $100 laptop, although no one has yet achieved such a low price. The cost of installing wireless computer networks also declined greatly in the past decade.

Regardless of the price of technology, the investments in creating effective laptop programs are large. The Australian government has recently committed Au$1.2 billion for its school technology program. In the U.S., one national program alone, the E-Rate program, has spent about US$20 billion since 1998 to help connect nearly all schools to the Internet (*5*).

Yet the cost of laptop programs consists of much more than the price of buying computers and connecting them to networks. Schools should consider the total cost of ownership (TCO), including training of teachers and administrators, technical support, software, replacement costs of aging equipment, and other items. In the United States, the direct and indirect costs of 1:1 programs per client computer are over $1000 annually (www.classroomtco.org/gartner_intro.html). Even in the developing world, where labor costs are lower, one recent estimate of the annual per-seat cost of a 1:1 classroom is more than $400 (*25*). Table 1 shows that the hardware itself composes only about one-third of the total in a developing nation, whereas training, service, and support account for more than half.

But it is a mistake to compare TCO to a baseline of zero. Almost no policy-makers suggest that all computers and Internet connections be removed from schools or that teachers need not be trained to make use of the resources available on the World Wide Web. Indeed, desktop computers have become far more prevalent in schools in many nations during the past decade (*26*). Thus, alternatives to laptop programs cost substantial money, too. Policy-makers also expect that laptop programs will reduce certain costs, such as those for textbooks and assessments (*27*).

The Future of Laptop Programs

Computers are different than other technologies used in schools because they are all-purpose machines. They can be used as a library, a way to model invisible phenomena, a communication device, a link to other tools (such as telescopes or online databases), a device students use to create knowledge artifacts in many media, and so forth. Computers' flexibility makes them uniquely powerful educational tools but also means that quality educational interventions or treatments cannot be realized simply by providing more computers.

Policy-makers and the public need to be clear about the educational and social goals for laptop programs (which will vary according to local needs and aims) and assure that the necessary elements are in place to reach those goals. Political leaders' beliefs that computer-based learning tools are powerful are well founded, as is shown by research as well as by the everyday experience of using the Internet. However, if the goal of laptop programs is to change educational goals; to improve patterns of teaching, learning, and assessment; and to help transform schools into more effective institutions; more needs to be done than acquire laptops and a corresponding technical infrastructure. Curricula need to be revised, better assessments developed, teachers must learn new approaches, and schools have to

support teachers as they learn to teach in new ways. As one study of more than two dozen countries' use of information and communication technology recommended, "policies that adopt a balanced, holistic approach catering for [the multiple changes needed] will be more successful than policies focusing on one or two strategic areas" (*24*).

Poorer nations face particularly challenging choices because large-scale technology installations are expensive, and their school systems are simultaneously trying to extend current education systems to reach large numbers of unschooled children (*28*) and trying to radically transform schooling. Policy-makers in some developing nations, such as India and parts of Latin America, believe that although computers and the Internet are important for schools, instead of funding 1:1 programs their best strategy for incorporating technology is to proceed at a slower pace, pilot testing different approaches in order to identify which programs effectively meet their needs (*29*). Computers are an increasingly important educational tool, but only as part of carefully designed policies affecting many aspects of education (*30*). A laptop program that does not seriously address the need for education reform is not an appropriate option for any school or nation.

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REVIEW

Online Education Today

A. Frank Mayadas,[1]* John Bourne,[2] Paul Bacsich[3]

Online education is established, growing, and here to stay. It is creating new opportunities for students and also for faculty, regulators of education, and the educational institutions themselves. Much of what is being learned by the practitioners will flow into the large numbers of blended courses that will be developed and delivered on most campuses. Some of what is being learned will certainly improve pedagogical approaches and possibly affect other important problems, such as the lengthening time to completion of a degree. Online education is already providing better access to education for many, and many more will benefit from this increased access in the coming years.

In a 1995 *Science* article, Eli Noam of Columbia University opined that the Internet would pave a difficult road ahead for traditional academic institutions; he wrote, "as one connects in new ways [the Internet], one also disconnects the old ways" (*1*). Thirteen years after Noam's article and 15 or so years after Internet usage began its rapid acceleration, online learning has become an important element in education, although it is not evenly distributed across institutions.

The term "online learning," however, obscures vast differences in methods supported by this educational approach. We limit this discussion to online education in traditional, regionally accredited, degree-granting institutions. Within this discussion, we include "blended courses," that is, those that feature some online elements but less face-to-face time than encountered in an equivalent traditional course. We do not discuss online education in the rapidly developing kindergarten through grade 12 environment, online corporate training, or the free educational resources (complete courses in many cases) being made available online by some universities, such as the Massachusetts Institute of Technology (MIT), Yale, Stanford, and a few others (*2*). These are widely accessed throughout the world but do not provide credit as courses or as partial fulfillment toward degree completion. These efforts can thus be thought of as use of the Internet to disseminate, free of charge, valuable, high-quality information, but not credentials,

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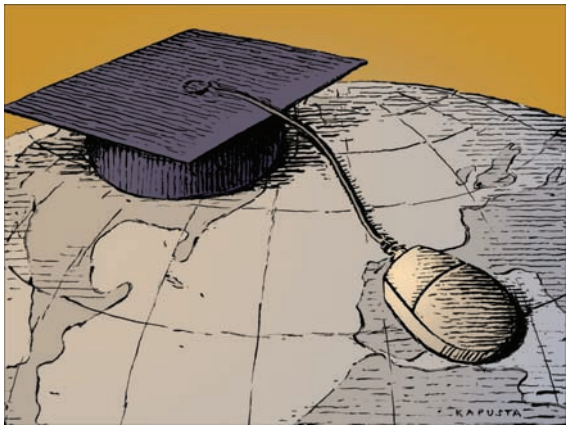
whereas the focus of this paper is on use of the Internet to provide access to educational credentials through use of the common tuition and fee mechanisms.

Generally, in corporate training, online learning means accessing short training modules that cover specific topics (e.g., quality practices, new product information, and diversity practices) available as self-study units on the corporate intranet. In this sense, the online aspect is providing an efficient distribution mechanism, replacing CD-ROMs or even printed manuals. In contrast, institutions of higher education offer their courses in quite a different form: In most cases, class cohorts are formed rather like traditional classes that start and end on specific days. These online classes are led by faculty members who most likely require participation from students on topics that are being covered, and discussions and exchanges of ideas among cohort members. A participant may seek help on a problem set or clarification of an assignment from classmates who are distant but online. This capability of student-to-student and student-to-faculty interaction, coupled with instant access to information resources worldwide on the Internet, plus the efficient distribution of class materials (readings, homework assignments, and possibly stored video lectures) distinguishes modern online education from older "distance education" models built around correspondence or television.

In this modern version, basic technology requirements for students anywhere in the world are conventional personal computers with a broadband connection to the Internet. Students and faculty engage in classes using course management software (*3*), and classes are conducted "asynchronously," that is, there is no need for students and faculty to assemble at the same time, as in traditional classroom instruction. Indeed, course management systems have become ubiquitous in higher education, used for both online and on-ground asynchronous instruction. Synchronous software is sometimes employed for simultaneous voice and text discussions, often as an optional class activity. Newer devices like iPods are being employed in a number of instances, and even virtual environments such as Second Life (*4*) are quite widely seen in connection with corporate or specialized lecture-style environments. For institutions that offer for-credit courses and degrees, however, newer methods of teaching are mainly at an early, experimental stage.

The annual Sloan Consortium survey of online education (*5*) provides an information base for positioning the online modality as an important element of education. The study reports the responses of chief academic officers at 2500 degree-granting institutions of higher education to the online teaching and learning landscape (the response represents more than 50% of all such institutions in the United States). The most recent survey, published in 2008, reports that 3.94 million students enrolled in at least one online course for the fall semester of 2007 in a wide range of disciplines (see Fig. 1). Annual enrollment increases have averaged just below 20% over the past 6 years of this study, leading to the result that today more than 20 to 25% of all students in U.S. colleges enroll in at least one online class. Many of these students are off-campus learners with a wide range of ages, work experience, and family circumstances; however, at a number of institutions, about half of the online enrollments are estimated to be full-time "traditional" students attracted to online courses for reasons of convenience or scheduling. Most are at public institutions—state universities, colleges, and community colleges—all of which offer at least some online education. Some of these institutions report large enrollments, for example, in the tens of thousands; community college enrollments alone account for about 50% of the 3.94 million students enrolled.

In contrast, only about half of the traditional private institutions provide any sort of for-credit online course offerings. Among those that do, however, are institutions such as Stanford and Johns Hopkins, both of which offer courses and some degree programs entirely online. Harvard and the University of California, Berkeley, also offer courses online, but mainly through their extension units; they offer no degree programs. Other elite schools, such as Princeton, Yale, and MIT, offer no online courses for credit or degree programs. In comparison, the enrollment at Pennsylvania State University's World Campus exceeds 20,000. The enrollment at the University of Massachusetts online unit (UMass Online) exceeds 35,000. Rio Salado Community College in Phoenix reports similar numbers. The University of Illinois, Springfield, a small institution, now has an online student population approximately equivalent to their on-campus enrollment. Overall, the highest growth rates are at community colleges and at for-profit organizations such as University of Phoenix, Kaplan, and Capella. It appears that, to this point, online education has not resulted in Noam's "dim circumstances" for the many institutions that have adopted online education. In fact, the fear that fewer faculty would be needed has been turned on its head: More faculty are being hired to service burgeoning online enrollments. Neither has online growth created a dim prognosis for highly endowed institutions, many of which have no online education program. It has, however, drawn the attention of other stakeholders, such as governments (both state and federal) and accreditors, now being forced to deal with an educational activity very different from traditional classroom teaching.



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Impact on Students

A primary driver for online education is the presupposition of faculty and university administrators that a sizable population of potential learners exists—typically, working adults who wish to obtain college credit and credentials but who cannot do so because of time restraints imposed by work, family, community responsibilities, or lack of proximity to a suitable educational institution. Faculty members and institutions expected the asynchronicity and distance-independence of online education to be an answer for this population. Largely, this assumption has proven to be true. We have no accurate national profile, however, of the age of the "average" online students and their demographics, nor are those facts likely to emerge in the near future; no national profile of "traditional students" exists either, but some inferences are possible from individual institutions that collect data about online students.

The University of Central Florida, a large metropolitan university in Orlando, has developed an excellent, regularly updated database of its online students. Their analysis (*6*) contains some surprises: Of 115,000 students enrolled in their blended online courses in the seven-semester period from summer 2004 to summer 2006, nearly 80% represented the so-called "millennial" generation (born after 1980); they dominated enrollments in lower- and upper-level undergraduate classes, and also in graduate classes. The remaining 20% comprised earlier generations, including a small number from the "matures," born before 1946.

Are the several million students who have taken online courses satisfied with their learning experience, and have they had a high-quality learning experience? Is online learning a doorway to high-quality education? These questions are difficult to answer because huge variances exist in instructors' teaching skills and experience, course organization, and in study materials for students. These elements provide variations similar to the differences in educational outcomes found in traditional classrooms. From data provided by individual institutions, we do see quite uniformly that grades and completion rates for well-designed online courses taught by experienced instructors tend to result in equivalent outcomes for both online and traditional students (*7*–*9*). In most cases, therefore, it appears that online

students receive an education equivalent in quality to what they would receive in traditional classes, and their drop-out rates appear to be about the same.

Impact on Faculty

The emergence of online education has had an impact on faculty as well, certainly on those who teach classes online, but also on those who are experimenting with Web-based elements blended into traditional classes. Eventually, we expect Internet use in courses to envelop all faculty, as blended approaches become the norm for college courses over the coming 5 to 10 years. In the United States, about 1.3 million faculty work in degree-granting postsecondary institutions; of those, about half are full time (*10*). The 3.94 million students taught online represent about 22% of the estimated total national student population. If the number of faculty is roughly proportional to the students enrolled, then approximately 300,000 faculty engage in online teaching in the United States today. Of those, some estimates place more than 100,000 in the adjunct category (*11*); that is, faculty who are not permanent employees of an institution. A commonly held misconception is that online faculty are all or mostly adjuncts. It would likely be fair to estimate that adjunct and permanent faculty of diverse types mirror the full-time/part-time proportions found in face-to-face teaching.

A number of institutions (*12*–*14*) conduct surveys of faculty satisfaction factors and attitudes toward online education. Across institutions, the results tend to be quite similar. Almost unanimously, online instructors assert that although preparing and teaching online courses is more time-intensive than classroom teaching, they plan to continue teaching in that modality for a variety of reasons: the flexibility of "anyplace, anytime teaching" for themselves and for their students, opportunity for professional growth, the option of teaching from home, and interactivity with students, which they report is of higher quality than classroom discussion. These faculty are also motivated by a strong conviction that the work they are doing is important to students who need flexible access to education, although they point out that online students need to be more self-disciplined. In addition to the data reported by a handful of institutions, a completed national survey of nearly 10,000 faculty members from a recent diverse sampling of 60 campuses by Seaman and Allen (*15*) confirms these conclusions. Economic issues also come into play for some faculty. Commonly, an on-ground itinerant faculty member who travels to three campuses a week may absorb sizeable transportation time and costs. Online instruction brightens this picture. By teaching online, those faculty can accommodate an additional course or two—a substantial bonus for an adjunct professor earning a living by teaching.

Because the professoriate is aging, not all faculty members wish to acquire the skills needed to engage with millennial students who befuddle them with wikis, blogs, Web casts, virtual worlds, and course management systems. To ease their transition to this new teaching agenda, many institutions provide support for instructors to transfer their courses to the online modality. Faculty members who subscribe to the mantra "I can't teach them if I can't look them in the eye" will slowly become obsolete except in some exclusive colleges. The millennials are changing the way teaching and learning must be approached. Mobile learning with podcasts, text messaging, and virtual worlds will be the future norm, giving faculty new tools through which to extend and enhance the educational experience.

Governments, Regulation, and Accreditors

Federal and state governments involve themselves in higher education for different reasons. The federal government is involved through its role in federal grant and loan programs such as Pell grants and the Federal Family Education Loan Program; state governments view their role to be licensing and accrediting institutions of higher education within their state boundaries, providing financial support for public institutions in their state, and, in an increasing number of cases, offering their own student aid programs. The states have traditionally asserted a right to impose rules and regulations on institutions that are located on their soil; that is, those with a "physical presence" within their state boundaries.

The role of the federal government in postsecondary education has largely been defined by the Higher Education Act of 1965 (HEA). Responsibility for administration of this law has fallen to the U.S. Department of Education, which delegates key functions and policies to other stakeholders, such as accreditation agencies and the colleges and universities themselves. The recent reauthorization of the HEA, the Higher Education Opportunity Act of 2008 (*16*), contains several specific provisions that indicate that the federal government is increasingly taking into account the importance of online education as an element in U.S. higher education.

Before 1996, the Department of Education treated asynchronous online learning as conventional correspondence study, and this resulted in a reduction of the federal financial aid available to individual online learners by more than half. In that year, the HEA was amended to separate "courses offered through telecommunications" from correspondence and to treat students enrolled in such courses as equivalent to those attending classes in person. However, reflecting a continuing unease with online learning, Congress limited the applicability of this provision to institutions that still offered a majority their courses in a conventional (that is, face to face) mode. Congress subsequently enacted, and the Department of Education implemented, the Distance Education Demonstration Project that allowed a limited number of colleges

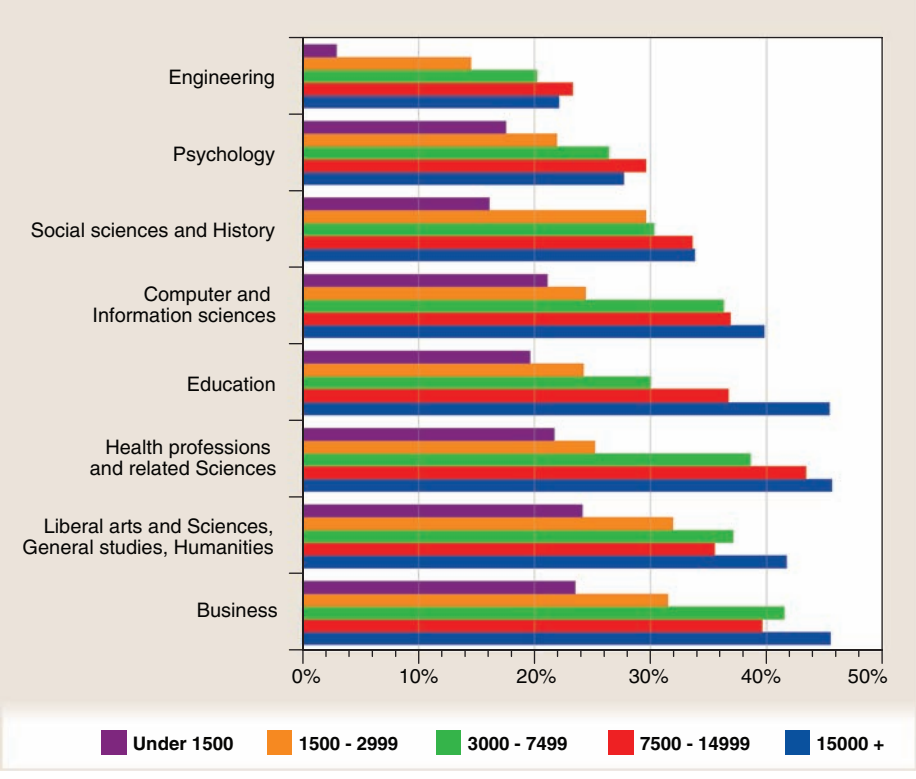

Fig. 1. Penetration of online programs by discipline and total enrollment, fall 2007. Data represent the results of the most recent Sloan Consortium national survey of all active, degree-granting institutions of higher education in the United States that are open to the public (*24*). "Penetration" refers to the fraction of all online degree programs offered by such institutions as a fraction of all degree programs in that discipline. [Reproduced by permission of the Sloan Consortium]

to offer more than 50% of their courses online, which opened the door to a few entirely online institutions. The 50% rule was rescinded in 2006, but the latest amendment to the HEA makes it clear that there is still residual discomfort with asynchronous learning. Although there is no longer any restriction on the ability of a university to offer its programs entirely online, it may only do so if it has secured specific approval from its accrediting commission (*17*), provided that the accrediting commission has itself been determined by the Department of Education to be qualified to evaluate an institution's distance learning offerings. One provision of the new law bears specifically on asynchronous learning. Institutions offering online programs must establish "processes through which the institution establishes that the student who registers in a distance education or correspondence education course or program is the same student who participates in and completes the program and receives the academic credit" (*18*). Congress has assigned responsibility for enforcing this requirement not to the Department of Education but to the accrediting commissions. Other issues in the law directly affecting online delivery of courses are covered in an excellent report from Dow Lohnes PLLC (*19*).

Although both Congress and the Department of Education have shown increasing interest in online learning, it is the individual states that have been most involved in the regulation of online education. In a recent survey (*19*), an increasing number of states are asserting what some would consider novel interpretations of "physical presence." For instance, it is common practice for online course providers to require a remote student to take a final examination under the supervision of a proctor, for example, a librarian or a local government official, or to meet with a discussion group of fellow students. Although both of these approaches are desirable adjuncts to effective online learning programs, some states are now asserting that these activities constitute a "physical presence" sufficient to require the institution to be licensed by the state in which the students reside. Indeed, some states have gone well beyond this, asserting that the mere fact of delivering the online program across its borders and enrolling its citizens is sufficient to require an institution to submit to its regulation. These examples show that both federal and state governments and accreditors are clearly being affected by the emerging world of online and blended instruction and that they are experimenting with ways to deal with this new world, particularly in balancing the need to protect consumers against unscrupulous purveyors of substandard programs and yet not interfere with the growth of this essential component of postsecondary education. The complexity of these issues is multiplied many times over when one considers the implications of online learning crossing international boundaries. Governments and quasipublic bodies charged with oversight of postsecondary education will need to develop considerably more sophistication as colleges and universities, both within the United States and globally, expand the scope and reach of their online programs.

Institutions

Finally, we turn to the question, Are traditional institutions of education facing a threat from the growth and increasing validation of online instruction? There is little evidence today to suggest much of a threat. For-profit institutions such as Phoenix, Kaplan, Capella, and Jones are successful, and their growth rates exceed those of the online programs in traditional institutions. They are clearly meeting a need. However, overall, online enrollments are still dominated by traditional institutions, and certainly all the public institutions and a number of private ones have acquired the skills, infrastructure, and faculty acceptance to allow them to compete effectively and to continue competing. Some evidence indicates that online enrollments in many of these institutions appear to be leveling off, and that is most likely an indication of internal decisions to maintain some arrived-at "balance" between classroom instruction and blended instruction and some overall ceiling on enrollments. Traditional institutions, especially public ones, do have some substantial advantages over for-profit institutions like the University of Phoenix, although it is clear that the distinction is blurring as the most successful for-profit institutions increase their focus on academic performance and as "traditional" schools learn how to compete more effectively in the marketplace, particularly in the context of leveraging name recognition at a local and regional level and, in some cases, at a national and international level, as well as the ability of public institutions in particular to offer considerably lower prices.

The institutions that have adopted online offerings on a large scale appear to be well positioned to avoid the "dim" future foreseen by Noam. They are adapting and can easily continue to adapt and prosper.

The leadership elements—presidents or chancellors—of the public institutions with large online enrollments are recognizing the strategic advantages of online and blended education and hence are treating this form of education as a strategic priority. Our discussion with the presidents or chancellors of Pennsylvania State University, University of Massachusetts, University of Central Florida, University of Southern Maine, University of Illinois, Springfield, and Rio Salado Community College indicate clearly that they are including online instruction as a strategic asset that is integral to the planning activities of their institutions. So, for instance, online possibilities can affect strategic decisions directed at addressing problems such as insufficient classroom space or reaching new markets (possibly including international students) to provide greater access to education. Some institutions are seeing strategic possibilities in online education to retard, and maybe reverse, the trend toward ever-lengthening time for a student to acquire a degree. Others are seeing possibilities for course-sharing and faculty-sharing among geographically separated institutions and for strengthening relationships among community colleges and baccalaureate and graduate institutions.

A number of select, highly endowed elite institutions do not see offering credit-bearing online courses and degree programs as a high priority, although they might make available free course materials, even the content of complete courses, as noted earlier. For these institutions, online teaching and credit-bearing offerings are not a necessary strategic or competitive tool. They do not appear to believe that their futures have been dimmed at all by the appearance of online education on a large scale at other institutions. These institutions, however, along with all others, will adopt and be affected by the more recent growth of blended education.

What of the less highly endowed institutions that have chosen not to involve themselves in online education? For these institutions, it appears the future may be more turbulent, perhaps even dim. Their income is largely dependent on students, and that supply of students may follow a downward path as online options proliferate from other, often distant, institutions.

Finally, a quick look at the situation outside the United States indicates that the story is less promising, even in Canada. In Europe, there have been a number of high-profile failures of online universities and a larger number of initiatives that never reached their full potential. (There have been failures in the United States, too, but few compared with the successes.) These include the UK e-University (*20*), the Scottish Interactive University (*21*), the Dutch Digital University (*22*), and the NHS University (*23*). Several others have dwindled more quietly, with no news emanating in English on the Web. Many reasons have been advanced for the far greater success of online education in the United States, greater than the higher gross domestic product alone should justify. Reasons suggested include the greater "travel to study" distances, a more "can do" culture, and more acceptance of private universities, both nonprofit and for-profit. Yet, in many European countries, initiatives continue—the Telematic Universities in Italy, the Campus Numériques in France, and the Swiss Virtual Campus collaboration (this latter example is coming to a planned end, not a failure). There is also an undercurrent of lower-profile but sound initiatives such as the private Hibernia College in Ireland; the U.K. universities of Derby, Leicester, Middlesex, Staffordshire, and Ulster; the collaboration of Liverpool University with Laureate Education Inc. to deliver master's programs; and, in addition, the various open universities across Europe rapidly reengineering themselves from distance learning

to online learning. Similar initiatives have arisen in the community college sector in several other countries and regions such as England, Wales, Bavaria, and Norway. So, although many U.S. providers are looking beyond the border and seeing very little competition, the global situation is likely to get considerably tougher in years to come, when competition for online students who live anywhere becomes as fierce as it is for traditional campus students.

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REVIEW

Opening Education

Marshall S. Smith

Spurred by the publication of Massachusetts Institute of Technology OpenCourseWare in 2002, the open educational resources (OER) movement, which has rapidly expanded and captured the imagination and energy of millions of creators and users throughout the world, now faces many opportunities and substantial challenges as it moves to become an integral part of the world's educational environment. The confluence of the Web and a spirit of sharing intellectual property have fueled a worldwide movement to make knowledge and education materials open to all for use. OER are content (courses, books, lesson plans, articles, etc.), tools (virtual laboratories, simulations, and games), and software that support learning and educational practice. OER are free on the Web, and most have licenses that allow copyright holders to retain ownership while providing specified rights for use in original and modified forms. At the least, OER have helped to level the distribution of knowledge across the world. A second promise of OER is to help transform educational practices. This article explores the history of and promises and challenges for OER.

A teacher in a Kenyan secondary school uses a free Web-based interactive simulation designed by a Nobel Prize–winning physicist (*1*). In a university in Xian, China, a student having trouble learning linear algebra goes for free help to a translated Web-based collection of course lectures from Massachusetts Institute of Technology (MIT) (*2*). And, in the United States, thousands of students go to a Web site that provides free homework help aligned with their state standards for most secondary school academic courses (*3*) (Fig. 1).

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These are examples of open educational resources (OER). OER include lesson plans, courses, textbooks, videos, podcasts, library collections, games, research journals and articles (often called open access), encyclopedias, dictionaries, simulations, and assessments and the open software that support their creation, hosting, and dissemination. Most OER are covered by flexible licenses that allow copyright holders to retain ownership of the materials while permitting users certain rights (*4*).

The power of OER comes from three rights fundamental to openness and sharing (*5*). OER users may be granted by the copyright holder of the resource some or all of the rights to (i) free access anytime, anywhere, to content or software that often has been available to only some because of firewalls, passwords, or proprietary rights; (ii) use the content by downloading, duplicating, and distributing it with or without restrictions; and (iii) reuse the content by translating or otherwise modifying it to accommodate local context and by combining it with other material and distributing the altered content, with or without restrictions.

Although OER are controversial, the affordances of the Web, together with growing interest and use of OER, make possible a leveling of access to knowledge and information around the world, as well as provide the potential for dramatic transformations of education practice. Students everywhere, enrolled or not, have free access to content and interactive instruction, as well as to networks of people with similar interests, enabling them to collaborate in the construction of knowledge and to learn at their own pace. The act of modifying an OER to meet language, cultural, or readiness requirements increases useful access and may be a creative learning endeavor. Open high-quality content and instruction can set standards of practice and, because of their quality, transparency, and availability, help improve the practice of teaching and learning throughout the world.

A Short History of OER

OER have been around almost as long as the Web, although not by name. During the mid-1990s, U.S. government libraries and museums posted examples of OER. Later in the decade, the U.S. Department of Education, the California State University, and European SchoolNet all provided substantial amounts of educational materials for free on the Web. In 1998, David Wiley, then a student at Brigham Young University,

created the first license for content that provided authors a way to maintain ownership of content while giving others the right to use it. Examples of open source software tools for creating and using open content appeared on the Web.

MIT faculty and administration decided in 2000 to make available on the Web the core content of all MIT courses (*6*). Named OpenCourseWare (OCW), it includes syllabi, reading lists, lecture notes, assessments, and video lectures: by 2007, 1800 MIT courses open on the Web were being accessed yearly by millions of students, teachers, and the general public.

MIT's commitment to "openness" sparked the imagination of people around the world, helping to spur dozens of other universities to post their own course materials freely on the Web and to form the OCW Consortium (*7*). In 2002, the United Nations Educational, Scientific, and Cultural Organization (UNESCO) proposed the name open educational resources, and in 2007 the Organization for Economic Cooperation and Development (OECD) published a study of the use and prevalence of OER in the developed world, concluding that "An apparently extraordinary trend has emerged" (*8*).

OER advocates believe that it will become a vibrant part of the educational and social fabric of the world. Lawrence Lessig, a professor of law at Stanford University, argues for the Web to be an open commons; if some people are restricted from knowledge while others are not, creativity will be constrained and inequalities heightened (*9*). However, there are questions often asked: How can OER survive if it gives away its products for free? Can the current momentum for OER be maintained and self-sustained? After all, the OER movement must survive in an environment that has a robust infrastructure to protect the private ownership of property and the maximization of financial return. For a movement with a very different ideology to coexist and grow, it, too, must have a supportive infrastructure.

Toward an Infrastructure for OER

An infrastructure provides an organizing and facilitating framework for the system it serves; in this case, the worldwide network of people and organizations that create and use OER. In recent years, a variety of studies and articles have been written about technology infrastructure issues (*10*). Most take a broad perspective, including not only pipes, standards, and software but also social, economic, and legal issues.

Technical. Effective use of technology and, therefore of OER, relies on a stable general technical infrastructure. This includes hardware, software, standards, and protocols. The rights to use and reuse content, however, may require software design characteristics different from those of software programs that operate in an environment without those rights. The need for open software tools for creating, recreating, and sharing the many forms of content is an example; without such applications and platforms, the rights to use and reuse are restricted to those able to afford proprietary tools. To facilitate full adaptation to new contexts and the addition of new functionality, open educational platforms and software should be modular and extensible (*11*). In addition, depending on its use, the success of OER may require additional standards and best practice models that establish criteria for quality and for meeting its promise of accessibility to everyone. For example, best practice protocols for making content openly accessible to the physically handicapped would be particularly useful. The field also will need to become comfortable with methodologies for evaluating materials and setting standards ranging from formal peer reviews and statistical trials to user judgments such as those used by Amazon.com and eBay.com.

Adaptive: social, cultural, political, and legal. Making intellectual property open on the Web often requires a major adaptive shift in the attitudes, norms, incentives, and legal orientation of organizations, groups, and individuals. Sharing is difficult in a society where competition and private enterprise are dominant.

Yet, even though the dominant values may weigh against OER, there are substantial social, cultural, and political movements in the developed and developing worlds that value openness and transparency, particularly in public transactions (*12*). Openness can be a way of organizing social activities that favors universal over restricted access, participatory over restricted decision-making, and collaborative over centralized production (*13*). Aspects of the Web, such as social networks, video sharing, wikis, and blogs facilitate an environment that supports independent learning and enhances information sharing, participative behavior, collaboration, and possibly even creativity. This (Fig. 2) is the world in which our children are growing up.

In this complex environment, our education institutions have mixed incentives for opening access to knowledge. Some university presidents see OCW and its transparency as a mechanism for improving the quality of courses and teaching. Professors who are comfortable with their course content post their material openly and are motivated by a desire to share, to further establish their reputation with their peers all over the world, and to improve their students' experiences. University or department policies to make research articles openly available are viewed as speeding the transmission of knowledge and facilitating greater opportunities for knowledge aggregation and partnerships. Many teachers have a thirst for seeing others' lesson plans and strategies and for sharing their own.

Working against OER, however, are financial concerns, authors' fears of exposing mediocre content, the weight of traditional practice, and legitimate reasons for protecting intellectual property. Course materials, such as books and videos, cost time, energy, and money to create, and authors may rightly expect compensation. Open content challenges conventional modes of publishing and distributing academic content. Some publishers and professional academic organizations believe they have a lot to lose as OER become more popular. These are trying problems that may sort themselves out over time. Instructors are becoming more comfortable with transparency and sharing, and publishers and academic organizations are testing different economic,

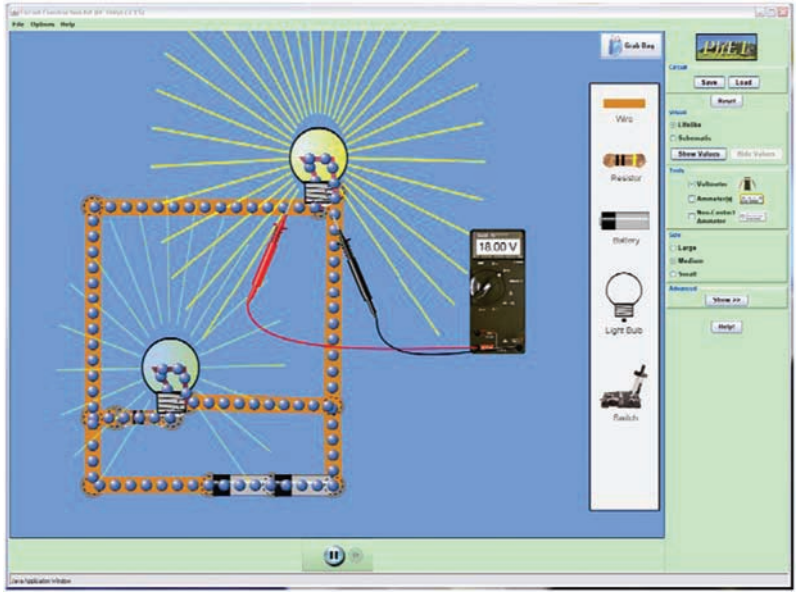

Fig. 1. This is a picture of an open interactive simulation of a Circuit Construction Kit that is used in classrooms from middle school to graduate school to teach basic electricity relationships. See (*1*) for Web site information.

service, and instructional models. But for now, tensions abide in many settings.

OER may have legal implications in a variety of areas, but the most obvious concerns are about intellectual property rights. Most works created after 1923 are under copyright and, although few people realize it, since 1989 in the United States copyrighted is the default condition for any creative work. A copyrighted item on the Web cannot be legally downloaded, printed, altered, or used in any fashion other than being read off the screen—unless permission is specifically granted by a license or statement of terms of use, or the item fits under an exclusion such as the "fair use" provisions that hold for educational institutions.

In 2001, building on Wiley's earlier work, Lessig started Creative Commons, an organization focused on designing easy-to-use licenses for individuals and institutions that allow them to maintain ownership of open content while providing users selected rights (*14*). Creative Commons' licenses change the copyright terms, allowing a range from no rights reserved—open sharing, reusing, and remixing—to some rights reserved. Creative Commons has provided legal and effective ways of making creative products open for others to use and reuse that, with Lessig's writing and advocacy, have given energy and credibility to an open content movement (*15*).

Financial. The dilemma of creating a sustainable organization or movement when the product is given freely is obvious. One sustainable model followed by some universities is to view OER as a part of its brand and the cost as a necessary part of its overhead. Many governments see opening their education content as a public good and responsibility. Other organizations look to private philanthropy or to government for help.

A few business models have been developed. Bloomsbury Academic, a new imprint of J. K. Rowlings's British publisher, will post its titles on the Web, downloadable and free, using Creative Commons licenses. It will sell print-on-demand books, as well as use normal channels. Other publishers, including National Academies Press, use a similar mixed model (*16*, *17*). Another approach, used when the traffic to an OER site is high, is to post advertisements to create a flow of income. A third model adds value to the OER by providing services to enhance the use of the OER. The Monterey Institute for Technology in Education (MITE), for example, takes open courses and adds value to them by charging for facilitating or providing installation, as well as for supporting communities of users and developers. Each of these financial models requires a substantial number of users who value the content.

The most powerful sustainability models, however, may not be rooted in financial rewards. Over time I expect to see multiple examples of models where groups of individuals and possibly organizations create virtual networks that are committed to creating, maintaining, and upgrading examples of OER. For example, many people and organizations are interested in ways to contribute to the public good. Engineers, such as those who have worked at night on Linux, or retired teachers and open universities in different nations are prime candidates for membership in such virtual organizations. Connexions, an OER commons and development platform created at Rice University, has a variety of open courses, some maintained by small communities of experts (*18*). Various Wiki applications, especially Wikipedia, are well-known examples.

Sustaining individual examples of OER is one challenge. Sustaining a viable OER movement is another. Two indicators are important to watch. First, networks of people and/or organizations pursuing common goals amplify social change, and the Web greatly facilitates the development of such networks. A rapidly growing number of networks of people and organizations actively support OER. The networks are local, national, and multinational, and they are located all over the world.

Fig. 2. Students are comfortable in turning to the Web for help. Open high-quality material from a trusted source is useful for students who need supplemental support outside of the classroom. This is a frame from a secondary school environmental sciences course at HippoCampus. See (*3*) for more information.

The second indicator stems from the fact that there are as yet few well-documented examples of powerful OER utility. OER will begin to come of age when there are multiple examples of its ability to improve the lives of substantial numbers of users—by increasing the flow of useful information, producing opportunities for collaboration and creativity, and enhancing teaching and learning.

Powerful Examples of OER

The Internet will change dramatically over the next decade as it incorporates such emerging technologies as geo- and semantic mapping, speech recognition, artificial intelligence (AI), data mining, and techniques that immerse learners in virtual worlds that engage multiple senses. The opportunities for influencing learning and teaching will expand dramatically. The power of OER to increase access and to allow use and reuse should both enhance and be enhanced by these innovations.

For now, however, our question is what current OER applications might be candidates for game changers in education. Many would consider OCW a candidate. Since MIT put up its site, more than 90 universities and colleges from 14 countries around the globe have adopted OCW and created a consortium (OCWC) to share best practices and course materials. As a result, more than 7800 courses are now available on the Web. Students and teachers across the world look to OCW to expand their understanding of courses they are taking or teaching, whereas nonstudents turn to OCW for self-learning (Fig. 3).

Another powerful application of OER lies in empowering users in the developing world to participate and, in a short time, control the creation and dissemination of important learning materials. The Commonwealth of Learning sponsors the Virtual University for Small States of the Commonwealth, in which representatives of 30 countries collaborate to produce vocational and academic content for learning inside and outside of education institutions (*19*). With assistance from the Open University of the United Kingdom, the Teacher Education in Sub-Saharan Africa network—16 public tertiary institutions in eight countries—develops and disseminates open teacher training materials to support public school teachers. As yet, we lack data about the effect of these efforts on teaching and learning. But the process of production, delivery, and use may be as important as the initial measurable effects on traditional educational outcomes.

The increasing number and quality of open textbooks and open textbook providers represents a third application that could prove to be powerful, this one directly facilitated by student and faculty concern over the high cost of commercial textbooks. A fourth possibility stems from a growing interest in the medical community and around developing-world users in exploiting OER for public health and medical treatment knowledge.

My fifth example is also in the formative stage. It is possible now to create an open library of effective, high-quality, Web-based courses for secondary school and college students (*20*, *21*). Experts in content areas, teaching, Web application development, and cognitive science would develop the courses. The courses would have multimedia, interactive simulations, and cooperative learning opportunities, and they would incorporate AI-supported feedback loops to personalize the instruction. They would also be modular and extensible.

Carnegie Mellon University's (CMU) 11 Open Learning Initiative (OLI) courses most closely conform to the model suggested here. The courses use intelligent tutoring systems, simulations, and corrective feedback loops and contain the same content as regular CMU lecture courses.

In the fall of 2007, OLI undertook an important randomized trial (*22*). Students in CMU's beginning statistics course were asked to choose whether they wanted to take the semester-long course with traditional lectures, weekly discussion sessions, and optional access to the statistics tutor software (the control group) or the same course with the tutoring software as the instructional medium, no lectures, two optional weekly discussion sessions, and only half as long to complete the entire course (the experimental treatment group). The volunteers for the experimental treatment were randomly assigned into experimental and control course sections. The students in the control and experimental sections took the same in-class exams and, before and after the courses, an externally developed and standardized assessment of introductory statistics. The results were statistically similar for the two groups for the in-class exams, whereas the experimental group made significantly larger gains on the standardized assessment, indicating that the experimental group learned better and in half the time.

CMU is following up this experiment with similar studies in other content areas and with students from other colleges. If the findings stand, they will challenge our conventional thinking. Do we need lecturers in many of our courses? Is the lecture an efficient way of delivering high-quality instruction? Is the semester sacrosanct, or should we be trying to determine what kinds of learning can be accelerated?

Now imagine a 21st century library whose holdings would be very-high-quality software courses built to the specifications suggested earlier and informed by the experiences of successful software course developers, such as those at CMU. The cost of creating a library of 50 such secondary school courses and 50 early college courses would be roughly $300 million, less than 2% of California's current budget deficit (for 2009–2010). Another $100 million would provide a powerful set of performance and multiple-choice end-of-course assessments to measure individual student success and a dozen rigorous experiments to assess the effectiveness of the courses. These would be fixed, one-time-only costs. Continuing costs of $50 million a year would be required to maintain, update, evaluate, and house the courses. When completed and made available, every person and school in the world

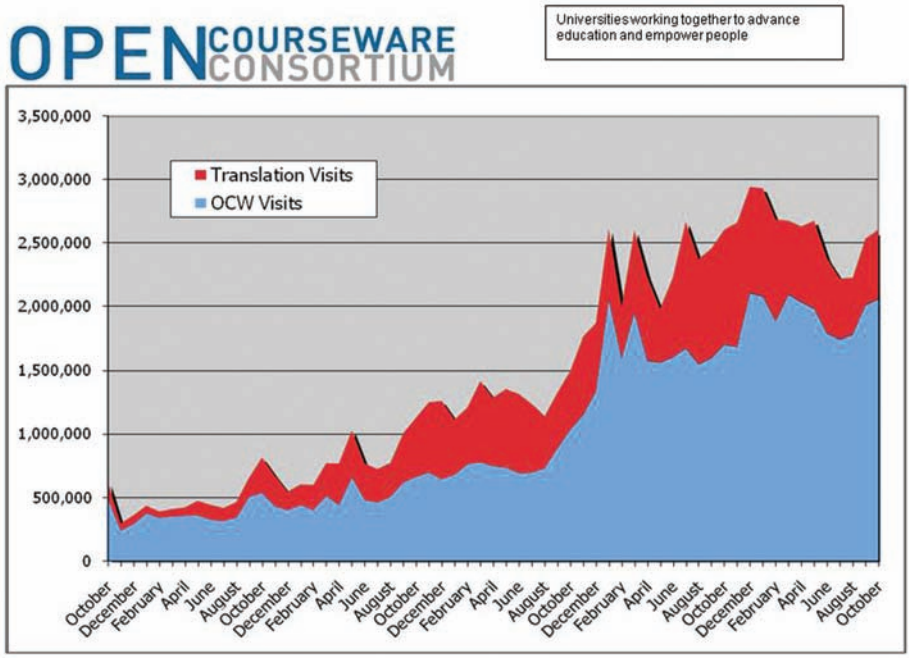

Fig. 3. This chart shows conservative estimates of the number of individual visits per month to OCWC sites from October 2003 to October 2008. The blue area represents visits to sites where the OCW is in the originally posted language, and the red area shows sites with translated versions of the OCW (*7*).

Fig. 4. One way of better understanding use is to go to the potential users with materials that have familiar designs and for advice about ways to improve the product. This photo shows Chinese students, in a middle school just outside of Xian, China, trying out an early version of an online, game-based, open learning course with cartoon characters, for teaching English to Chinese speakers.

would have access to an entire repertoire of high-quality high school courses (including elective and vocational courses) and an equivalent coverage for the first 2 years of many college programs.

Making the courses open rather than proprietary has several advantages. First, the courses would be immediately available to everyone. Second, the open nature of the courses would give institutions and individuals the rights to modify, adapt, and customize the courses for their purposes. Third, fully open materials would allow organizations to build professional services around the courses—such as professional development—providing a financial source for sustainability. Lastly, for non-enrolled students, a service could offer a fee-based proctored assessment. If students pass the assessment, the same service or a local school might reward them with credit, thereby replacing seat time with learning performance as the criterion for success.

This relatively small investment could have immediate ramifications in a nation with a troubled economy, a retiring teacher workforce, and a great demand for more highly skilled workers. On the other hand, successful implementation would have to overcome the weight of powerful cultural and social norms and practices. For example, widespread use would require a significant change in the role of some teachers from presenter to mentor.

This project would not be a universal solution. There are many skills and concepts that are better learned in classrooms taught by caring and competent teachers or in laboratories or in face-to-face groups of peers.

But the project could make a dramatic contribution beyond its own direct impact by establishing a standard for many instructional settings because of the quality of its content and its use of strategies for providing feedback and support for students. The capacity to quickly update a open Web-based course with new knowledge or based on new understandings of what parts of the instruction work or do not work to effectively teach students is another powerful argument for implementing the project.

Building Momentum

Although there have been many successes, the OER movement continues to face a substantial number of challenges. Two areas are important to the ultimate sustainability and the impact of OER on teaching and learning. Both require research.

The first has to do with the extent of use of OER in the developed and developing worlds. Without significant use, there will not be sustainability. But we do not know what the demand is for OER, how to use supply to increase demand, how to make use more probable, how best to measure use, or what we might consider to be little or great amounts of use. These questions pose complex and probably expensive design, measurement, and analysis problems (Fig. 4).

The other challenge concerns the question of effectiveness. One aspect of this question is easy. OER has expanded access to knowledge both by being open and by allowing adaptation to various contexts, although we are not sure about the extent of the expansion. But we do not have evidence, for example, about whether the act of modifying materials has special educational value. And, with a few exceptions, we know little about the effectiveness of individual OER applications in causing their users to learn.

To some extent the latter question reflects our lack of understanding about the effectiveness of technology applications in education. OER is an enabler. An OER application by its nature is more widely accessible and, if modified or otherwise adapted, potentially more appropriate for a target audience than a commercial application, but otherwise it is not technically different from such an application. We need research on effectiveness of using OER materials, but, if we knew more about commercial technology applications' effects on teaching and learning, we would also know more about the potential of OER.

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Nanodiamonds in the Younger Dryas Boundary Sediment Layer

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The Younger Dryas (YD) was a 1300-year-long interval during which deglacial warming in the Northern Hemisphere was interrupted by a return to cold conditions. Its cause remains unclear. Recently, it was hypothesized that multiple cometary airbursts barraged North America at the onset of the YD at ≈12.9 ± 0.1 thousand calendar years before the present (cal. yr B.P.) (*1*), triggering massive environmental changes, abrupt Pleistocene megafaunal extinctions, and the disappearance of the Clovis cultural assemblage (*2*). The YD boundary (YDB) is a distinctive layer containing above-background amounts of magnetic microspherules, iridium, and other impact proxies found with evidence for intense wildfire (*2*). It often occurs immediately beneath an organic-rich layer reported in unconsolidated terrestrial deposits across North America (*3*). A YDB peak in the abundance of magnetic microspherules has been independently confirmed at Murray Springs, Arizona (*3*). Because traditional impact markers, such as high-pressure-modified (shocked) minerals, breccias, tektites, and visible craters appear to be absent from 12.9-thousand-cal.-yr-B.P. North American sediments, one explanation for the observed evidence is an impact by a previously fragmented body, for example, Comet LINEAR (*4*), that produced a cluster of widespread air shocks, each analogous to the Tunguska impact in 1908 (*5*).

We report that nanometer-sized impact diamonds are abundant at multiple locations across North America in YDB sediments dating to 12.9 ± 0.1 thousand cal. yr B.P. Nanodiamonds (NDs) are associated with known impacts, during which they may arrive inside the impactor or form through shock metamorphism (*6*). We found subrounded, spherical, and octahedral crystallites, ranging in size from 2 to 300 nm, distributed within carbon spherules, suggesting crystallization from the amorphous carbon matrix. Selected area electron diffraction patterns (SADPs) display d-spacings typical of cubic diamonds (2.06, 1.26, and 1.08 Å) and often produce additional "forbidden" reflections indexed at 1.78, 1.04, and 0.796 Å, consistent with the n-diamond polymorph (*7*) known to occur in meteorites (*8*). Diamonds within carbon spherules peaked in the YDB at glacial Lake Hind, MB, Canada (Fig. 1A) and other localities across North America (Fig. 1 and fig. S1), estimated to range from ≈10 to 3700 parts per billion (ppb) by weight, amounting to >1 billion diamonds per cm^3, with the highest concentrations overlapping the range for the Cretaceous-Tertiary impact (*6*).

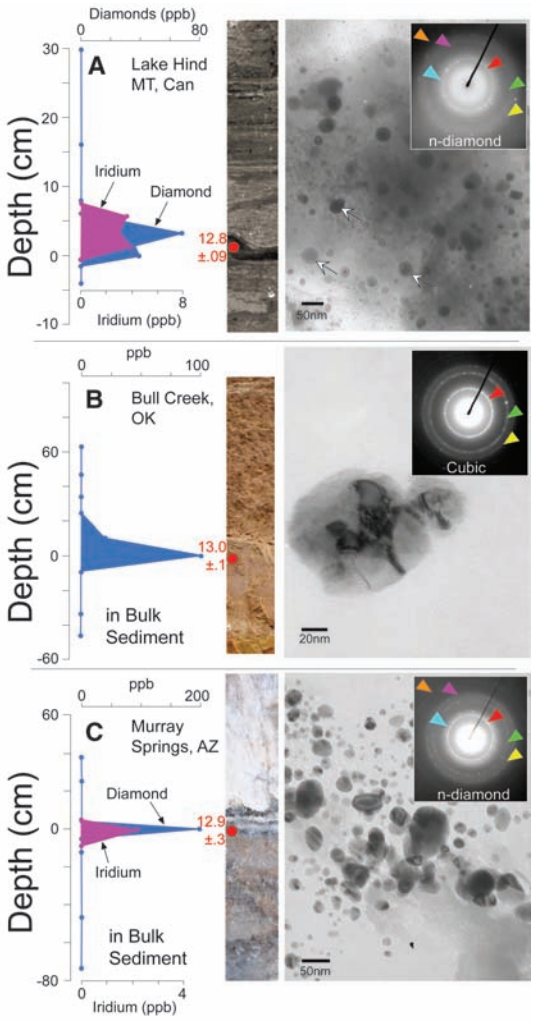

Fig. 1. TEM photomicrographs and SADPs of YDB NDs from (**A**) Lake Hind, MB, Canada; (**B**) Bull Creek, Oklahoma; and (**C**) Murray Springs, Arizona. Stratigraphic profiles on left show NDs only in the YDB and peaking with above-background iridium amounts at Lake Hind and Murray Springs (*1*). Red dots on stratigraphic profiles (center) show age estimates based on available calibrated radiocarbon dates (thousand cal. yr B.P.). Arrows on the photomicrograph from Lake Hind highlight examples of diamonds within the amorphous carbon matrix of a spherule. Color-coded arrows on SADPs (upper right corners) are indexed d-spacings (Å): red indicates 2.06; blue, 1.78; green, 1.26; yellow, 1.08; purple, 1.04; and orange, 0.796.

We also have found NDs outside carbon spherules in bulk sediments at Murray Springs, Arizona, and Bull Creek, Oklahoma. Transmission electron microscopy (TEM) images and SADPs indicate that n-diamonds have abundances ranging from ≈100 to 200 ppb in YDB bulk sediments at both localities. In addition, we recovered typical cubic diamonds in samples from Bull Creek, Oklahoma, which are more angular and range in size from 1 to 50 nm. NDs were not detected above or below the YDB layer at any site tested.

Cubic diamonds form under high temperature-pressure regimes, whereas n-diamonds crystallize under lower temperature-pressure conditions (*7*). Both form outside the range of Earth's surficial processes but may crystallize during cosmic impacts (*6*). These data support the hypothesis that a swarm of comets or carbonaceous chondrites produced multiple air shocks and possible surface impacts at ≈12.9 ± 0.1 thousand cal. yr B.P. (*2*). Such a rare event would have had abrupt environmental consequences and severe repercussions for plants, animals, and humans in North America.

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Supporting Online Material

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Fig. S1
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Glucosinolate Metabolites Required for an *Arabidopsis* Innate Immune Response

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The perception of pathogen or microbe-associated molecular pattern molecules by plants triggers a basal defense response analogous to animal innate immunity and is defined partly by the deposition of the glucan polymer callose at the cell wall at the site of pathogen contact. Transcriptional and metabolic profiling in *Arabidopsis* mutants, coupled with the monitoring of pathogen-triggered callose deposition, have identified major roles in pathogen response for the plant hormone ethylene and the secondary metabolite 4-methoxy-indol-3-ylmethylglucosinolate. Two genes, *PEN2* and *PEN3*, are also necessary for resistance to pathogens and are required for both callose deposition and glucosinolate activation, suggesting that the pathogen-triggered callose response is required for resistance to microbial pathogens. Our study shows that well-studied plant metabolites, previously identified as important in avoiding damage by herbivores, are also required as a component of the plant defense response against microbial pathogens.

Although plants are in continual contact with potential pathogens, a successful infection is rare. The ability of a particular plant species to prevent the successful colonization by a given pathogen species is referred to as nonhost resistance (*1*–*3*). The molecular basis of nonhost resistance is poorly understood, but presumably relies on both constitutive barriers and inducible responses. Inducible defenses include the surveillance along the cell membrane from multiple pattern recognition receptors that respond to highly conserved microbe-associated molecular pattern (MAMP) molecules such as bacterial flagellin or peptidoglycan (*4*, *5*). MAMP recognition triggers the activation of mitogen-activated protein kinases (MAPKs) and various hormone signaling pathways (*6*). This signaling starts a cascade that activates a variety of defense responses including callose deposition, programmed cell death, production and accumulation of antimicrobial reactive oxygen species, and induction of phytoalexins and other secondary metabolites such as the indolic antimicrobial compound camalexin (3-thiazol-2′yl-indole) and glucosinolates (1-thio-β-D-glucosides) (*3*), which are found in cruciferous plants, including *Arabidopsis thaliana*. These plants also constitutively synthesize and store glucosinolates, which are converted by endogenous *S*-glycosyl hydrolases (myrosinases) into compounds that function as insect feeding and/or oviposition stimulants or deterrents (*7*). However, glucosinolates and their breakdown products have also been identified as potential antimicrobials (*8*, *9*).

Despite detailed characterization of MAMP recognition and various hormone-mediated signaling pathways in plant defense response, relatively little is known about the host mechanisms that connect the perception of a particular pathogen to the downstream signaling pathways that lead to activation of specific immune responses.

Flg22-induced callose response requires FLS2, PMR4, Et signaling, and MYB51. *Arabidopsis* genes induced in response to treatment with Flg22, a synthetic 22–amino acid polypeptide that corresponds to a highly conserved region of eubacterial flagellin, include those involved in ethylene (Et)–mediated defense hormone signaling and in indole glucosinolate biosynthesis (table S1A). We performed a phenotypic assay for Flg22-induced callose responses in *Arabidopsis* seedlings under the same conditions as those of previous transcriptional profiling studies (*10*). Callose is a β(1,3) glucan polymer that strengthens and dams weak or compromised sections of plant cell walls at the site of pathogen attack. A callose-response assay was used to identify particular Flg22-induced genes involved in callose deposition. Aniline blue staining was used to detect callose, and we observed deposits on the cotyledons of *Arabidopsis* seedlings treated with ≥1 μM Flg22 (Fig. 1B) that were absent in water-treated plants (Fig. 1A). Mutants lacking the functional Flg22 receptor encoded by the *FLS2* gene (*11*) or the functional callose synthase encoded by the *PMR4* gene (*12*) did not respond to Flg22 treatment (Fig. 1, C and D), demonstrating that the appearance of these fluorescent deposits was a consequence of MAMP perception and subsequent callose synthesis. A more sensitive assay of callose staining in the *pmr4-1* mutant revealed faint fluorescent flecks (fig. S2A), suggesting that another callose synthase plays a minor role in response to Flg22. The callose response was also elicited by other MAMPs and was not specific to Flg22 (SOM Text and fig. S2B). The perception of Flg22 in roots requires both the Flg22 receptor FLS2 and its receptor complex partner, BAK1 (*13*, *14*). In contrast, the Flg22-induced callose response in cotyledons does not require BAK1 (table S1B), suggesting that the immune requirement for BAK1 in the FLS2 receptor complex is tissue-specific.

We screened a collection of *Arabidopsis* defense hormone-related mutants, as well as mutants defective in various Flg22-inducible transcription factors (see table S1B for all mutants screened), and found that *etr1-1*, *etr1-3*, *ein2-1*, and *ein2-5* mutants in the Et signaling pathway, as well as two transcription factor mutants *myb51-1* and *myb51-2*, were impaired in the Flg22-induced callose response (Fig. 1, E to G, and fig. S2, C to E). ETR1, an Et receptor, and EIN2, a membrane protein, are required for Et perception and signaling, respectively (*15*, *16*). MYB51 is involved in the transcriptional activation of indole glucosinolate (IGS) biosynthetic genes (*17*).

MYB51 is downstream of Et signaling and upstream of IGS biosynthesis. Figure 1, A to G, shows that the Et signaling pathway and the MYB51 transcription factor may function in the callose-forming pathway. We examined the expression of candidate genes in Flg22-treated wild-type and mutant seedlings and observed that the expression of the Et-responsive transcription factor *ERF1* (which is up-regulated by Flg22; table S1A) is significantly reduced in *etr1-1* and *ein2-1* mutants but not in *myb51-1* and *myb51-2* mutants (Table 1). This indicates that Et signaling is intact in *myb51* mutants. In contrast, Flg22-induced expression of *MYB51* is reduced in *etr1-1* and *ein2-1* mutants (Table 1), indicating that Et signaling is required for the full induction of MYB51 and ERF1 in response to Flg22 and that MYB51 is downstream of Et signaling for the callose response. Furthermore, Flg22-induced expression of all known IGS biosynthetic genes (*CYP79B2*, *CYP79B3*, *CYP83B1*, *SUR1*, *UGT74B1*, and *AtST5a*; fig. S1) was significantly reduced in *myb51* mutants (Table 1), indicating that MYB51 up-regulates IGS biosynthesis in response to Flg22. A homolog of MYB51, ATR1/MYB34, also regulates IGS abundance (*18*), but Flg22-elicited callose deposition was not affected in *atr1-3* mutant seedlings (Fig. 1H), and when treated with Flg22, *ATR1/MYB34* expression was down-regulated (table S1A) (*6*).

Indole glucosinolates are derived from tryptophan, the biosynthesis of which is defense-regulated by Et-induced expression of *ASA1*. ASA1 catalyzes the first and rate-limiting step in the tryptophan biosynthetic pathway (*19*, *20*). *ASA1* gene expression was also induced by Flg22 treatment (table S1A), and this up-regulation was dependent on MYB51 (Table 1). Because *ASA1*

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expression is Et-inducible, these data suggest that MYB51 probably mediates at least some transcriptional responses to Et signaling.

IGS biosynthesis is required for Flg22-induced callose response. The drastically reduced expression of IGS biosynthetic genes in *myb51* mutants suggested that IGS biosynthesis may be required for Flg22-induced callose deposition. Therefore, mutants defective in IGS biosynthesis or accumulation were tested for the callose response to Flg22. The IGS biosynthetic double mutant *cyp79B2 cyp79B3*, which completely lacks IGS (*21*), as well as mutants *atr4-1/cyp83B1* and *ugt74B1-2*, were impaired in the induction of the callose response (Fig. 1, J to L). Furthermore, *tfl2* mutants, which have reduced IGS amounts in their leaves (*22*, *23*), were also impaired in Flg22-induced callose response (table S1B and fig. S2F).

In contrast to mutations that affect the biosynthesis of indole glucosinolates, the aliphatic glucosinolate biosynthetic mutant *ref2/cyp83A1* (*24*) exhibited a wild-type response to Flg22 (Fig. 1M), indicating that aliphatic glucosinolates do not play a major role in callose accumulation. The hypothesis that biosynthesis and accumulation of indole glucosinolates are specifically required for the Flg22-induced callose response is consistent with the down-regulation of *CYP83A1* expression in response to Flg22 (table S1A) (*6*), as well as the expression of *MYB28* and *MYB29* (table S1A) (*6*), which regulate the aliphatic glucosinolate biosynthetic pathway (*25*–*27*). Finally, the tryptophan biosynthetic mutant *trp3-1* (*28*) was also found to have a greatly reduced callose response to Flg22 (Fig. 1N), consistent with the requirement of tryptophan for IGS biosynthesis.

Flg22 also induces the expression of *CYP71A13* and *PAD3* (table S1A) (*29*, *30*), which are genes involved in the biosynthesis of the indole phytoalexin, camalexin. Like IGS, camalexin requires CYP79B2 and CYP79B3 for biosynthesis; however, the camalexin biosynthetic mutants *cyp71A13-1*, *cyp71A13-3*, and *pad3-1* exhibited a wild-type callose response to Flg22 (Fig. 1, O and P, and fig. S2G), showing that camalexin is not required for the callose response.

4-Methoxy-I3G is required for the Flg22-induced callose response. Our data indicate that the synthesis of IGS is required for callose deposition in response to Flg22 and suggest that IGS functions as a signal or coactivator downstream of MAMP responses. Because of the large number of cytochrome P450s involved in IGS biosynthesis, we searched for other Flg22-inducible cytochrome P450 genes and found one with unassigned function, *CYP81F2* (table S1A). The mutants *cyp81F2-1* and *cyp81F2-2* showed a complete loss of callose deposition in response to Flg22 (Fig. 1I and fig. S2H), suggesting that *CYP81F2* might also be involved in IGS biosynthesis. Unlike the characterized IGS biosynthetic genes *CYP79B2*, *CYP79B3*, *CYP83B1, SUR1*, *UGT74B1*, and *AtST5a* (fig. S1), the induced expression of *CYP81F2* after 3 hours of Flg22 treatment was independent of Et signaling and MYB51 (Table 1). At 6 hours of Flg22 treatment, however, continued *CYP81F2* expression became dependent on Et signaling but remained independent of MYB51 (Table 1).

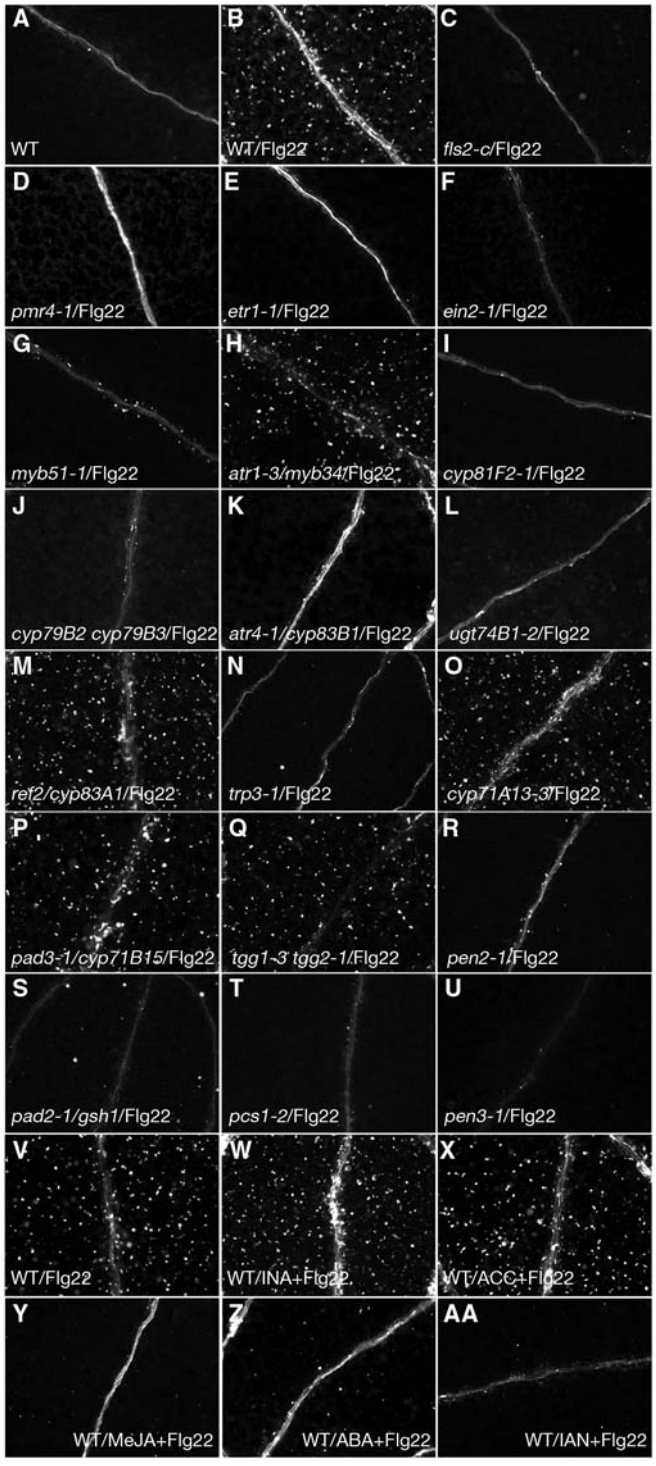

Fig. 1. IGS biosynthesis and hydrolysis are required for Flg22-induced callose formation. (**A** to **U**) Cotyledons of seedlings treated with water (A) or Flg22 (B to U). (A and B) Wild type. (C) *fls2-c*. (D) *pmr4-1*; traditional staining method. (E) *etr1-1*. (F) *ein2-1*. (G) *myb51-1*. (H) *atr1-3/myb34*. (I) *cyp81F2-1*. (J) *cyp79B2 cyp79B3*. (K) *atr4-1/cyp83B1*. (L) *ugt74B1-2*. (M) *ref2/cyp83A1*. (N) *trp3-1*. (O) *cyp71A13-3*. (P) *pad3-1/cyp71B15*. (Q) *tgg1-3 tgg2-1*. (R) *pen2-1*. (S) *pad2-1/gsh1*. (T) *pcs1-2*. (U) *pen3-1*. (**V** to **AA**) Cotyledons pretreated with water (V), INA (W), ACC (X), MeJA (Y), or ABA (Z) for 24 hours and then treated with Flg22 (V to Z) or simultaneously treated with IAN and Flg22 (AA). Shown are representative examples of 40 to 60 cotyledons from two independent experiments per genotype.

To further ascertain the biochemical function of CYP81F2, we carried out IGS metabolic profiling experiments on a variety of mutants (see below) to look for correlations between callose-deficient phenotypes and abundances of the three known IGS species [indol-3-ylmethylglucosinolate (I3G), 4-methoxy-I3G, and 1-methoxy-I3G]. For all tested genotypes, Flg22 treatment caused a significant reduction in I3G (Fig. 2A), a counterintuitive result given that Flg22 treatment activates the expression of IGS biosynthetic genes. This result suggested that Flg22 also activates the expression of myrosinase enzyme(s) (*S*-glycosyl hydrolases) that catalyze the hydrolysis of IGS (see below).

In wild-type seedlings, Flg22 treatment produced no effect on 4-methoxy-I3G abundance; however, in Flg22-treated *myb51-1* and *ein2-1* mutants, 4-methoxy-I3G abundances were significantly reduced compared to those in wild-type seedlings

Table 1. Expression analysis of IGS biosynthetic and hydrolytic genes. MYB51 is downstream of Et signaling and upstream of IGS biosynthesis. Ratio of mRNA abundance in mutant relative to wild-type plants after Flg22 treatment as determined by quantitative reverse transcription–polymerase chain reaction (qRT-PCR). Data are from three replicate samples with pairwise comparisons to wild-type plants. ND, not determined. HAT, hours after treatment with Flg22.

Genotype	HAT	PAMP-inducible gene												
		PEN2	*PCS1*	*PEN3*	*CYP81F2*	*ERF1*	*MYB51*	*ASA1*	*CYP79B2*	*CYP79B3*	*CYP83B1*	*SUR1*	*UGT74B1*	*AtST5a*
etr1-1	3	0.73	1.01	0.67	0.56	0.07*	0.25*	0.08*	0.06*	0.77	0.14*	ND	ND	ND
	6	0.97	1.10	0.96	0.31*	0.10*	0.17*	0.10*	0.04*	0.88	0.19*	ND	ND	ND
ein2-1	3	1.04	1.07	0.91	0.80	0.11*	0.31*	0.20*	0.13*	1.12	0.54	ND	ND	ND
	6	0.85	0.80	1.29	0.36*	0.05*	0.13*	0.13*	0.09*	0.75	0.40*	ND	ND	ND
myb51-1	3	1.24	1.16	1.39	1.41	0.99	0.00*	0.25*	0.26*	0.07*	0.09*	0.37*	0.18*	0.12*
	6	0.86	1.31	0.81	0.62	0.83	0.06*	0.24*	0.18*	0.06*	0.18*	0.36*	0.27*	0.19*
myb51-2	3	1.24	1.18	1.61	1.62	0.86	0.13*	0.32	0.29*	0.12*	0.13*	0.43*	0.22*	0.19*
	6	1.09	0.83	0.91	0.74	0.86	0.00*	0.17*	0.07*	0.05*	0.08*	0.28*	0.14*	0.12*

*$P < 0.01$, two-tailed t test; false discovery rate < 0.05.

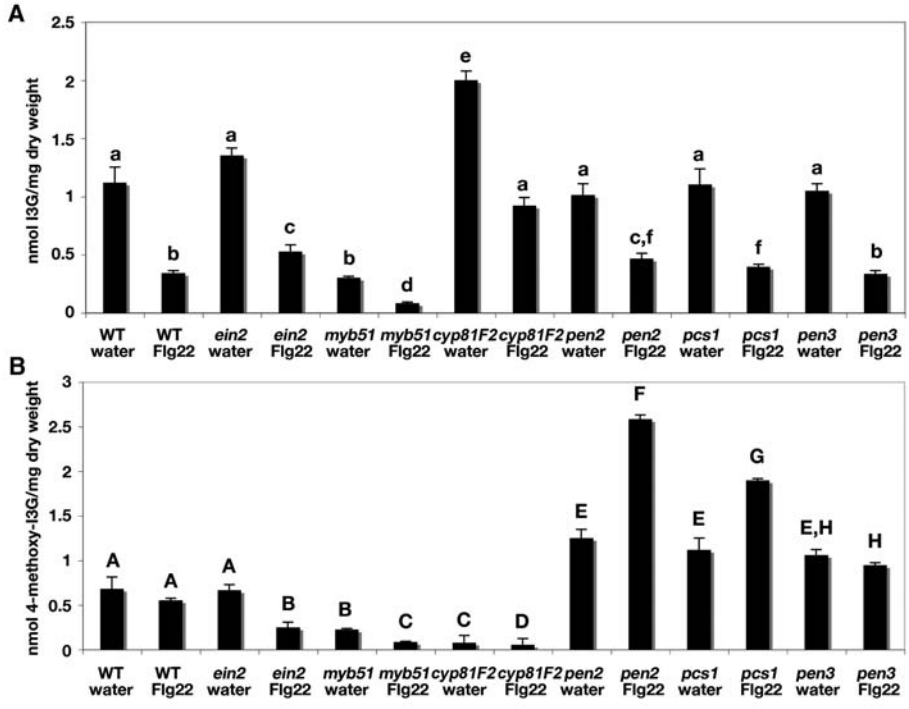

Fig. 2. Blocking IGS biosynthesis or hydrolysis depletes or elevates 4-methoxy-I3G amounts, respectively. Upon Flg22 elicitation, I3G amounts (**A**) are reduced in wild-type and mutant seedlings, and 4-methoxy-I3G amounts (**B**) are reduced in *ein2-1, myb51-1*, and *cyp81F2-1* mutants, and elevated in *pen2-1*, *cad1-3* (a null *pcs1* allele), and *pen3-1* mutants. Data represent the mean ± SD of four replicate samples. Different letters above the bars denote statistically significant differences ($P < 0.01$, two-tailed t test).

(Fig. 2B). These results suggest that the perception of Flg22 triggers both the biosynthesis and subsequent hydrolysis of 4-methoxy-I3G. Furthermore, 4-methoxy-I3G amounts were extremely low in the *cyp81F2-1* mutant in control (water) and Flg22 treatments (Fig. 2B), suggesting that CYP81F2 may produce 4-methoxy-I3G via the 4-methoxylation of I3G. No consistent effect on 1-methoxy-I3G abundance was observed after Flg22 treatments (fig. S3), suggesting that it is not required for the Flg22-induced callose response. Therefore, we hypothesize that Flg22 perception activates the biosynthesis of MYB51- and CYP81F2-dependent 4-methoxy-I3G, accompanied by a Flg22-triggered hydrolysis of 4-methoxy-I3G into callose-eliciting compound(s).

PEN2 is the putative myrosinase involved in Flg22-triggered IGS breakdown. The Flg22-elicited reduction in I3G abundance suggests that the Flg22-triggered IGS biosynthesis may also be accompanied by IGS hydrolysis and that IGS hydrolytic products may be required for callose synthesis. IGS hydrolysis is brought about by myrosinase enzymes and their associated modifiers, and ascorbate is an essential cofactor for myrosinases (*31*). Consistent with the hypothesis that IGS hydrolysis is required for Flg22-induced callose deposition, the ascorbate-deficient mutants *vtc1-1* and *vtc2-1*, which contain 10 to 30% wild-type amounts of ascorbic acid (*32*), exhibited a greatly reduced callose response to Flg22 (Fig. 4G and fig. S2I). However, the double-mutant *tgg1-3 tgg2-1*, carrying lesions in the two characterized foliar myrosinases in *Arabidopsis* (*33*), exhibited a wild-type callose response to Flg22 (Fig. 1Q). A search for predicted glycosyl hydrolases with a Flg22-inducible expression profile identified *PEN2* (table S1A). Corresponding mutants exhibit enhanced penetration by the nonadaptive fungal pathogen *Blumeria graminis* f. sp. *hordei* (*34*, *35*). Both *pen2-1* and *pen2-2* mutants exhibited a loss of the callose response to Flg22 (Fig. 1R and fig. S2J), indicating that PEN2 is the putative myrosinase enzyme involved in the Flg22-induced hydrolysis of IGS [see also (*36*) for direct biochemical evidence supporting this hypothesis].

In contrast to the *myb51-1* and *cyp81F2-1* mutants, the IGS profile of the *pen2-1* mutant shows increased accumulation of 4-methoxy-I3G upon Flg22 treatment (Fig. 2B), supporting the hypothesis that PEN2 acts as a myrosinase catalyzing the hydrolysis of 4-methoxy-I3G. Furthermore, in the absence of PEN2, Flg22-treated plants exhibit up-regulation of IGS biosynthesis and 4-methoxylation, and the substrate 4-methoxy-I3G accumulates. The correlation between reduced and/or depleted amounts of 4-methoxy-I3G in the *cyp81F2-1* mutant and accumulation of 4-methoxy-I3G in the *pen2-1* mutant and the callose-deficient phenotypes of both the *cyp81F2-1* and *pen2-1* mutants suggest that a hydrolytic product of 4-methoxy-I3G functions as a signaling molecule or coactivator for callose deposition.

Our results suggest not only that the IGS biosynthetic pathway is required for callose deposition but also that the IGS biosynthetic pathway may be under feedback inhibition by elevated amounts of 4-methoxy-I3G and that this feedback regulation occurs at the level of *MYB51* expression, consistent with previous reports that glucosinolates regulate their biosynthesis by feedback inhibition of relevant transcription factors (*37*): In the *pen2-1* mutant, Flg22-induced expression of *MYB51* and IGS biosynthetic genes was greatly reduced after Flg22 elicitation (Table 2). *PEN2* expression is independent of Et signaling and MYB51 (Table 2), indicating that Flg22-elicited activation of IGS breakdown is independent of IGS biosynthesis. Mutants of the modifiers of myrosinase-catalyzed reactions, *esp* and *esm1* (*38*, *39*), as well as the

Columbia-0 (Col-0) *ESP*-overexpressing transgenic line (Col-0 is a natural *esp* mutant), exhibited a wild-type callose response to Flg22 (fig. S2, K and L, and table S1B), suggesting that other, as yet unidentified associated modifier proteins may catalyze MAMP-triggered glucosinolate activation in conjunction with PEN2. Consistent with these results, recent work has shown the presence of an ESP-independent nitrile-forming IGS activation in *Arabidopsis* (*40*).

PCS1 is required for Flg22-triggered IGS activation. Glutathione may function in IGS biosynthesis (*41*), and consistent with this, glutathione biosynthetic mutants (*pad2-1* and *cad2-1*) (*42*, *43*) exhibited reduced callose response to Flg22 (Fig. 1S and fig. S2M). The *PAD2* (or *GSH1*) gene encodes a γ-glutamylcysteine synthetase (*42*), which catalyzes the first committed step in the synthesis of the tripeptide glutathione (GSH). Glutathione is a precursor for a class of heavy-metal–chelating glutathione polymers known as phytochelatins (*44*). Microarray data of Flg22-inducible expression profiles identified a phytochelatin synthase gene, *PCS1* (table S1A), whose corresponding mutants (*pcs1-1*, *pcs1-2*, and *cad1-3*) were all impaired in the callose response to Flg22 (Fig. 1T, fig. S2N, and table S1B). Moreover, the transcriptional and IGS profiles of the *pcs1* mutant resemble those of the *pen2* mutant (Fig. 2B), suggesting that it too is involved in the breakdown of 4-methoxy-I3G. Like *pen2*, the *pcs1* mutant also exhibited reduced Flg22-induced expression of *MYB51* and IGS biosynthetic genes (Table 2), supporting the conclusion that accumulation of IGS is involved in feedback inhibition of IGS biosynthesis at the level of *MYB51* expression. Also, like *PEN2*, the Flg22-induced expression of *PCS1* is independent of Et signaling and MYB51 (Table 1).

PEN3 is also required for Flg22-induced IGS activation. Phytochelatin synthases require the cofactor cadmium for enzymatic activity (*44*) and may be involved in the transport and sequestration of cadmium into the vacuole (*43*, *45*). ABC-type transporters in plants and yeast also transport and sequester cadmium into the vacuole and may work in concert with phytochelatins (*46*–*48*). We searched the Flg22-elicited transcriptional profiling data and identified one ABC transporter, *PEN3/AtPDR8* (table S1A) (*49*, *50*), whose corresponding mutants (*pen3-1* and *atpdr8-2/pen3*) were also impaired in the callose response to Flg22 (Fig. 1U and fig. S2O). Furthermore, like the *pen2* and *pcs1* mutants, the *pen3* mutant accumulated 4-methoxy-I3G upon Flg22 treatment (Fig. 2B), suggesting that it too is involved in IGS breakdown. However, unlike the *pen2* and *pcs1* mutants, the *pen3* mutant did not exhibit diminished *MYB51* expression after 6 hours of Flg22 treatment (Table 2). PEN3 localizes to the plasma membrane, not the vacuole (*49*, *50*), and is involved in the extrusion of cadmium out of the cell (*51*), which may provide a targeting mechanism to direct cadmium-chelating phytochelatin-bound complexes of IGS hydrolytic products toward the plasma membrane/cell wall where the callose synthase PMR4 resides. These observations may explain the lack of presumptive feedback inhibition of *MYB51* expression in the *pen3-1* mutant and why increased amounts of 4-methoxy-I3G in the *pen3-1* mutant are not as high as those in the *pen2-1* and *pcs1-2* mutants (Fig. 2B).

4-Methoxy-I3G rescues callose formation. Callose formation was induced by 100 μM 4-methoxy-I3G in conjunction with Flg22 treatment in mutants defective in IGS biosynthesis or 4-methoxylation of I3G (Fig. 3, D to F) but not in mutants defective in IGS hydrolysis (Fig. 3, G to I), Flg22 perception (Fig. 3B), or callose synthesis (Fig. 3C). This supports the hypothesis that 4-methoxy-I3G is involved as a signaling molecule in, or a potential coactivator of, callose deposition. I3G had no effect on callose-deficient mutants (Fig. 3), indicating that it is not involved in Flg22-induced callose response. Identifying breakdown products of 4-methoxy-I3G associated with the Flg22-induced callose response will be technically challenging as these compounds may be unstable and it is not feasible to purify large quantities of them from *Arabidopsis*. A presumed IGS hydrolytic product, 4-methoxy-indole-3-acetonitrile (4-methoxy-IAN), has been purified from Chinese cabbage, a close relative of *Arabidopsis*, but neither 4-methoxy-IAN nor the endogenous IGS hydrolytic products 4-methoxy-indole-3-carboxylate and

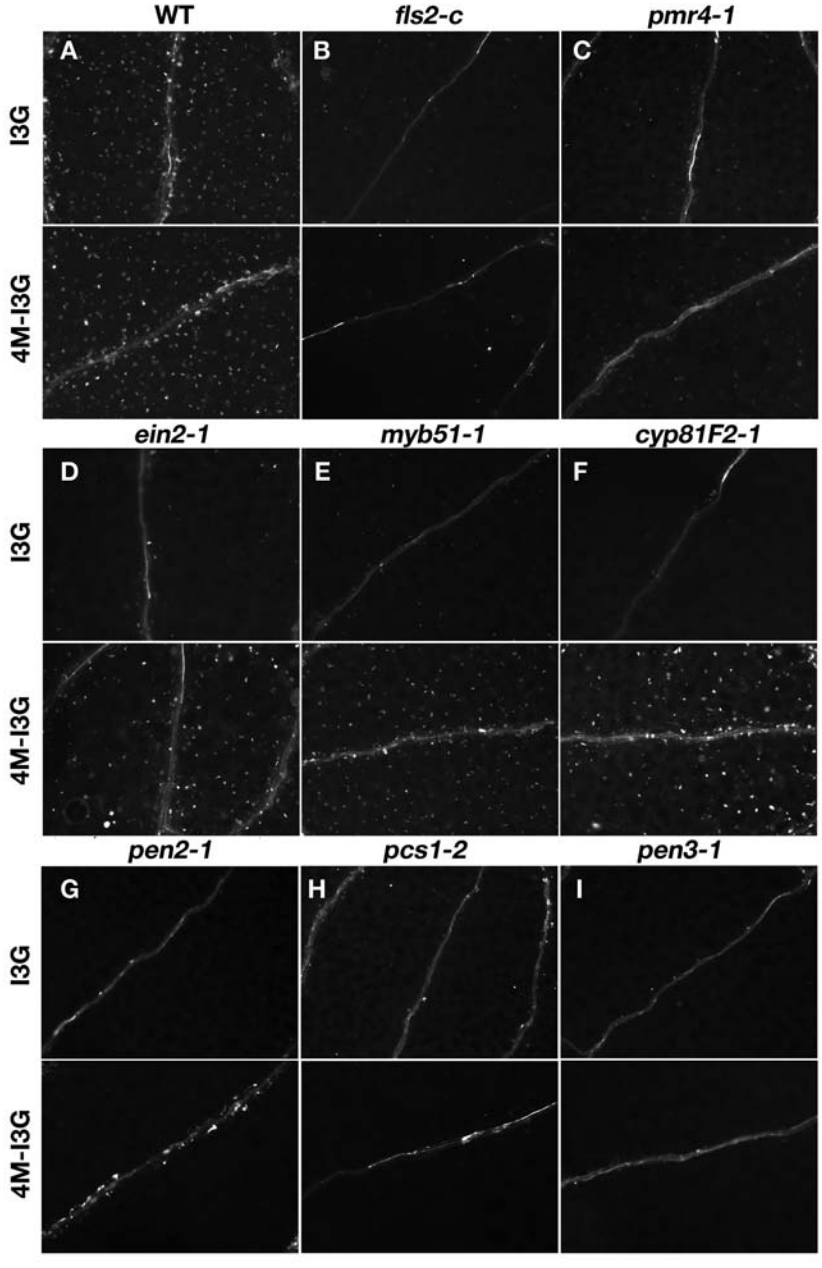

Fig. 3. 4-Methoxy-I3G induces callose formation in IGS biosynthetic mutants. (**A** to **I**) Cotyledons of seedlings simultaneously treated with Flg22 and I3G or 4-methoxy-I3G (4M-I3G). (A) Wild type. (B) *fls2-c*. (C) *pmr4-1*. (D) *ein2-1*. (E) *myb51-1*. (F) *cyp81F2-1*. (G) *pen2-1*. (H) *cad1-3/pcs1*. (I) *pen3-1*.

methyl-4-methoxy-indole-3-carboxylate rescued callose-deficient mutants (table S1B), suggesting that they probably are not directly involved in callose deposition.

The parallel SA-dependent IGS hydrolytic pathway. Microbes can trigger callose formation in *Arabidopsis* leaves via the plant hormone salicylic acid (SA)–dependent pathway (*52*), suggesting that there are multiple signaling pathways in MAMP-induced callose formation. We added hormones to rescue callose-deficient mutants and found that pretreatment with SA or the functional SA analog 2,6-dichloro-isonicotinic acid (INA) (*53*) rescued the callose-deficient phenotype of the *pen2*, *pcs1*, and ascorbate-deficient *vtc* mutants (Fig. 4, B and C, E and F, and H and I, respectively). This suggests that SA compensates for deficient PEN2 myrosinase activity and that PCS1 activity is necessary for PEN2 function. This SA-mediated rescue was not seen with any other described callose mutants (Fig. 4, J to M), and the transcriptional and IGS profiles of *pen2* and *pcs1* mutants were unchanged (Table 2 and fig. S4A). No other hormones were found to rescue callose-deficient mutants (table S1B). Because the double mutants *pen2-2 pen2-like* [PEN2-like (At3g60120; table S1A)] and *pcs1-1 pcs2-1* [PCS2 (*54*)] exhibited a Flg22-triggered callose response in the presence of SA (Fig. 4, N and O), this removes the possibility of functional redundancy. An SA-mediated pathway apparently can bypass the requirement of PEN2 and PCS1 in IGS hydrolysis through an as yet unknown mechanism.

Glucosinolate-dependent callose deposition restricts bacterial growth. A published report has shown that PMR4-dependent callose deposition contributes to MAMP-induced growth suppression of the type III secretion system–deficient bacterial pathogen *Pseudomomas syringae* PtoDC3000*hrcC* (*55*). In our seedling growth assay, the Flg22 receptor mutant *fls2-c*, the IGS biosynthetic mutants *ein2-1* and *cyp81F2-1*, and the IGS hydrolytic mutant *pen2-1* were slightly but significantly more susceptible to wild-type PtoDC3000 (fig. S5), suggesting that glucosinolate-dependent callose deposition probably contributes to MAMP-induced growth suppression of PtoDC3000. This is in accord with the finding that PtoDC3000 actively suppresses pathogen-activated callose deposition (*56*). This growth suppression was not observed in the *pmr4-1* mutant (fig. S5), probably because of the increased SA amounts in the mutant (*12*).

Suppressing the Flg22-triggered callose response. Defense-related plant hormones [SA, methyljasmonate (MeJA), abscisic acid (ABA), and the Et precursor 1-aminocyclopropane-1-carboxylic acid (ACC)] and the common IGS hydrolytic product IAN did not induce callose formation in the absence of Flg22. In the presence of Flg22, pretreatment with SA or ACC had no effect on the callose response (Fig. 1, W and X). In contrast, pretreatment with ABA or MeJA or treatment with IAN completely suppressed the Flg22-induced callose response (Fig. 1, Y to AA). ABA, MeJA, and IAN may differ in their modes of callose suppression. By 6 hours after Flg22 elicitation, ABA pretreatment greatly reduced Flg22-induced expression of *ERF1*, *MYB51*, and *CYP81F2*, which depend on Et signaling for full activation (Table 3), suggesting that ABA antagonizes the Et signaling triggered by Flg22. This is consistent with previously reported antagonistic interactions between ABA and Et signaling pathways (*57*, *58*).

In contrast to ABA, MeJA pretreatment transiently reduced transcript abundance of *MYB51* and *CYP81F2*, but after 6 hours of Flg22 treatment, gene expression returned to wild-type levels (Table 3). MeJA and JA induce I3G and 1-methoxy-I3G but not 4-methoxy-I3G production in mature plants (*59*–*61*), and we also observed this in seedlings (fig. S4). It is possible that MeJA blocks callose deposition by inducing the *N*-methoxylation pathway, which could predominate over the Flg22-induced 4-methoxylation pathway, thereby reducing the production of 4-methoxy-I3G required for callose formation. However, this does not appear to be the case because 4-methoxy-I3G abundances were unchanged in both the wild-type and the 4-

Table 2. *pen2* and *pcs1* mutants exhibit feedback inhibition at the level of *MYB51* expression. Ratio of mRNA abundance in mutant and/or INA-pretreated relative to water-pretreated wild-type plants after Flg22 treatment as determined by qRT-PCR. HAT, hours after treatment with Flg22.

Genotype/ chemical	HAT	PAMP-inducible gene												
		PEN2	*PCS1*	*PEN3*	*CYP81F2*	*ERF1*	*MYB51*	*ASA1*	*CYP79B2*	*CYP79B3*	*CYP83B1*	*SUR1*	*UGT74B1*	*AtST5a*
pen2-1	3	0.17*†	0.77	0.89	1.16	1.06	0.48*†	0.25*†	0.30	0.10*†	0.20*†	0.39*†	0.21*†	0.17*†
	6	0.13*†	1.10	0.60	0.52*†	0.42	0.40*†	0.19*†	0.15*†	0.34*†	0.28*†	0.37*†	0.19*†	0.24*†
pen2-1/INA	3	0.19*‡	0.55	0.98	0.96	1.05	0.52*‡	0.51*‡	0.46*‡	0.09*‡	0.31*‡	0.60*‡	0.29*‡	0.28*‡
	6	0.22*‡	1.45	0.54*	0.74*‡	0.51*‡	0.59*‡	0.40*‡	0.43*‡	0.38*‡	0.35*‡	0.66	0.33*‡	0.41*‡
pcs1-2	3	1.12	0.08*†	0.89	1.49*†	0.81	0.67*†	0.32*†	0.19*†	0.29*†	0.32*†	0.48*†	0.30*†	0.24*†
	6	1.00	0.22*†	0.94	1.21	0.63	0.80	0.49*†	0.41*†	0.17*†	0.50	0.64*†	0.54*†	0.51*†
pcs1-2/INA	3	0.89	0.12*‡	0.98	1.97	1.26	0.77*‡	0.76*‡	1.04*‡	0.27*‡	0.46*‡	0.79*‡	0.43*‡	0.46*‡
	6	0.86	0.25*‡	0.91	1.08	1.10	0.88	0.79	0.49*‡	0.23*‡	0.67	0.89	0.62	0.56*‡
WT/INA	3	0.78	0.86	0.98	1.57*†	0.90	1.85*†	2.69*†	6.12*†	1.29	1.80*†	2.04*†	2.05*†	1.81*†
	6	0.72	1.31	0.83	0.99	0.88	1.17	1.33	1.64	1.69	1.14	1.14	1.00	0.97
pen3-1	3	1.19	1.04	1.09	0.82	1.40	0.40*†	0.32*†	0.14*†	0.19*†	0.30*†	0.42*†	0.26*†	0.24*†
	6	1.20	1.46	1.15	1.10	1.21	0.85	0.46	0.53	0.39	1.30	0.86	0.97	0.96

*P < 0.01, two-tailed t test; false discovery rate < 0.05. †Pairwise comparisons to water-pretreated wild-type plants. ‡Pairwise comparisons to INA-pretreated wild-type plants.

Table 3. Suppressors of Flg22-induced callose response differ in their modes of inhibition. Ratio of mRNA abundance in suppressor-pretreated relative to water-pretreated wild-type plants after Flg22 treatment as determined by qRT-PCR. Pairwise comparisons to water pretreatment. HAP, hours after pretreatment with ABA, IAN, or MeJA; HAT, hours after treatment with Flg22.

Suppressor	HAP	HAT	PAMP-inducible gene								
			PEN2	*PCS1*	*PEN3*	*CYP81F2*	*ERF1*	*MYB51*	*ASA1*	*CYP79B2*	*CYP83B1*
ABA	24	3	1.33	0.97	1.42	1.06	1.81	0.61	0.65	0.61	0.83
	24	6	1.08	0.81	0.60	0.35*	0.32*	0.33*	0.15*	0.22*	0.29*
IAN	0	3	0.81	1.17	0.93	0.76	0.86	0.57*	0.56*	0.14*	0.56*
	0	6	0.99	1.13	1.28	0.25*	1.03	0.44*	0.38*	0.02*	0.14*
MeJA	24	3	0.66	0.65	0.77	0.65*	1.64	0.47*	0.71	0.24*	0.56
	24	6	0.87	0.90	0.95	0.96	1.55*	0.82	1.86*	1.01	0.60

*P < 0.01, two-tailed t test; false discovery rate < 0.05.

methoxy-I3G–accumulating *pen2* mutant upon MeJA and Flg22 treatment (fig. S4B), suggesting that there is no appreciable substrate competition between the two methoxylation pathways and that the mode of MeJA suppression occurs downstream of methoxylation, probably at the level of IGS hydrolysis.

IGS biosynthetic pathway is inhibited by IAN feeding. IAN treatment immediately suppressed Flg22-induced expression of *MYB51* and MYB51-regulated IGS biosynthetic genes (Table 3). By 6 hours after Flg22 elicitation, *CYP81F2* expression also decreased, but *ERF1* expression remained at wild-type levels (Table 3), suggesting that Et signaling is not affected in IAN-treated plants. Because IAN is a common IGS hydrolytic product, the suppressive effect of exogenous IAN treatment suggests that the IGS biosynthetic pathway is under feedback inhibition by the accumulation of IGS hydrolytic products, although the identity of the actual regulatory metabolite remains unknown. Expression in IAN-treated wild-type plants resembles that of putative IGS breakdown mutants *pen2* and *pcs1* at the level of *MYB51* expression (Table 2), suggesting that the IGS biosynthetic pathway is under feedback inhibition by IGS accumulation and its hydrolytic products. Other IGS hydrolytic products—4-methoxy-IAN, methyl-4-methoxy-indole-3-carboxylate, and methyl-indole-3-carboxylate—also suppressed the Flg22-induced callose response (table S1B), further supporting the conclusion that IGS biosynthesis is under feedback inhibition by its hydrolytic products.

Conclusions. Our study of the MAMP-triggered callose defense response, a classic innate immune response to both adapted and nonadapted pathogens, shows that the essential and ubiquitous compounds glutathione and ascorbate, the transported metal ion cadmium, and the secondary metabolite 4-methoxy-indol-3-ylmethylglucosinolate are all required for callose deposition. We showed that the Flg22-triggered callose response in *Arabidopsis* seedlings requires the concomitant induction of three pathways: Et- and MYB51-dependent I3G biosynthesis; CYP81F2-dependent 4-methoxylation of I3G; and the PEN2-, PCS1-, and PEN3-mediated hydrolysis of 4-methoxy-I3G (Fig. 5). The core IGS biosynthetic pathway is under feedback inhibition at the level of *MYB51* expression by the accumulation of IGS and IGS hydrolytic products. The defense hormones ABA and MeJA suppress the callose response by antagonizing Et signaling and IGS breakdown, respectively, whereas an SA-dependent pathway can bypass the hydrolytic requirement of PEN2 and PCS1. The role of glucosinolate hydrolysis in insect resistance has been studied for more than 100 years. Here we have identified a new role for this metabolic pathway, linking the endogenous hydrolytic products of glucosinolates to MAMP-mediated defense responses as potential signaling molecules. Further exploration of the species- and genus-specific effects of these molecules, including their potential role as compounds with direct antimicrobial activity (*36*), will be needed to determine their multiple functions in plant-microbe interactions.

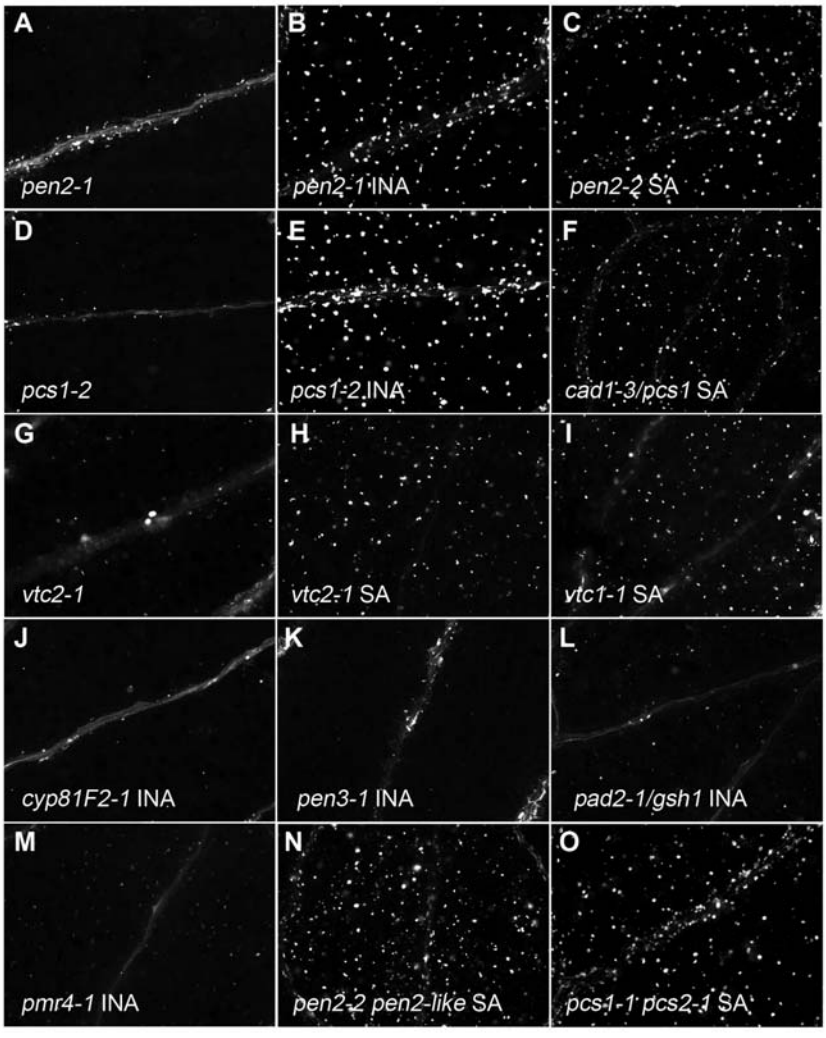

Fig. 4. SA pretreatment rescues Flg22-induced callose formation in *pen2* and *pcs1* mutants. (**A** to **O**) Cotyledons of seedlings pretreated with water (A and D), INA (B, E, and J to M), or SA (C, F, H and I, and N and O) for 24 hours and treated with Flg22. (A and B) *pen2-1*. (C) *pen2-2*. (D and E) *pcs1-2*. (F) *cad1-3/pcs1*. (G and H) *vtc2-1*. (I) *vtc1-1*. (J) *cyp81F2-1*. (K) *pen3-1*. (L) *pad2-1/gsh1*. (M) *pmr4-1*. (N) *pen2-2 pen2-like*. (O) *pcs1-1 pcs2-1*. Shown are representative examples of 40 to 60 cotyledons from two independent experiments.

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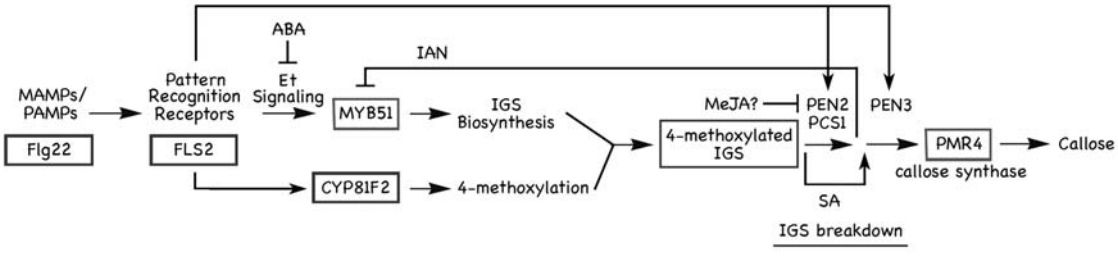

Fig. 5. A model of MAMP-activated callose deposition in *Arabidopsis*.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1164627/DC1
Materials and Methods
Figs. S1 to S5
Table S1
References

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A Glucosinolate Metabolism Pathway in Living Plant Cells Mediates Broad-Spectrum Antifungal Defense

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Selection pressure exerted by insects and microorganisms shapes the diversity of plant secondary metabolites. We identified a metabolic pathway for glucosinolates, known insect deterrents, that differs from the pathway activated by chewing insects. This pathway is active in living plant cells, may contribute to glucosinolate turnover, and has been recruited for broad-spectrum antifungal defense responses. The *Arabidopsis CYP81F2* gene encodes a P450 monooxygenase that is essential for the pathogen-induced accumulation of 4-methoxyindol-3-ylmethylglucosinolate, which in turn is activated by the atypical PEN2 myrosinase (a type of β-thioglucoside glucohydrolase) for antifungal defense. We propose that reiterated enzymatic cycles, controlling the generation of toxic molecules and their detoxification, enable the recruitment of glucosinolates in defense responses.

Flowering plants synthesize and accumulate a vast array of structurally diversified small molecules known as secondary metabolites (*1*). Each particular compound class is usually restricted to a narrow phylogenetic lineage, the result of genetic adaptations enabling or restricting interactions with other organisms. Although the chemical diversification of several secondary metabolite classes is driven by microbes and insects (*1*), it is often difficult to prove their presumed antimicrobial or insect-deterring functions in a whole-organism context. Among the notable exceptions are camalexin, an inducible *Arabidopsis* antimicrobial (phytoalexin) (*2*) and glucosinolates (Fig. 1A), Capparales-specific (which includes the Brassicaceae) thio-glucosides known to deter insects (*3*). This function of glucosinolates requires their tissue damage–triggered activation by specialized β-thioglucoside glucohydrolases (TGGs, also called myrosinases) (*4*) compartmentalized either in specialized myrosin cells in the phloem parenchyma (*5*) or in stomata cells (*6*).

Arabidopsis is immune to nonadapted powdery mildew fungi, such as *Blumeria graminis* and *Erysiphe pisi*, that colonize grass and pea species, respectively. During these interactions, fungal pathogenesis is terminated coincident with the switch from surface to invasive growth by two parallel pathways of induced preinvasive defense responses. The *Arabidopsis* PEN1 syntaxin resides in the plasma membrane and forms hetero-oligomeric complexes for vesicle-mediated secretory defense together with the adaptor SNAP33 and endomembrane-anchored VAMP721/722 (*7*, *8*). PEN2, a deduced family 1 glycosyl hydrolase (F1GH), and the plasma membrane-resident PEN3 ATP (adenosine triphosphate)–binding cassette (ABC) transporter act in a second path-

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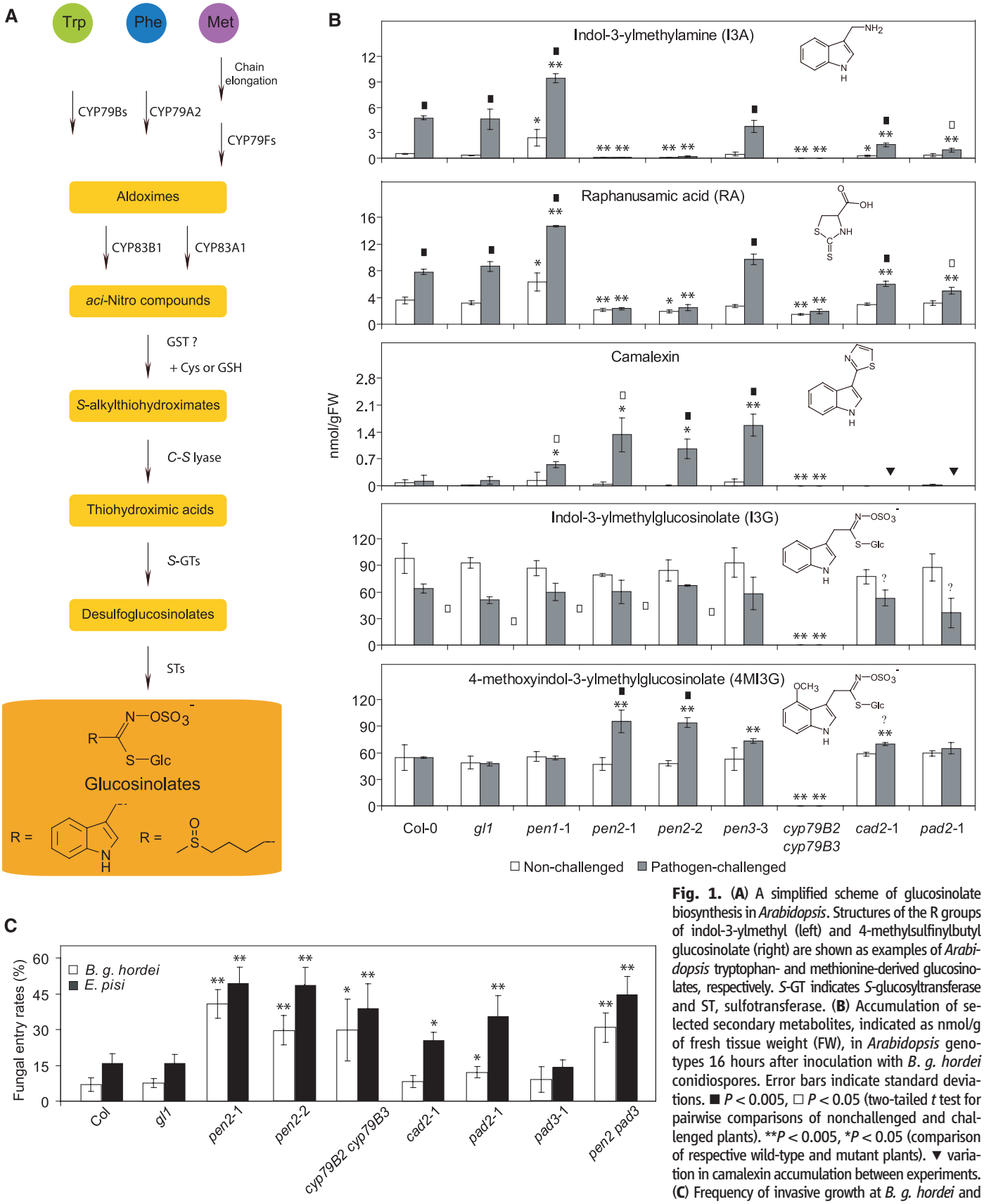

Fig. 1. (**A**) A simplified scheme of glucosinolate biosynthesis in *Arabidopsis*. Structures of the R groups of indol-3-ylmethyl (left) and 4-methylsulfinylbutyl glucosinolate (right) are shown as examples of *Arabidopsis* tryptophan- and methionine-derived glucosinolates, respectively. *S*-GT indicates *S*-glucosyltransferase and ST, sulfotransferase. (**B**) Accumulation of selected secondary metabolites, indicated as nmol/g of fresh tissue weight (FW), in *Arabidopsis* genotypes 16 hours after inoculation with *B. g. hordei* conidiospores. Error bars indicate standard deviations. ■ $P < 0.005$, □ $P < 0.05$ (two-tailed *t* test for pairwise comparisons of nonchallenged and challenged plants). $^{**}P < 0.005$, $^{*}P < 0.05$ (comparison of respective wild-type and mutant plants). ▼ variation in camalexin accumulation between experiments. (**C**) Frequency of invasive growth at *B. g. hordei* and *E. pisi* interaction sites on *Arabidopsis* genotypes scored 48 or 72 hours, respectively, after inoculation with conidiospores. Error bars, SD. $^{**}P < 0.001$, $^{*}P < 0.01$ (two-tailed *t* test for pairwise comparisons of respective wild-type and mutant plants).

way and have been implicated in the cytoplasmic synthesis and transport of unknown small molecules across the plasma membrane (*9*, *10*). The biochemical pathway underlying PEN2- and PEN3-dependent defense is of particular interest because this pathway restricts the growth of a broader spectrum of pathogens, including the nonadapted oomycete *Phytophthora infestans*, the adapted powdery mildews *Golovinomyces orontii* and *G. cichoracearum*, and the necrotrophic fungus *Plectosphaerella cucumerina* (*9*, *10*). In addition, *PEN2* and *PEN3* were recently shown to be required for the extracellular accumulation of the glucan polymer callose, mediated by the glucan synthaselike enzyme PMR4/GSL5, in response to treatment with a microbe-associated molecular pattern (MAMP) derived from bacterial flagellin (*11*).

***pen2* plants fail to accumulate an indole and a cysteine metabolite.** PEN2 is 1 of 42 annotated F1GHs in the *Arabidopsis* Col-0 genome (*12*). These enzymes usually catalyze the hydrolysis of a β-glycosidic or a thio-β-glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate (so-called aglycone) residue (*4*, *12*, *13*). To identify PEN2 candidate substrate(s) and product(s), we performed comparative metabolite profiling experiments with use of leaf extracts of wild-type strains (Col-0 and *gl1*), *pen1*-1 null mutant, two independent *pen2* mutant lines (*pen2*-1 and *pen2*-2), and *pen3*-3 null mutants (*14*). These experiments revealed a specific defect in the pathogen (*B. g. hordei*)-inducible accumulation of two compounds in *pen2* tissue: a novel metabolite with spectral properties similar to indole derivatives and a putative cysteine derivative, raphanusamic acid (RA), whose function and biosynthetic origin is unclear (*15*). We purified the former, and its structure was identified on the basis of mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques as indol-3-ylmethylamine (I3A) (Fig. 1B and fig. S1) (*14*). The indole core structure suggested a tryptophan biosynthetic origin. We investigated *cyp79B2 cyp79B3* double mutants defective in the P450 monooxygenase-catalyzed conversion of tryptophan to indole-3-acetaldoxime, a precursor of most known tryptophan-derived metabolites (*16*, *17*). *cyp79B2 cyp79B3* plants fail to accumulate detectable I3A quantities (Fig. 1B), confirming that tryptophan is the biosynthetic I3A precursor. Moreover, the presumed cysteine derivative RA was no longer pathogen-inducible in *cyp79B2 cyp79B3* leaves (Fig. 1B), demonstrating that coaccumulation of structurally unrelated RA and I3A is dependent on an intact tryptophan metabolism.

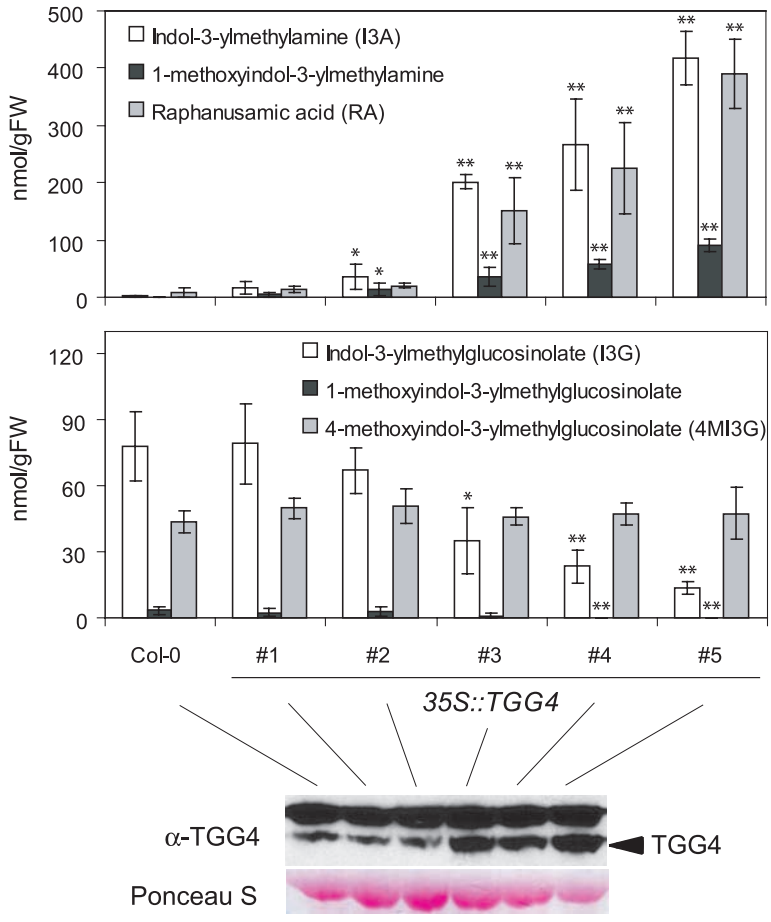

Fig. 2. Accumulation of selected secondary metabolites and immunodetection of TGG4 myrosinase in independent *Arabidopsis* transgenic lines constitutively expressing *TGG4*. Error bars, SD. $^{**}P < 0.001$; $^{*}P < 0.01$ (two-tailed *t* test for pairwise comparisons of Col-0 and respective transgenic plants).

Arabidopsis PAD2, encoding γ-glutamylcysteine synthetase (γ-ECS), has previously been linked to the biotic stress-induced accumulation of the phytochemicals glucosinolates and camalexin, which require cysteine incorporation into their core structure (*18*, *19*). PAD2 catalyzes the first committed step of glutathione biosynthesis, and *pad2*-1 mutants contain ~20% of the amount of glutathione present in wild-type plants, accumulate reduced amounts of these phytochemicals upon biotic stress, and are susceptible to infection by bacteria and oomycete pathogens, as well as herbivory by generalist insects (*18*, *19*). We examined the impact of the glutathione/cysteine metabolism on the pathogen-induced accumulation of the presumed cysteine derivative RA. In comparison with wild-type and *pen2* plants, both *pad2*-1 and an independent mutant allele, designated *cad2*-1 (*18*, *20*), produced not only intermediate amounts of RA but also of I3A after *B. g. hordei* inoculation (Fig. 1B), suggesting that glutathione is a potential cysteine donor in the biosynthesis of RA and linking RA and I3A accumulation.

We identified three additional tryptophan-derived compounds whose quantities were altered upon pathogen challenge. Camalexin accumulated to higher concentrations in *pen1*, *pen2*, and *pen3* mutants relative to concentrations in wild type (Fig. 1B), suggesting that its inducible biosynthesis is an indirect effect resulting from both elevated *B. g. hordei* entry rates and induction of death of invaded host epidermal cells (*9*, *21*). The other two compounds, according to structure analysis, were indol-3-ylmethylglucosinolate (I3G) and 4-methoxyindol-3-ylmethylglucosinolate (4MI3G) (SOM text). I3G was reduced 16 hours after *B. g. hordei* inoculation, whereas 4MI3G accumulated in *pen2* inoculated leaves at higher quantities than any other line (Fig. 1B).

Tryptophan-derived indolics are essential for pre- and postinvasion defense. Inoculation of *cyp79B2 cyp79B3* mutant plants with nonadapted *B. g. hordei* or *E. pisi* revealed infection phenotypes indistinguishable from those of *pen2* plants, whereas infected *cad2*-1 and *pad2*-1 plants exhibited moderate fungal entry rates between those of resistant wild-type and those of defense-compromised *pen2* plants (Fig. 1C). This suggests that *CYP79B2/B3* and *PAD2* function in preinvasion resistance, revealing a positive correlation between I3A and RA accumulation and plant defense. However, because *cyp79B2 cyp79B3* plants fail to accumulate multiple groups of indole-type secondary products, including camalexin and indole-type glucosinolates (*16*, *17*), we lack demonstration of a direct role of I3A and RA in *Arabidopsis* defense responses. Of note, we detected extensive epiphytic hyphal growth of *E. pisi* on leaves of *cyp79B2 cyp79B3* plants compared with growth on wild-type and *pen2* leaves (fig. S2). We therefore tested the *pad3*-1 mutant (*22*), which is specifically compromised in camalexin biosynthesis, and a *pen2 pad3* double mutant. *E. pisi* infection phenotypes on *pad3* plants were indistinguishable from that of wild-type, whereas *pen2 pad3* plants

resembled *cyp79B2 cyp79B3* plants in entry rates and epiphytic hyphal growth (Fig. 1C and fig. S2). This suggests that the infection phenotype of *cyp79B2 cyp79B3* plants results from the absence of both PEN2-generated products and camalexin. On the basis of these data, we propose that PEN2 and PAD3 act sequentially during pre- and postinvasive defenses, respectively. This would explain why camalexin amounts are elevated in mutants defective in preinvasion resistance [such as *pen1* and *pen3* (Fig. 1B)], because these plants have a compensatory postinvasive and cell death–associated defense that is lacking in wild type (*9*).

Myrosinase misexpression supports the existence of a novel glucosinolate metabolism pathway. Our data suggest an unexpected role of glucosinolates in fungal defense (Fig. 1B and figs. 1C and S2). We generated *Arabidopsis* lines expressing the root myrosinase *At*TGG4 (*12*, *23*) constitutively. Previously reported in vitro hydrolysis end products of I3G (*24*, *25*) were either undetectable (e.g., indol-3-ylcarbinol) or present at low abundance (e.g., indol-3-ylacetonitrile) in these lines (fig. S3A). Instead, transgenic *35S::TGG4* expressing lines showed a constitutive hyperaccumulation of I3A and RA (Fig. 2). Increasing amounts of both compounds correlated with TGG4 protein abundance and a concomitant depletion of I3G (Fig. 2).

Hyperaccumulation of I3A and RA was independent of PEN2 activity because *At*TGG4 overexpression in the *pen2*-1 background resulted in similar biochemical phenotypes (fig. S3B). Taken together, the metabolite profiles of both pathogen-challenged wild-type plants (Fig. 1B) and *35S::TGG4* lines (Fig. 2) suggest that myrosinase-dependent hydrolysis of glucosinolates occurs in vivo in living plant cells and generate different end products from those reported from studies of in vitro hydrolysis and damage by chewing insects (*24*, *25*). This also suggests that the amine I3A and RA are proxies of in vivo indole glucosinolate metabolism rather than specific markers of PEN2 activity.

We detected 1-methoxyindol-3-ylmethylamine (Fig. 2) (*14*) in *35S::TGG4* plants when looking for structure variants of indole-type and/or methionine-derived aliphatic glucosinolates (a glucosinolate subclass, Fig. 1A) subject to in vivo hydrolysis. We postulated that this molecule could be derived from a low abundance 1-methoxyindol-3-ylmethylglucosinolate in *Arabidopsis* leaves (*26*). Liquid chromatography/mass spectrometry (LC/MS) also revealed the presence of additional amines in extracts from *35S::TGG4* plants that correspond in their side chain structure to *Arabidopsis* aliphatic glucosinolates (fig. S3C) (*14*). These observations suggest that both indole and aliphatic glucosinolates undergo metabolism in vivo with a concomitant buildup of the respective amines.

Amounts of one of the two methoxylated indole glucosinolates, 4MI3G, remained unaffected in tested transgenic lines (Fig. 2), and the corresponding 4-methoxyindol-3-ylmethylamine (4MI3A) was barely detectable by LC/MS (*14*).

PEN2 is an atypical myrosinase. The detection of a novel glucosinolate metabolism pathway in intact tissue together with the identification of I3A and RA as pathogen-inducible and PEN2-dependent metabolites predicted that PEN2 functions as a myrosinase. This was unexpected because the enzyme possesses an acid/base catalyst glutamic acid in its catalytic cleft (Glu^{183}, E183) (*9*), characteristic for β-*O*-glycosylhydrolases but not myrosinases (*4*, *12*). We heterologously expressed an epitope-tagged fusion protein lacking 64 residues from the C-terminal region, which is possibly critical for in planta subcellular PEN2 localization (*9*). This vector, PEN2Δ-*Strep*, permitted high protein expression in *Pichia pastoris*. Affinity chromatography–purified PEN2Δ-*Strep* (fig. S4A) showed a pH optimum around 6 (fig. S4B) and cleaved in vitro both *S*-glucosides (I3G and 4MI3G) and, at ~10-fold lower maximum reaction rate and ~fivefold lower Michaelis constant, a model *O*-glucoside (4-methyl-umbelliferyl-β-D-*O*-glucoside, 4MUG) (fig. S4C). We tested the in vitro activity of a site-directed mutant (Glu^{183}→Asp^{183}), $PEN2_{E183D}$Δ-*Strep*, in order to test these molecules as physiologically relevant substrates. The stable, full-length $PEN2_{E183D}$ variant was inactive in vivo because it failed to rescue impaired entry resistance to *B. g. hordei* in the *pen2* background (*9*). Remarkably, $PEN2_{E183D}$Δ-*Strep* failed to convert the glucosinolate I3G but retained β-*O*-glycosylhydrolase activity in the presence of 4MUG (fig. S4, A and C), suggesting that PEN2 acts as myrosinase in vivo and implicating glucosinolate-derived products as antifungal defense compounds. Furthermore, RA was no longer pathogen-inducible in the *cyp79B2 cyp79B3* mutant lines (Fig. 1B) despite the accumulation of aliphatic glucosinolates (*16*), indicating that PEN2 cleaves indole glucosinolates in planta preferentially, if not exclusively.

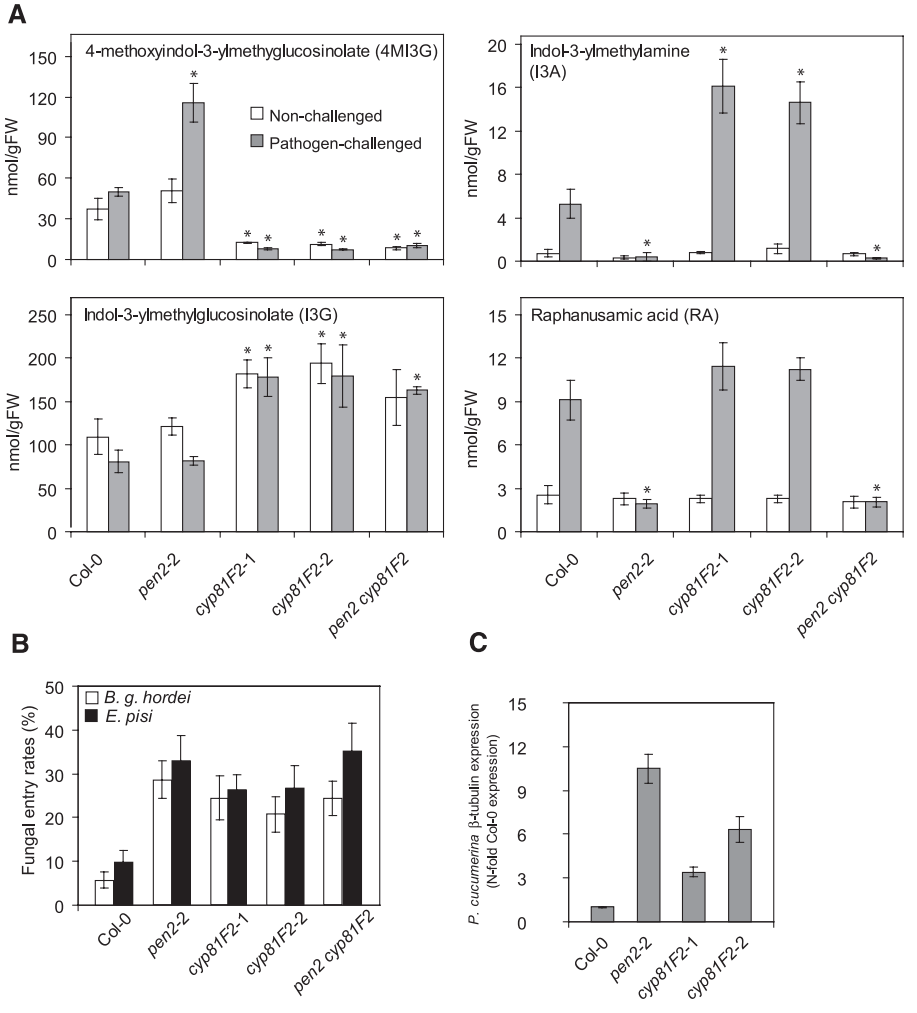

Fig. 3. (**A**) Accumulation of selected secondary metabolites in *Arabidopsis* genotypes 16 hours after inoculation with *B. g. hordei* conidiospores. Error bars, SD; *$P < 0.005$ (two-tailed *t* test for pairwise comparisons of Col-0 and respective mutant plants). (**B**) Frequency of invasive growth at *B. g. hordei* and *E. pisi* interaction sites scored 48 or 72 hours after inoculation. Error bars, SD; all differences between Col-0 and tested mutant lines are significant at $P < 0.001$ (two-tailed *t* test). (**C**) Growth of *P. cucumerina* as determined by real-time quantitative reverse transcriptase polymerase chain reaction of *P. cucumerina* β-tubulin 3 days after inoculation with spores. Error bars, SD; all differences between Col-0 and tested mutant lines are significant at $P < 0.05$ (two-tailed *t* test).

Although amines and RA have not been considered as glucosinolate hydrolysis products to date (*24*, *25*), the myrosinase hydrolysis product of glucosinolate sinigrin, allylisothiocyanate, decomposes in the presence of nucleophilic reagents to an amine (*27*). This and the fact that reduced glutathione is one of the most abundant plant nucleophiles (*28*) suggested a possible metabolic link between I3G and I3A/RA by which glucosinolate-derived isothiocyanate and glutathione form a dithiocarbamate adduct that is later converted to the respective amine and RA (fig. S5A). This pathway is likely because the formation of corresponding adducts occurs (i) nonenzymatically (*29*), (ii) enzymatically, driven by glutathione-*S*-transferases (GSTs) in vitro (*30*), or (iii) in vivo in mammals after consuming glucosinolates or isothiocyanates in Brassicaceae vegetables (*31*).

Biosynthesis and cleavage of 4MI3G is essential for plant defense. It is likely that I3G and 4MI3G are directly metabolically connected as uptake of I3G by detached *cyp79B2 cyp79B3* leaves restores the accumulation of 4MI3G (*32*). If so, then the fungus-induced depletion of I3G and the concomitant *pen2* mutation-dependent increase in 4MI3G (Fig. 1B) suggest that the latter glucosinolate is critical for fungal defense. We reasoned that conversion of I3G to 4MI3G may involve the hydroxylation of the indole core by cytochrome P450 monooxygenases and that the corresponding gene(s) might be coexpressed with *PEN2*. Microarray data sets (*33*) in *Arabidopsis* identified five P450 monooxygenase genes that coexpressed with *PEN2*, *PEN3*, and *CYP83B1* (r = 0.4; the latter gene encodes a P450 monooxygenase required for glucosinolate biosynthesis). Transferred-DNA insertions in the *At5g57220* gene, encoding CYP81F2, showed reduced 4MI3G amounts (Fig. 3A), linking CYP81F2 with 4MI3G biosynthesis. Both *cyp81F2* mutant lines showed impaired entry resistance to *B. g. hordei* and *E. pisi* and were more susceptible to *P. cucumerina* (Fig. 3B and C), reminiscent of the *pen2* infection phenotypes (*9*). We also generated *cyp81F2 pen2* double mutants in which the resistance phenotype was indistinguishable from that in the single mutants (Fig. 3B). This suggests that *CYP81F2* and *PEN2* act in a common pathway. Moreover, although PEN2 can hydrolyze both unsubstituted and methoxylated indole glucosinolates (fig. S4C), only the compound(s) derived from 4MI3G is important for the restriction of fungal growth. Retained accumulation of I3A and RA in *cyp81F2* plants excludes their direct function in preinvasion resistance. Despite analyses supported with a synthetic reference compound (*14*), we did not detect pathogen-inducible accumulation of 4MI3A (the predicted product derived from 4MI3G). This suggests an alternative biochemical route for the processing of the 4-methoxylated indole dithiocarbamate adduct (Fig. 4A and fig. S5). The structural similarity of this intermediate with the core structure of a major group of Brassicaceae phytoalexins (*34*) supports an already-proposed close metabolic link between these compounds (*35*, *36*) and indole glucosinolates, pointing at them as possible antimicrobial end products of the CYP81F2/PEN2 pathway and as coactivators of MAMP-triggered PMR4/GSL5-dependent callose formation (*11*).

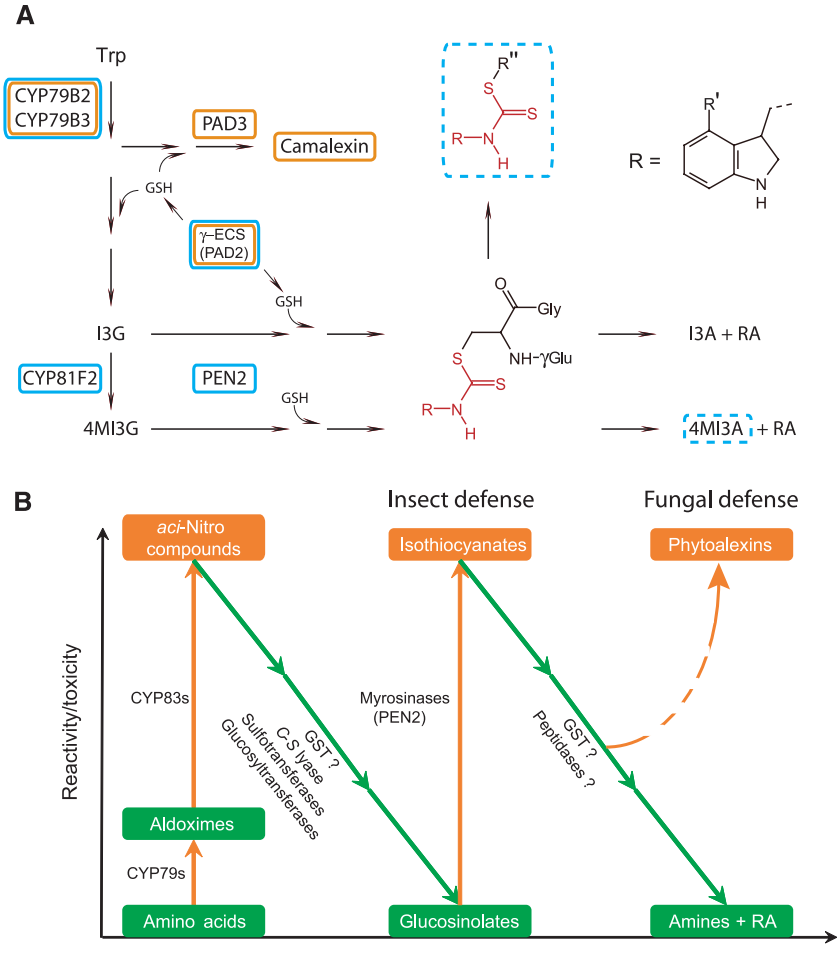

Fig. 4. (**A**) Involvement of tryptophan-derived metabolites in *Arabidopsis* defense against powdery mildews. Components critical for termination of pre- and postinvasive fungal growth are highlighted with blue and orange frames, respectively. The matching structure fragments of the isothiocyanate-glutathione adduct and some of the Brassicaceae phytoalexins are highlighted in red; for detailed structures, see fig. S5B. Dashed frames denote putative metabolites derived from 4MI3G. (**B**) Model for reiterated enzymatic cycles controlling the generation of small toxic molecules and their detoxification in *Arabidopsis*.

Conclusions. Our study revealed a PEN2-driven glucosinolate metabolism pathway that is fundamentally different from insect interactions with plants in the Brassicaceae family (*24*, *25*). Unlike the passive mode of glycoside activation by myrosinases and other F1GHs (*13*), PEN2-dependent indole glucosinolate hydrolysis is an active process involving directed movement to, and concentration of, the peroxisome associated enzyme to the cell periphery at fungal entry sites (*9*), which likely generates high local concentrations of hydrolysis product(s). Our work questions the specificity of *O*- and *S*-glycosylhydrolases (*4*, *12*) and reveals different glucosinolate end products relative to those produced after insect-mediated tissue destruction (fig. S5A). This discrepancy results most likely from the in vivo conjugation of the glucosinolate hydrolysis product, isothiocyanate, with glutathione to form a dithiocarbamate adduct, which is subject to further processing (Fig. 4A and fig. S5). Because this metabolism pathway occurs only in living cells, the engagement of specific enzymatic activities, or even specialized enzymatic complexes, appears likely (*37*). Moreover, the strict requirement of a particular aglycone structure (4MI3G) in the tested plant-fungus interactions contrasts with the limited structural specificity of glucosinolates in deterring insects (*3*, *32*).

The glucosinolate pathway originates from the biosynthesis of cyanogenic glucosides and most likely was initiated by the appearance of CYP83 enzymes that convert acetaldoximes to reactive *aci*-nitro compounds (Figs. 1A and 4B) (*37*). Because most of the enzymes acting downstream of CYP83s represent classes involved in detoxification, like GSTs, glucosyltransferases, and sulfotransferases (*3*, *19*), the glucosinolate pathway may have evolved from routes triggered by the appearance of toxic CYP83 product(s) (*38*). In parallel or subsequently, a class of F1GHs emerged that is capable of cleaving glucosinolates for insect defense and turnover. However, because the direct

products of myrosinase activity are toxic, another detoxification cascade involving glutathione conjugation was required, yielding RA and amines (Fig. 4B). This pathway was most likely later recruited into plant defense against microbial intruders. The relatively recent evolution of PEN2 is supported by its unique exon-intron structure (*12*) and also explains the retention of an ancestral function: cleaving *O*-glucosides (fig. S4). This suggests that also one of the most closely related proteins to PEN2 in *Arabidopsis*, the root-specific PYK10 enzyme (*39*), which restricts colonization by a soil-borne fungus (*40*), may act as a second generation myrosinase. The seven PEN2-like F1GHs in the *Arabidopsis* genome suggests that glucosinolate hydrolysis has been repeatedly engaged in plant defense or glucosinolate turnover and may have given rise to a diverse array of phytochemicals.

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Figs. S1 to S5
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REPORTS

Mott Insulating State in Ultraclean Carbon Nanotubes

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The Mott insulating state is a manifestation of strong electron interactions in nominally metallic systems. Using transport spectroscopy, we showed that an energy gap exists in nominally metallic carbon nanotubes and occurs in addition to the band gap in small–band-gap nanotubes, indicating that carbon nanotubes are never metallic. This gap has a magnitude of ~10 to 100 milli–electron volts and a nanotube radius (r) dependence of ~1/r, which is in good agreement with predictions for a nanotube Mott insulating state. We also observed neutral excitations within the gap, as predicted for this state. Our results underscore nanotubes' exceptional capabilities for use in studying correlated electron phenomena in one dimension.

According to the quantum theory of solids, materials can be either metallic or band insulators. However, this theory breaks down in metals at half-filling of energy bands, in which strong Coulomb repulsion makes it energetically favorable for electrons to localize, one electron per atomic site, to form a Mott insulator (*1*). This state is known to be antiferromagnetically ordered in higher dimensions and has been observed in a variety of bulk systems, including thin films (*2*), nanobeams (*3*), and optical lattices (*4*). The one-dimensional (1D) analog of this state has no long-range magnetic order and can form a spin liquid with gapped spin excitations (*5*). The presence of a spin gap in some classes of spin liquids is believed to be related to the emergence of high-temperature superconductivity in cuprate oxides (*6*), motivating a search for such systems. Theoretical work (*7*–*12*) predicts that carbon nanotubes are a realization of a gapped spin liquid Mott insulator.

Experiments on bulk quasi-1D Mott insulating systems (*5*) typically use chemical doping, which introduces additional disorder. Carbon nanotubes offer the opportunity to study electronic phenomena without interference from disorder by using electric-field doping. Recently, the fabrication of ultraclean nanotube devices (*13*) has facilitated the observation of long-predicted phenomena, such as Wigner crystallization (*14*) in large–band-gap nanotubes, as well as spin-orbit coupling (*15*), and may produce favorable conditions for observing a tunable 1D Mott insulator in an individual nanostructure.

We show that the energy gaps exhibited by carbon-nanotube field-effect devices made from small–band-gap and nominally metallic nanotubes cannot be accounted for using noninteracting electron pictures but agree well with predictions for a spin-liquid Mott insulating state in carbon nanotubes. These finite-sized samples act as quantum dots, and Coulomb peaks corresponding to a single electron or hole pair at the band edges are observable. We tuned the magnitude of the energy gap by applying an axial magnetic field (*16*). By tracking the first electron- and hole-addition

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energies with transport spectroscopy, we accurately estimated the magnitude of the gap as it was tuned.

Previous work has shown the presence of energy gaps in carbon nanotubes. Indeed, depending on their radius and chirality, two thirds of nanotubes are expected to be semiconducting because of the boundary conditions that cause the allowed wave vectors in the Brillouin zone to miss the gapless Dirac points. For the remaining one third of tubes that are expected to be metallic in a zone-folding picture, the displacement of the Dirac points in the Brillouin zone of graphene by symmetry-breaking mechanisms, such as strains, twists, or curvature (*17*), can open a band gap (*17*), producing so-called small–band-gap nanotubes. Scanning tunneling microscopy studies of small–band-gap nanotubes have attributed energy gaps to curvature (*18*); however, these studies were performed on metal substrates, which screen electron-electron interactions. Previous transport experiments on nanotubes, in which interactions were not screened, have shown gaps in the range of ~10 to 100 meV (*19*, *20*), but their origin was not investigated. To assess the role of curvature in producing the observed gaps in transport studies, we first studied armchair nanotubes, which are predicted to be metallic by band-structure calculations and protected by symmetry from having curvature-induced gaps (*17*).

Figure 1A, right, shows the conductance G versus gate voltage V_g for two 500-nm segments each on two armchair nanotubes, fabricated and characterized by Rayleigh scattering measurements (Fig. 1A, left) according to (*21*). All segments show a dip that is characteristic of an energy gap (*19*). Similar behavior has been observed in ~90 devices fabricated on five separate armchair nanotubes. Thus, curvature cannot fully account for the observed gaps in transport experiments. Nevertheless, gaps of similar magnitude or larger in tubes could also occur, for example, because of twists or strains produced during the device fabrication (*16*), making the origin of the observed gaps in transport studies unclear.

To address the origin of energy gaps in metallic nanotubes, we fabricated extremely clean, as-grown, suspended devices that were free from disorder due to chemical processing or the substrate. These samples, discussed exclusively in the remainder of this report, were fabricated by chemical vapor deposition growth over Pt electrodes (*14*, *13*) and presumed to have random chirality (*22*). The growth substrate was a Si_3N_4 layer on Si that has a trench between the two electrodes that enables the nanotubes to be freely suspended. Additionally, varying ratios of Fe/Mo catalyst salts were used to vary the average nanotube radii (*23*). The scanning electron microscope image (Fig. 1B) of a representative device shows a measurement geometry similar to that used earlier (*14*). After growth, individual single-walled nanotube devices were selected based on their room-temperature high-bias transport characteristics (*14*).

Figure 1C shows G versus gate voltage V_g for a representative device D1 (length, ~100 nm) at several different temperatures. The device shows a dip similar to that in Fig. 1A. As the temperature is lowered, the minimum conductance tends toward 0. Figure 1D shows a plot of G in color scale versus V_g and bias voltage V for D1 at 1.5 K. The gap corresponds to the large diamond marked with the red dotted lines. For the electrons, smaller diamonds marked by the blue dotted lines indicate a Coulomb blockade with a charging energy U of ~40 meV. On the hole side, the conductance is somewhat larger, and the features are reminiscent of the Fabry-Perot interference behavior previously observed in strongly coupled nanotube quantum dots (*24*). These regular features are indicative of extremely clean quantum dots. Energy gaps were observed in all clean samples studied.

The magnitude of the gaps in single-walled nanotubes can be modulated with an axial magnetic field (*16*). Figure 2A shows a plot of G versus V_g and axial magnetic field B for device D2 with a length of 200 nm at 1.5 K. As with D1, the hole side is more conductive than the electron side, and the two are separated by an energy gap. On application of the field, the lowest quantum energy levels (namely, the first electron and hole state) do not undergo crossings and can be used to accurately determine the gap. At low fields, the Coulomb peaks for the electrons shift their energy E with a slope $dE/dB = \alpha dV_g/dB = 0.79$ meV T^{-1} (α, the gate-efficiency factor) and with a similar magnitude but opposite sign for the holes. The gap therefore decreases as the magnetic field is increased from $B = 0$. However, at a critical magnetic field $B_c \sim 4$ T, the gap stops closing and begins to open again. At this field, we also often see an approximately horizontal conductance ridge extending across the gap in the G versus B and V_g plot.

This gap includes a contribution U from the charging energy as well as a term ΔE arising because of discrete energy levels of the 1D electron system. Both of these contributions arise from the finite size of the nanotube and would vanish in an infinite tube. To find the magnitude of the intrinsic gap that would occur for a very long nanotube, the contributions from U and ΔE must be subtracted. We therefore subtracted the measured energy (≈12 meV for D2) obtained from Coulomb blockade diamonds from the measured energy of the gap. The inset to Fig. 3B shows the corrected gap versus B. This gap does not reach 0 when $B = B_c$ but instead reaches a minimum value of $\Delta_{min} \approx 37$ meV. One can directly reduce finite-sized effects by studying longer devices. Indeed, as shown in Figs. 2B and 2C for devices D3 and D4 of lengths 500 nm and 2 μm, respectively, the minimum gap dominates the charging energy and confirms the generality of the observed behavior.

In a noninteracting electron picture (*17*), perturbations can cause the quantized transverse electron wave vector to miss the zero-gap Dirac point of graphene's band structure. This opens a gap in

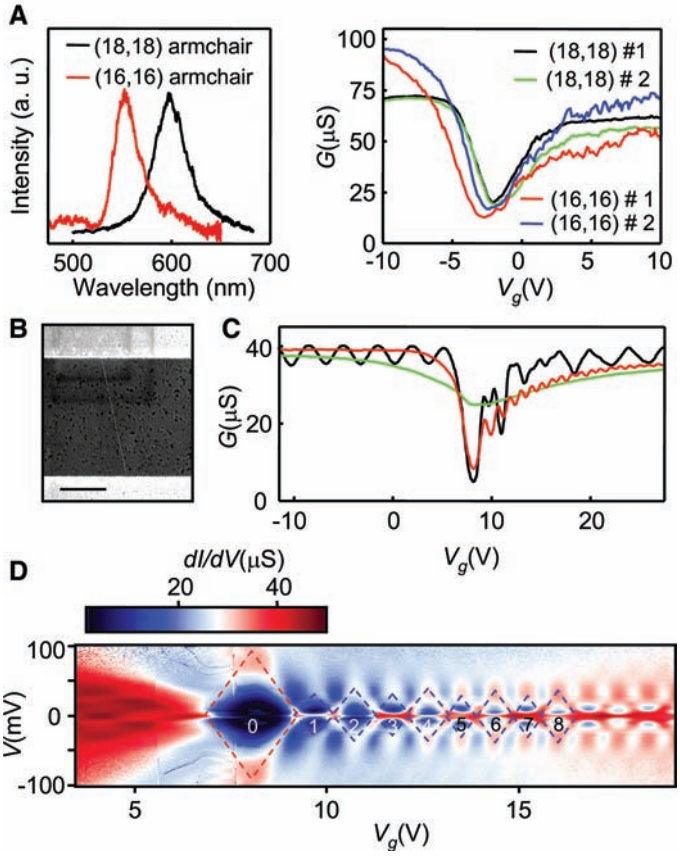

Fig. 1. (**A**) Rayleigh scattering spectra (left) and room-temperature transport characteristics (right) of two armchair nanotubes. (**B**) Scanning electron microscope image of a representative suspended nanotube device. Scale bar, 2 μm. (**C**) G versus V_g for D1 at temperatures $T = 1.5$ K (black), 150 K (red), and 300 K (green). (**D**) Color plot of dI/dV versus V_g and source-drain bias V for D1. The numbers indicate the number of charge carriers in the blockaded state.

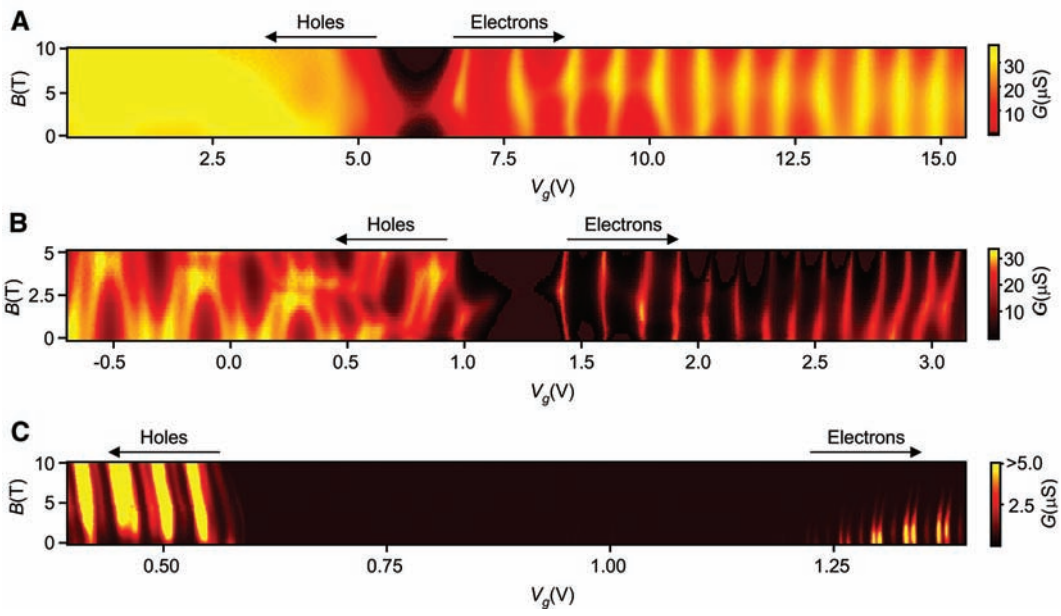

Fig. 2. Color plot of G versus V_g and B for three devices: (**A**) D2, L ~ 200 nm; (**B**) D3, L ~ 500 nm; (**C**) D4, L ~ 2 μm. The electron and hole states are indicated for each device. The first electron and hole energy levels do not undergo crossings and can be used to study the gap. The evolution of higher charge states has been studied using quantum-dot spectroscopy (*25*, *15*). In (C), the first few electrons are not visible in the linear conductance plot. However, their position is known from nonlinear transport experiments.

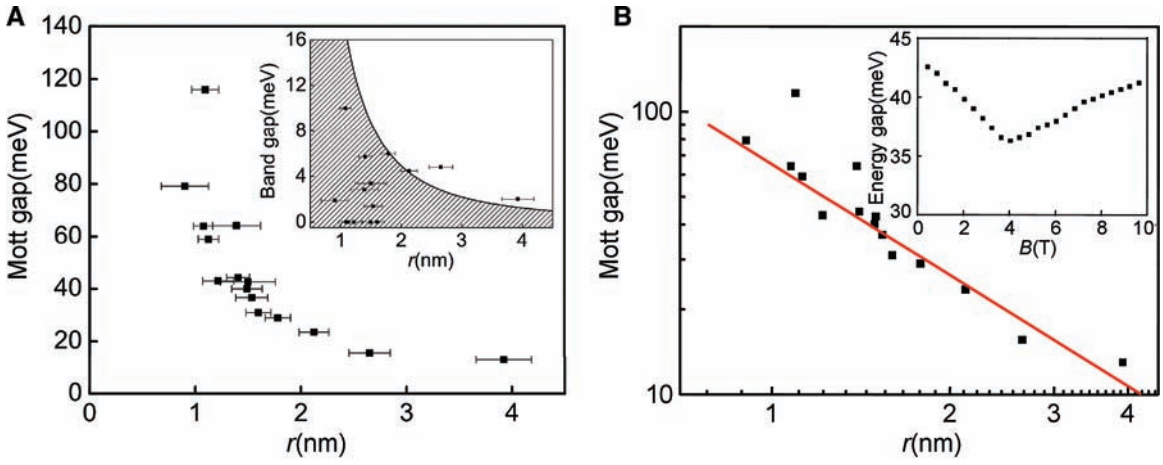

Fig. 3. (**A**) Variation of the gap Δ_{min} with nanotube radius for 15 devices. The largest contribution to error bars comes from pixel size in Fig. 2 and the uncertainty of nanotube alignment with magnetic field (±20°). (Inset) Plot of Δ_{sp} versus r. The shaded region corresponds to the prediction for curvature-induced gaps (*17*) depending on chirality. (**B**) Log-log plot of Δ_{min} with r. (Inset) Variation of energy gap with B for D2.

the nanotube band structure (*17*). The axial magnetic field shifts the transverse wave vector (*16*), sweeping the quantization through the Dirac point for a large-enough field. However, the gap would be 0 at the minimum rather than the finite value we observed. This contradiction with experiment, along with the observed energy gap in armchair nanotubes, rules out curvature, strain, or twist as a possible mechanism for Δ_{min}.

To gain further insight into the origin of this gap, we have investigated how Δ_{min} depends on the nanotube radius r. The radius was obtained by measuring the slope of the Coulomb peak evolution in the magnetic field and using the α obtained from nonlinear transport measurements on each device to determine $dE/dB = \alpha dV_g/dB$. This can be related to the radius via the orbital magnetic moment μ_{orb} by using the relation $dE/dB = \mu_{orb} \pm \mu_Z = \pm evr_F/2 \pm \mu_Z$, where μ_Z is the electron spin magnetic moment, e is the electric charge, and v_F is the Fermi velocity. This procedure has been previously used to estimate nanotube radii (*14*–*16*, *25*). A plot of Δ_{min} versus r for 15 devices (Fig. 3A) follows an approximate $1/r$ relation. The Fig. 3A inset shows the value of the additional gap that occurs at $B = 0$ as compared with when $B = B_c$, taken to be the single-particle band gap Δ_{sp}. Unlike the minimum gap plotted in Fig. 3, the main panel, Δ_{sp} shows no trend with r. However, it falls within the theoretical prediction for a curvature-induced gap (Fig. 3A, shaded region) (*17*), which is given by $\approx 20 \cos(3\theta)/r^2$ meV, where r is in nanometers and $0 < \theta < 30°$ is the range of nanotube chiral angles (*18*).

Another possibility for Δ_{min} is the Peierls instability, which opens an energy gap in 1D systems. However, because of stiff C-C bonds, such a gap for nanotubes is expected to be ~1 meV for a 1-nm-diameter nanotube (*17*), and recent calculations give $\Delta_{peierls} = 0.26/r^3$ meV, where r is in nanometers (*26*). Theoretical calculations thus are 2 orders of magnitude smaller than the observed gap and have a different r dependence for Δ_{min} than we observed.

Having ruled out these noninteracting electron pictures, we considered the influence of electron-electron interactions. In the simplest picture, electron-electron interactions are considered in the forward-scattering approximation, producing a Tomonaga-Luttinger liquid state governed by the long-range part of the Coulomb interaction, which retains the metallic behavior (*17*). However, at half-filling of energy bands, Umklapp scattering via short-ranged Coulomb interactions is a viable backward-scattering mechanism. These interactions arise from the confinement of the electron wave functions because of the nanotubes' wrapped geometry. Confinement to a finite length L could also potentially produce a gap because of the enhancement of backscattering by Friedel oscillations. However, these should show a $1/L$ dependence to the gap (*27*), which was not observed (fig. S1), ruling out this mechanism as the primary source of a gap in our devices. Finally, an existing Umkapp-induced gap could potentially be made larger by such a mechanism; however, further theoretical and experimental work will be required to fully explore this possibility.

Upon considering Umklapp scattering, theory (*7*–*12*) predicts the formation of a Mott energy gap in metallic nanotubes at half-filling, rendering the metallic state into a Mott insulator. As

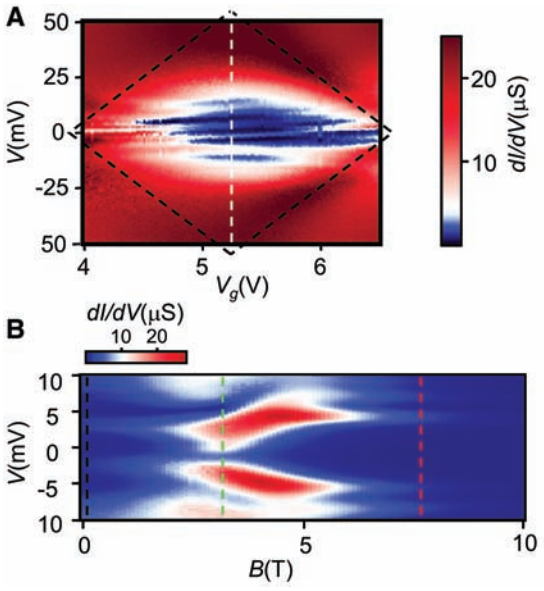

Fig. 4. (**A**) Color plot of *dI*/*dV* versus gate voltage V_g and source-drain bias *V* for D5. The diamond indicated by black dashed lines corresponds to the energy gap. (**B**) Color plot of *dI*/*dV* versus magnetic field *B* and source-drain bias *V* in the middle of the gap [V_g corresponding to the white dashed line in (A)]. The green dashed line corresponds to B_c, where the energy gap is at the minimum. (**C**) *dI*/*dV* versus *V* at three different magnetic fields in (B) corresponding to the black, green, and red dashed lines. The green curve (scaled down by a factor of 10 and offset for clarity) taken at $B = B_c$ shows a Kondo-like peak at zero bias that could account for the ridges observed, for example, in Fig. 2, A and B, at $B = B_c$.

discussed in (*7*) and (*9*), one can establish equivalence between a nanotube and a two-chain version of the 1D Hubbard model (a two-leg ladder). The equivalent on-site interaction in this model is the average over atoms along the circumference of the nanotube, giving a universal $\sim 1/r^a$ scaling to the energy gap in all nominally metallic tubes with scaling exponent *a* (*10*), for which long-range Coulomb forces are taken into account. In particular, the renormalization-group calculation (*10*) suggests that the long-range part of the Coulomb interaction modifies the Mott gap scaling with *r* so that $a = 1/(1 - g)$, where *g* is the Luttinger parameter, taken to be ~0.2 to 0.3 for nanotubes (*17*).

In Fig. 3B, the measured Δ_{min} is plotted against *r* on a log-log scale. The data falls on a straight line with a slope of $\sim -1.3 \pm 0.15$, which yields an estimate for $g \sim 0.13$ to 0.3, in agreement with the expected range of values (*28*). The slope reveals that the data follow the curve $\Delta = \beta r^{-1.3}$, with $\beta = 60$ meV nm$^{-1.3}$. This value for β is in agreement with the theoretically estimated range of 10 to 100 meV nm$^{-1.3}$ (*10*).

We turned to neutral excitations that were observed within the gap. Figure 4A shows a plot of *dI*/*dV* versus V_g and *V* for a device D5 of length 200 nm. The gap region is indicated by the dashed lines. A number of features approximately independent of gate voltage appear in the gap. These features arise from inelastic cotunneling (*29*), in which a tunneling electron leaves behind a neutral excitation on the nanotube. The bias at which they occur directly yields the energies *eV* of these excitations. Figure 4B shows a plot of *dI*/*dV* versus *V* and *B* for V_g in the middle of the gap. B_c for this device is indicated by the vertical dashed green line. Near B_c, the features corresponding to the lowest energy excitations acquire a slope with an energy shift of $\mu_{ex} \sim 2$ meV T^{-1}, indicating that they are of electronic origin rather than from, for example, phonons. The presence of such features are not expected to occur in non-interacting electron pictures, in which the minimum excitation energy is the band gap. Similar features were observed in all strongly coupled devices from which data was taken.

Neutral massive (gapped) excitations of the Mott insulator in a metallic nanotube are characterized by several quantum numbers, such as spin and vorticity (*11*, *10*). Symmetry-breaking mechanisms can also give rise to charge and neutral gaps (*30*). Neutral gaps in both pictures are separated in energy from the charge gap by the Luttinger parameter *g*. Although the interplay between these two mechanisms of a realistic metallic tube has not been considered theoretically, it is likely that the gapped neutral excitations will generally persist even in the presence of a single-particle gap. This is consistent with our observations (Fig. 4) because the neutral excitations remain gapped for any magnetic field. The lowest-energy neutral gap is predicted to be a fraction (0.26) of the Mott gap for D5 (*10*). We observed the ratio to be ~0.2, which is in good agreement with theory. This measured separation of scales between the neutral and Mott gaps is also consistent with the value *g* of the Luttinger parameter for nanotubes.

At the critical field B_c, the symmetry-breaking Δ_{sp} is likely to be compensated by the field. This regime may still be more complex than that considered in (*11*) because the gap vanishes for only one of the two Dirac points. Furthermore, away from $B = B_c$, it is likely that the gap in the neutral sector will increase mostly because of single-particle terms of the kind considered in (*30*), which scale as $erv_F(B - B_c)$ and are consistent in magnitude with the observed μ_{ex}.

Our observation of a universal gap, together with two independent measures of the Luttinger parameter, provides strong evidence for the 1D Mott state in nanotubes. This realization in an individual nanostructure facilitates a model system for testing theories of strong electron interactions (*11*, *30*, *31*), which may be important for gaining insight into the mechanism of high T_c materials. Finally, the Mott gap for sub-nanometer–diameter nanotubes can be much greater than the characteristic thermal energy at room temperature and could be combined with techniques for the growth of small-diameter devices to yield room-temperature transistors.

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Supporting Online Material
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Fig. S1

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Broadband Invisibility by Non-Euclidean Cloaking

Ulf Leonhardt[1,2]* and Tomáš Tyc[2,3]

Invisibility and negative refraction are both applications of transformation optics where the material of a device performs a coordinate transformation for electromagnetic fields. The device creates the illusion that light propagates through empty flat space, whereas in physical space, light is bent around a hidden interior or seems to run backward in space or time. All of the previous proposals for invisibility require materials with extreme properties. Here we show that transformation optics of a curved, non-Euclidean space (such as the surface of a virtual sphere) relax these requirements and can lead to invisibility in a broad band of the spectrum.

Geometry has always played a distinguished role in optics (*1*), but direct optical applications of differential geometry are rather recent (*2*–*4*). Most notably, electromagnetic cloaking devices (*5*) are inspired by ideas of transformation optics (*6*–*10*), whereby transparent materials mimic coordinate transformations, forcing light to follow curved coordinates. The coordinates may enclose a hidden space, making the interior invisible and the act of cloaking undetectable. Another application of transformation optics (*3*, *4*) is negative refraction (*11*, *12*), where light follows coordinates that run backward in space (*2*) or time (*13*). One can also create optical analogs of the event horizon (*2*, *3*, *14*) and perhaps even electromagnetic wormholes (*15*). The key to engineering practical implementations of ideas that normally belong to general relativity (*2*–*4*) is the application of modern metamaterials (*16*–*19*). In metamaterials, man-made subwavelength structures generate unusual electromagnetic and optical properties. Metamaterials are potentially very versatile, but they are still subject to fundamental limits.

Take, for instance, the cloaking device (*10*) with the coordinate transformation illustrated in Fig. 1. The coordinates of physical space (Fig. 1B) are curved transformations of straight Cartesian coordinates in a virtual space that we call electromagnetic space (*2*) (Fig. 1A). This space is empty, so light follows straight lines that appear curved in physical space. If the coordinate transformation expands one point in electromagnetic space to an extended volume in physical space, anything in the "interior of the point" is invisible, as shown in Fig. 1B. However, Fig. 1 also reveals a fundamental problem of such cloaking devices. In electromagnetic space, light passes a point in infinitely short time, but in physical space the point has become an extended region. Thus, light must propagate along the inner lining of the cloak at infinite speed (*2*). In materials, including metamaterials, the phase velocity (*1*) of light may approach infinity, but only at discrete frequencies that correspond to resonances of the material's constituents. Light with different frequencies (different colors) would not be cloaked but instead be distorted. Furthermore, the group velocity (*1*) tends to be zero at resonances: Light pulses would become glued to the device instead of traveling around it (*20*). Therefore, turning invisibility from a tantalizing idea into a practical broadband device requires a different approach.

So far, transformation optics have mostly applied concepts of only Euclidean, flat space, the curved light rays being mere coordinate transformations of a space that is inherently flat. Here we explain how concepts of non-Euclidean geometry (i.e., of intrinsically curved space) could pave the way to broadband invisibility. In curved space, light may propagate along closed loops or may avoid some regions altogether. Most transparent materials act as if they would curve the geometry of light (*3*); light focused by a lens, refracted in a water droplet, or bent in a mirage perceives space as being curved, in general. Transformation media where the perceived space is inherently flat are the exceptions (*3*). However, to

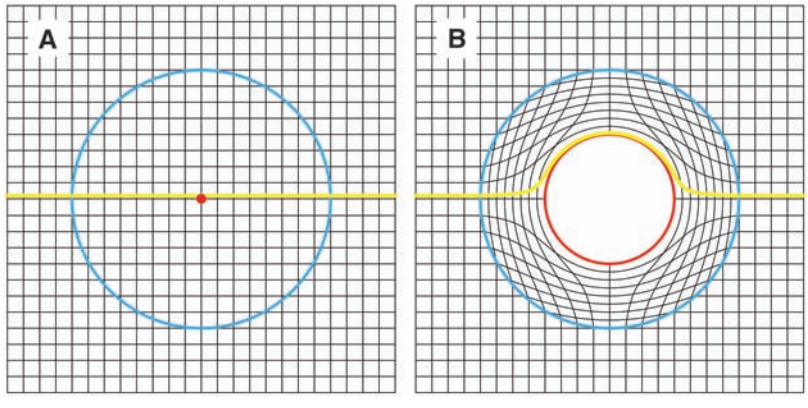

Fig. 1. Euclidean cloaking device (*10*). The device performs a coordinate transformation from the virtual space (**A**) to physical space (**B**). The virtual space is empty and flat (Euclidean). Because the curved coordinate lines of physical space are transformations of straight lines, physical space is Euclidean as well. The device creates the illusion that light propagates through flat space that is empty, apart from one point that, in physical space, has been expanded to finite size. The interior of the expanded point is hidden. Light, however, passes a point in infinitely short time. So, in physical space, the speed of light in the material of the device must approach infinity, which severely limits the use of Euclidean cloaking (*10*).

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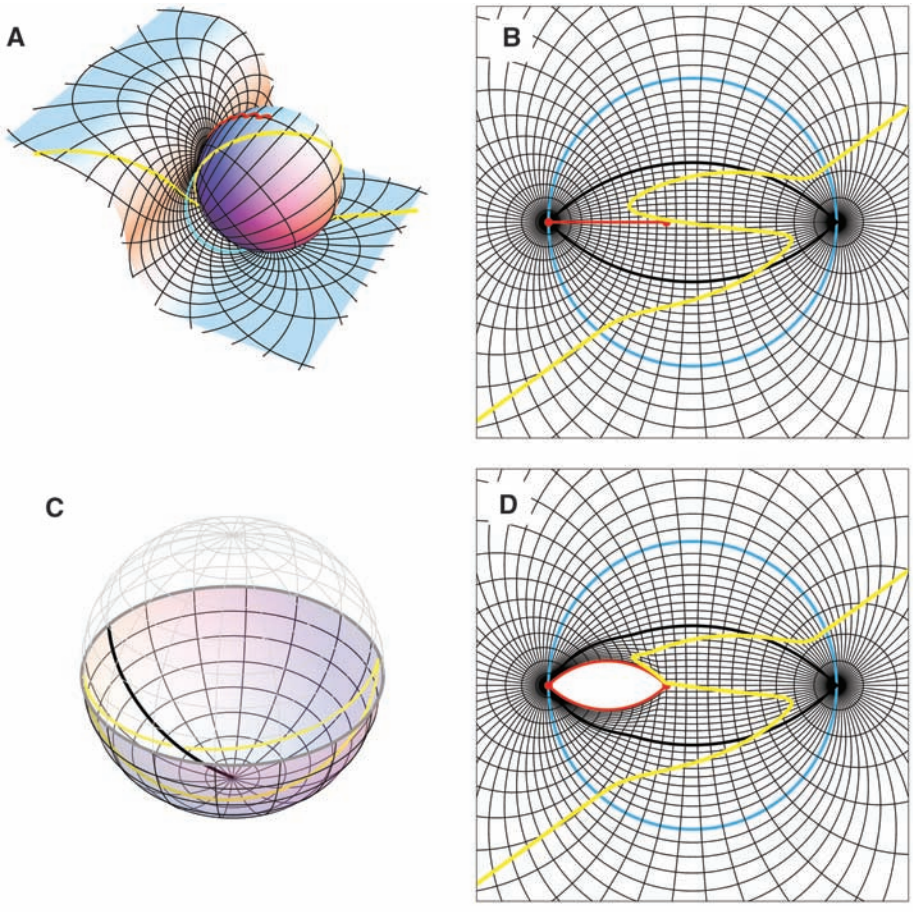

Fig. 2. Non-Euclidean cloaking device in two dimensions. The device creates the illusion shown in (**A**): Light propagates through a virtual space that consists of a plane and the surface of a sphere, a curved space, which touch along a line. Some incident light rays venture from the plane to the sphere; they return after one loop and continue in the same direction. Note that the rays never cross the red zigzag line on the sphere. Plane and sphere carry a coordinate grid that is mapped onto physical space (**B**). The magenta circle defines the boundary of the device. Its interior has been expanded to make space for the grid of the sphere. In particular, the line where plane and sphere touch has been opened like an eye (thick black lines) to include the sphere. This is not a cloaking device yet, but one could place a mirror around the equator of the virtual sphere (**C**), making the northern hemisphere invisible and creating the same illusion as shown in (A). (**D**) Alternatively, one could expand the red line that light never crosses to create a hidden space.

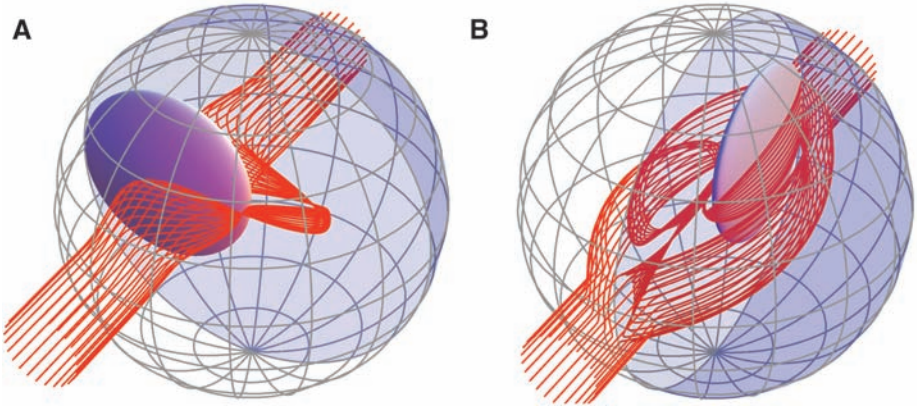

Fig. 3. 3D cloaking. One can extrapolate the ideas illustrated in Fig. 2 to 3D space, replacing the plane by flat space and the sphere by a hypersphere. The lentil-shaped object indicates the hidden interior of the device, and the partly shaded grid denotes the boundary of the invisibility device. For better contrast, light rays are shown in red. (**A**) Rays are bent around the invisible region. (**B**) In three dimensions, some rays turn out to perform two loops in hyperspace that appear in physical space as light wrapped around the invisible interior.

achieve invisibility, it is necessary to curve the geometry in specific ways.

We explain our ideas with pictures, the complete calculations behind the pictures being described in the supporting online material (*21*). As three-dimensional (3D) curved space is difficult to visualize, we first explain our concept on a 2D example and then extend this case to three dimensions. Figure 2A shows the archetype of a non-Euclidean space (the surface of a sphere) combined with a Euclidean space (the plane) that touches the sphere like a piece of paper partially wrapped around a globe. Both the plane and the sphere carry a coordinate grid that we map onto physical space (the plane shown in Fig. 2B). The entrance to the sphere (i.e., the line where the globe touches the plane) has been opened like an eye in the physical plane to make space for the grid of the sphere. In mathematical terminology, electromagnetic space consists of two branches, plane and sphere, that are connected at a branch cut. Although the globe has been flattened in physical space, the exterior curvature of the sphere is maintained as intrinsic curvature.

As there is a one-to-one correspondence between light propagation in the physical plane (Fig. 2B) and in electromagnetic space (Fig. 2A), we discuss the optics in electromagnetic space. Light rays follow geodesics (*3*), lines of shortest or longest path (*1*, *3*). The geodesics on the sphere are the great circles. Light entering the sphere through the branch cut performs a loop and leaves in the same direction as before; the sphere is invisible but it does not make anything else invisible yet. However, if we place a mirror around the equator of the globe (Fig. 2C), light is reflected twice, creating the illusion of following a great circle, yet never reaching the northern hemisphere. Anything placed inside the corresponding area in physical space is invisible. A more elegant option instead of hiding behind a mirror is the creation of an invisible space that light naturally avoids (*22*). For example, the light circles on the sphere never cross the red zigzag shown in Fig. 2A. Imagine we open the zigzag like a zip in physical space (Fig. 2D). Anything inside this region is hidden, and the act of hiding is not detectable on the light rays: We have a cloaking device. On the other hand, light performs loops on the sphere, which takes time. Measuring time delays or examining the phase fronts of light rays could reveal the presence of the cloaking device. This imperfection (*9*, *22*) is the price to pay for practical invisibility, whereas perfect invisibility (*10*) is not practical.

The implementation of our idea does not demand extreme optical properties such as infinities or zeros of the speed of light, for the following reason: In electromagnetic space, light propagates at the speed of light in vacuum. Physical space represents a deformed image of electromagnetic space; the speed of light follows this deformation. Expressed in quantitative terms, if an infinitesimal line element in electromagnetic space is n times longer than its image in physical

space, then the refractive index in the corresponding direction in physical space is n. Figure 2 as well as calculations (*21*) show that the ratio of the line elements is neither infinite nor zero. Even at a branch point the spatial deformation in any direction is finite, because here the coordinate grid is only compressed in angular direction by a finite factor, in contrast to optical conformal mapping (*9*). Furthermore, the spatial deformations are gradual, for avoiding reflections at boundaries (*23*).

Figure 3 illustrates the extension of our idea to three dimensions. Instead of the 2D surface of the globe of Fig. 2A, we use the 3D surface of a 4D sphere (a hypersphere). Such a geometry is realized (*24*, *25*) in Maxwell's fish eye (*1*, *26*). Inside the cloaking device, we inflate a 2D surface, the branch cut in 3D, like a balloon to make space for the 3D surface of the hypersphere. Again, at this point the cloak is invisible but does not hide anything yet. Then we open another spatial branch on the "zip" of the hypersphere to create a hidden interior. The branch cuts are curved surfaces in electromagnetic space, which is the only important difference when compared with the 2D case. Some light rays may pierce the entrance to the hypersphere twice; they perform two loops in the non-Euclidean branch. In physical space, light is wrapped around the invisible interior in such cases (Fig. 3B). We calculated the required electromagnetic properties (*21*) and found that the electric permittivity ranges from 0.28 to 31.2 for our specific example. One could give the cloaking device any desired shape by further coordinate transformations, which would change the requirements on the optical properties of the material. As a rule, the larger the cloaked fraction of the total volume of the device, the stronger the optics of the material must be, but the required speed of light will always remain finite.

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Control of Self-Assembly of DNA Tubules Through Integration of Gold Nanoparticles

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The assembly of nanoparticles into three-dimensional (3D) architectures could allow for greater control of the interactions between these particles or with molecules. DNA tubes are known to form through either self-association of multi-helix DNA bundle structures or closing up of 2D DNA tile lattices. By the attachment of single-stranded DNA to gold nanoparticles, nanotubes of various 3D architectures can form, ranging in shape from stacked rings to single spirals, double spirals, and nested spirals. The nanoparticles are active elements that control the preference for specific tube conformations through size-dependent steric repulsion effects. For example, we can control the tube assembly to favor stacked-ring structures using 10-nanometer gold nanoparticles. Electron tomography revealed a left-handed chirality in the spiral tubes, double-wall tube features, and conformational transitions between tubes.

Nanoparticles can exhibit distinctive electronic, magnetic, and photonic properties (*1*), and their assembly into well-defined one-dimensional (1D), 2D, and 3D architectures with geometric controls could add to their functionality. DNA-mediated assembly of nanoparticles is an attractive way to organize both metallic and semiconducting nanoparticles into periodic or discrete 1D and 2D structures (*1*–*14*) through the programmable base-pairing interactions and the ability to construct branched DNA nanostructures of various geometries. Recent success in using DNA as a molecular glue to direct gold nanoparticles (AuNPs) into periodic 3D crystalline lattices further demonstrates the power of DNA as building blocks for 3D nanoengineering (*15*, *16*).

Here, we report a group of complex 3D geometric architectures of AuNPs created using DNA tile-mediated self-assembly. These are tubular nanostructures with various conformations and chiralities resembling those of carbon nanotubes. The nanoparticle tube assembly can be engineered both by the underlying DNA tile scaffolds and the nanoparticles themselves. Previous work in structural DNA nanotechnology has shown that DNA tubes can form through either the self-association of multi-helix DNA bundle structures or the closing up of 2D DNA tile lattices (*17*–*26*). The forces that drive tube formation have been attributed to the intrinsic curvature of the tile-array (*21*) and the thermodynamic requirement to lower the free energy of the system by minimizing the number of unpaired sticky ends (*22*). The intrinsic dimensional anisotropicity of the DNA tiles also plays an important role in the kinetic control of the tube growth (*26*).

In all of the above studies, the true 3D conformations of DNA tubes have never been revealed in detail because of limitations in microscopic imaging techniques; deposition of the samples on a surface for atomic force microscope (AFM) or transmission electron microscope (TEM) imaging usually causes flattening and sometimes opening of the tubes. This limitation has prevented a comprehensive understanding of the structural features of DNA nanotubes. For example, the handedness of the chiral tubes can be better revealed with 3D structural characteriza-

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tion of the samples. Furthermore, there has been no report to explore the use of DNA tiles to control the assembly of AuNPs into tubular architectures, which may lead to interesting properties for nanoelectronics and photonics applications.

We considered the incorporation of AuNPs into a planar DNA tile array by conjugating each AuNP with a single DNA strand. We propose that AuNPs lined up on the DNA array will have systematic steric and electrostatic repulsion effects that will favor DNA tube formation. In addition, we rationalize that varying the size of the AuNPs in such constructs could help control the tube conformation. The use of metallic NPs provides an effective image-enhancement method to probe the 3D DNA nanostructures with electron microscopy because of their high electrical contrast.

An array system formed from four double-crossover (DX) DNA tiles (*27*) was used in the current study (Fig. 1, A and B). In the first design, we modified two out of the four component tiles (*28*). The central strand in the A tile was conjugated with a thiol group, which was then linked to a 5-nm AuNP in a 1:1 ratio (*28*) so that, when self-assembled, each A tile carried a AuNP on one side of the tile (shown as the top side). The C tile was modified with a DNA stem loop extending out of the tile surface toward the bottom side (Fig. 1, A and B). As illustrated in Fig. 1C, these four tiles were designed to self-assemble into a 2D array through sticky-end associations, with the A tiles forming parallel lines of AuNPs that are all located at the top side of the array and the C tiles forming parallel lines of stem loops at the bottom side of the array. The designed periodicity between the neighboring A tiles is expected to be ~64 nm when the tiles are closely packed side by side. Additionally, the intrinsic curvature of the array is expected to be cancelled because the A and C tiles face one direction whereas the B and D tiles face the opposite direction (*20*–*22*, *26*). However, in the presence of the 5-nm AuNPs, which have diameters that are comparable or even greater than the center-to-center distances of the neighboring A tiles within the parallel stripes (4 to 5 nm), the strong electrostatic and steric repulsions between the neighboring AuNPs force the 2D arrays to curl up to avoid direct contact between the particles. This curling will lead to tube formation with the particles displayed on the outer surface of the tubes.

The stem loops on the C tiles in this design were placed on surfaces opposite from the AuNPs as a counterforce to resist tube formation (that is, to increase the energy barrier for bending the 2D array). However, because the AuNPs are much larger than the DNA stem loops, their forces are not perfectly counterbalanced. The tile arrays still have a tendency to curl up into tubes with the stem loops wrapped inside and the AuNPs displayed on the outside.

There are a few different possible ways for the edge tiles to associate in the tube formation (Fig. 1D). When the edge tiles at one side of the array that associate with the corresponding edge tiles at the opposite side of the array are within the same lines, a tube displaying stacked rings of AuNPs will form. When the corresponding edge tiles that associate are at neighboring lines (with 1 line offset), tubes displaying a single spiral of nanoparticles will result. Depending on the sign of the offset ($n \rightarrow n + 1$ lines, or $n \rightarrow n - 1$ lines), the spiral can potentially display either a left-hand or right-hand chirality. Similarly, when the corresponding edge tiles that associate are at alternating lines ($n \rightarrow n + 2$ lines, or $n \rightarrow n - 2$ lines), tubes displaying double spirals of nanoparticles will result. When the corresponding edge tiles are at lines with a larger interval ($\Delta n \geq 3$ lines), spiral tubes will be nested.

Varieties of tubes with different conformations were observed from the above design (Figs. 1E and 2A and fig. S2) (*28*). The results of statistical image analysis are shown in Fig. 2E (red bars). The enthalpy changes of the formation of the spiral tubes and the stacked-ring tubes are similar because the same number of base pairings is satisfied per unit of tile. The free energy changes differ by the bending energy because the tubes have different diameters and hence curvatures, and an extra twisting energy for the spiral tube to form. The transition between the two forms of tubes requires a large activation-energy barrier (simultaneously breaking many sticky-end pairs and reforming all of the sticky-end pairs at distance a few tiles away). Thus, the distribution of tube-product conformations can be considered the result of the differences in the bending energy and twisting energy. From the broad distribution of the different tube conformations in this sample (a significant percentage of the resultant tubes are single and multiple spiral tubes), we can deduce that with the presence of a stem loop, the energy required to twist the tile array is relatively small as compared with the energy required to bend the tile array.

To gain control over the type of tube conformation formed, we removed the DNA stem loop in the C tile and placed differently sized AuNPs on the A tile in a series of experiments (Fig. 2, B to D). First, after removing the stem loop but retaining the 5-nm AuNPs, the resulting tubes (Fig. 2B and figs. S3 and S6) (*28*) displayed a different distribution of the tube conformation (Fig. 2E, light blue bars), in which more stacked rings (>55%) than single-spiral tubes (45%) were

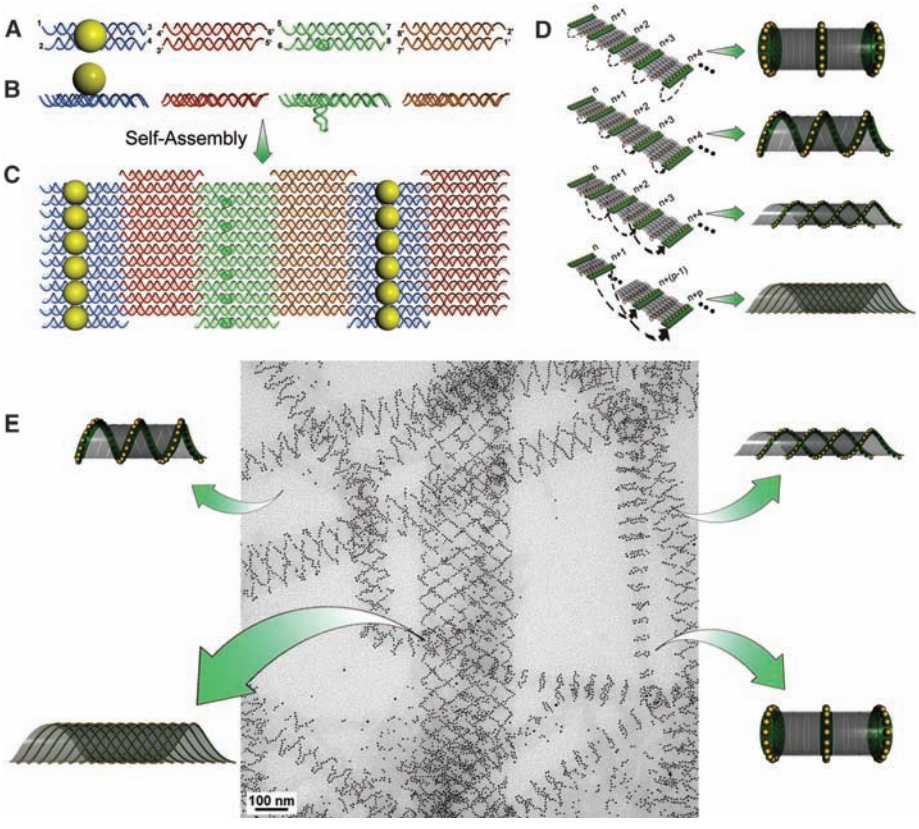

Fig. 1. The design of a DNA tile system for the formation of a variety of tubular structures carrying 5-nm AuNPs. (**A** and **B**) Top and side view of the four DX tiles (A tile, blue; B tile, red; C tile, green; and D tile, brown). The A tile carries a 5-nm AuNP on the top of the tile. The C tile carries a DNA stem loop pointing downward. (**C**) The four different tiles are designed to self-assemble into a 2D array displaying parallel lines of AuNPs. (**D**) Possible ways for the corresponding edge tiles on opposite sides of the 2D array to associate and lead to formation of tubes displaying patterns of AuNPs in stacked rings, single spirals, double spirals, and nested spiral tubes. (**E**) The different tube conformations were observed in a single TEM image.

formed and no double-spiral or nested-spiral tubes were observed. Deleting the stem loop removed a substantial part of the counterforce that resisted the bending of the tile array. Thus, the array had a greater tendency to curl up and the tubes had a smaller diameter (table S1, diameter analysis) (*28*). As the diameter of the tube gets smaller, the twisting energy increases, especially for the multiple-spiral tubes, which explains why more stacked-ring tubes and few or no multiple-spiral tubes were obtained for this construct.

As a control experiment, when we deleted both the stem loop from the C tile and the AuNP on the A tile so that the curving forces on both sides of the arrays were balanced, 2D arrays (single-layer ribbons, 300 to 500 nm in width and a few micrometers long) were the dominant morphology (fig. S19, AFM images) (*28*), although coexistence of tubes was also observed, similar to those previously reported (*20*–*22*). This control experiment supports our argument that the AuNPs act as an active component in the tube formation: In the tile arrays with AuNPs on one side, the repulsion between the AuNPs can cause an overall bending of the 2D array to minimize the repulsion and promote tube formation.

When larger sized AuNPs (diameters of 10 and 15 nm) were used, a majority of the tubes formed were in the stacked-ring conformation. This distribution change can be explained with the same bending-energy-and-twisting-energy argument. The repulsive forces exerted by the larger sized AuNPs further promote the curving of the tile array into smaller-diameter tubes (table S1, diameter analysis) (*28*), and the increased energy required for twisting into spirals causes the stacked ring conformations to be favored. Indeed, for the 10-nm particles, 92% of the tubes were stacked rings with only ~7% of single spirals [Fig. 2, C and E (orange bars), and figs. S4 and S7] (*28*). Only one double-spiral but no nested-spiral tube was observed. For the 15-nm particles, the same trend prevailed [Fig. 2, D and E (dark blue bars), and figs. S5 and S8] (*28*).

In the arrays, the widths of the AuNPs (10 and 15 nm) were much greater than those of the tiles (4 to 5 nm for undistorted DX tiles), which led to extreme crowding of the AuNPs along the ring or along the spiral lines if the tiles remained closely packed. The DNA tiles were not perfectly rigid. For the DX tiles, the four arms bearing sticky ends can all swing around the two crossover points within a limited range. Thus, the repulsion between the AuNPs can induce expansion in the direction perpendicular to the axis of the tube and concurrent shrinking along the parallel direction. This distortion resulted in a decreased periodical length (table S1) (*28*), similar to the effect of stretching a meshed net in one direction.

The above TEM images were only 2D projections of the 3D structures. From the parallel closed double lines, ellipsoidal rings, and occasional asymmetric zigzags observed in these images, we can deduce that these are true tubular structures with tube axes not perfectly perpendicular to the electron beam. In order to gain full appreciation of their 3D structural architectures, electron cryotomography was used to image these tubes. The native conformations of the tubes were better preserved by embedding them in vitreous ice. The samples were imaged at a series of tilted angles and then aligned and back-projected to reconstruct their 3D conformations (Fig. 3, A to D, figs. S9 to S14, and movies S1 to S7) (*28*). The stacked-ring tubes were clearly observed to be closed circular rings aligned in parallel. A number of single- and double-spiral tubes observed from different samples were revealed to be all left-handed. Figure 1D illustrates how this observation is counterintuitive and infers that both right- and left-handed tubes are equally possible. The preference of this left-handed chirality can be explained by the tendency of relaxed or underwound right-handed DNA double helices to form left-handed supercoils. This left-handed super-helicity may exist in each DNA tile and accumulates as the tiles self-assemble into the tile arrays. Thus, a left-handed twist naturally exists in the tile array, so

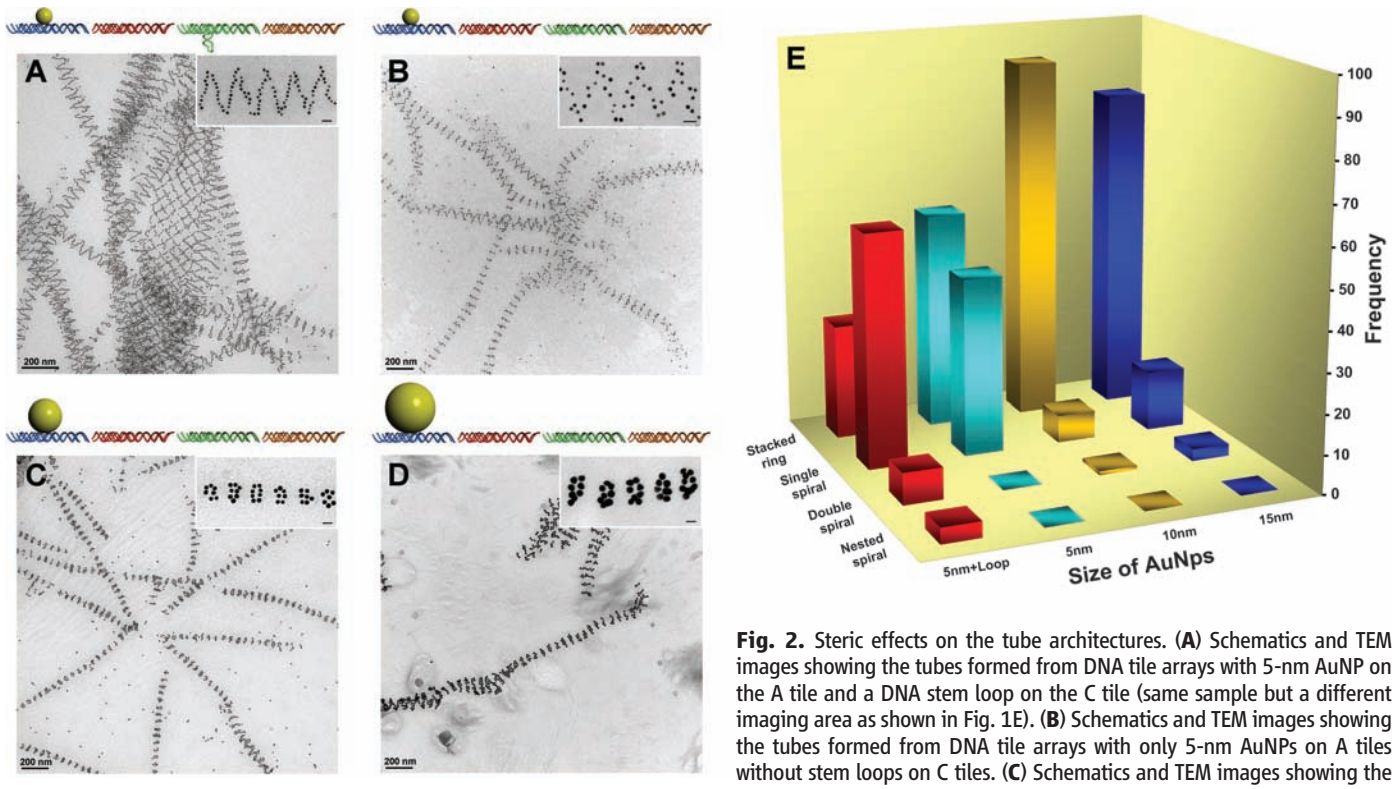

Fig. 2. Steric effects on the tube architectures. (**A**) Schematics and TEM images showing the tubes formed from DNA tile arrays with 5-nm AuNP on the A tile and a DNA stem loop on the C tile (same sample but a different imaging area as shown in Fig. 1E). (**B**) Schematics and TEM images showing the tubes formed from DNA tile arrays with only 5-nm AuNPs on A tiles without stem loops on C tiles. (**C**) Schematics and TEM images showing the tubes formed from DNA tile arrays with 10-nm AuNPs on A tiles. (**D**) Schematics and TEM images showing the tubes formed from DNA tile arrays with 15-nm AuNPs on A tiles. These TEM images are 2D projections of flattened tubular structures. (**E**) Histogram showing the distribution of tube types observed for the four samples from (A) to (D). One hundred tubes were randomly counted and analyzed from nonoverlapping images for each sample. Additional images are shown in (*28*). Each image contains a magnified representative tube from each sample. The scale bars in the inserts are all 20 nm.

that the superstructure formed prefers to have a left-handed chirality.

In addition to the left-handed chirality, we also observed an interesting double-walled DNA nanotube (Fig. 3C) formed by a single-spiral AuNP nanotube inside of a double-spiral AuNP nanotube; their periodicities are well aligned. A closer examination of this image showed that the right ends of the two tubes share a common layer, which may indicate that the growth of an internal or external secondary tube can be initiated from a primary tube defect.

Various types of tube conformation transitions were evident in both TEM and tomograms (Fig. 3D and figs. S15 and S16) (*28*). For example, continuous transitions from stacked rings to spiral tubes and vice versa can be discerned. Splitting of a single tube into two tubes of smaller diameters was also observed. Such transitions can involve any type of tube structure and can be explained either as conformational transitions during the tube growth or end-to-end joining of different tubes during and/or after the growth. This end-to-end joining is thermodynamically driven by the reduction in the number of unpaired sticky ends existing at the ends of the tubes after the nucleation stage. This type of tube-end joining can still occur with only partially matched sticky ends for the end tiles. In addition, the flexibility of the tile arrays and the presence of defects can also induce transitions from one type of tube to another during tube growth.

To increase the complexity of the 3D architecture, we placed 5- and 10-nm particles on opposite sides of the array on the A and C tiles, respectively. Electron tomographic images (Fig. 4)

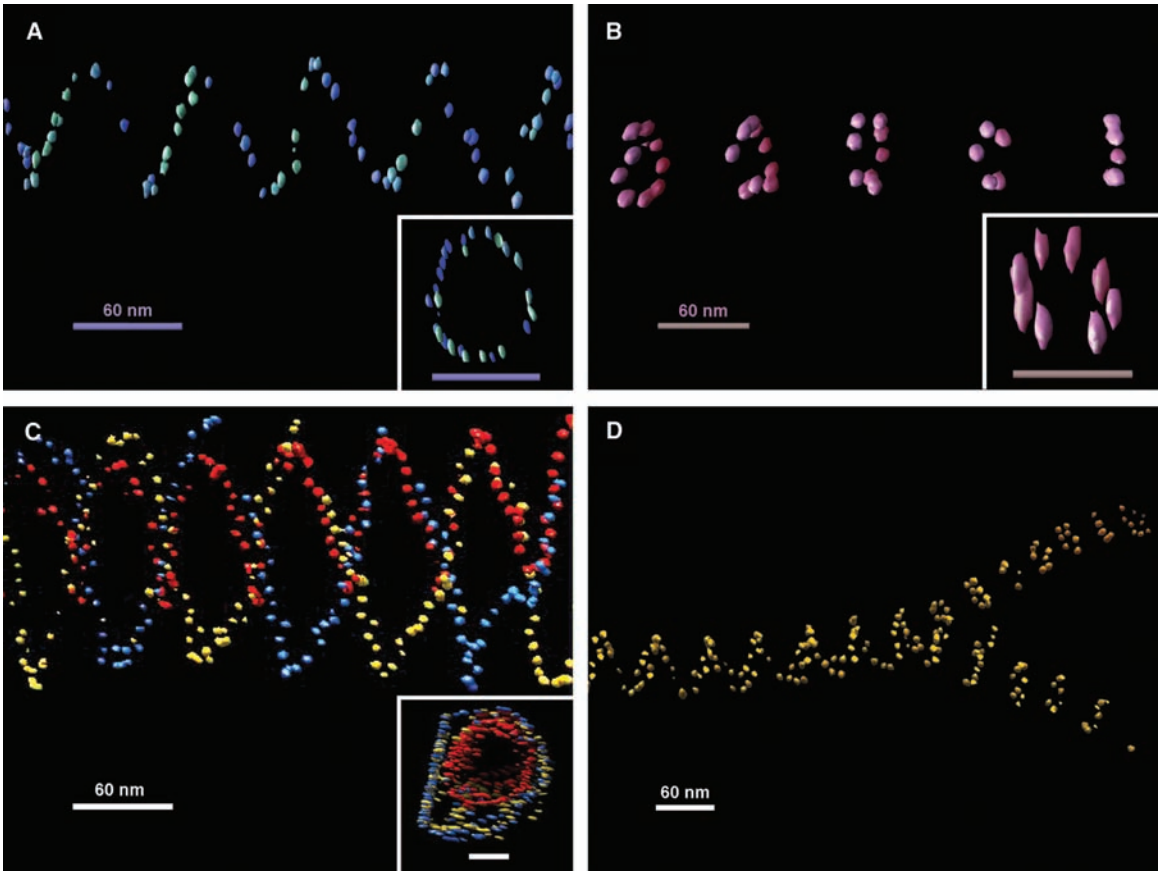

Fig. 3. Representative 3D structures of nanoparticle tubes reconstructed from cryoelectron tomographic imaging. (**A**) One view of the tomogram of a single-spiral tube of 5-nm AuNPs. The inset shows a top view from the axis of two helical turns of the spiral tube; scale bar, 60 nm. (**B**) Tomogram of a stacked-ring tube of 5-nm particles. The inset shows a top view from the axis of a single ring from the stacked-ring tube; scale bar, 60 nm. (**C**) Tomogram of a double-spiral tube of 5-nm AuNPs with a single spiral of 5-nm nanoparticles inside each coded with a different color. The inset shows a top view from the axis of the double-wall spiral tube; scale bar, 60 nm. (**D**) Tomograph showing the splitting of a wider single-spiral tube into two narrower stacked-ring tubes of 10-nm AuNPs. All of the spiral tubes show a left-handed chirality. A weakly colored depth cue was applied to each view. The elongated appearance of the gold bead in the top views of the tubes is an effect of limited tilts in the tomography data collection. Movies of electron tomographic reconstruction corresponding to these structures are available in (*28*).

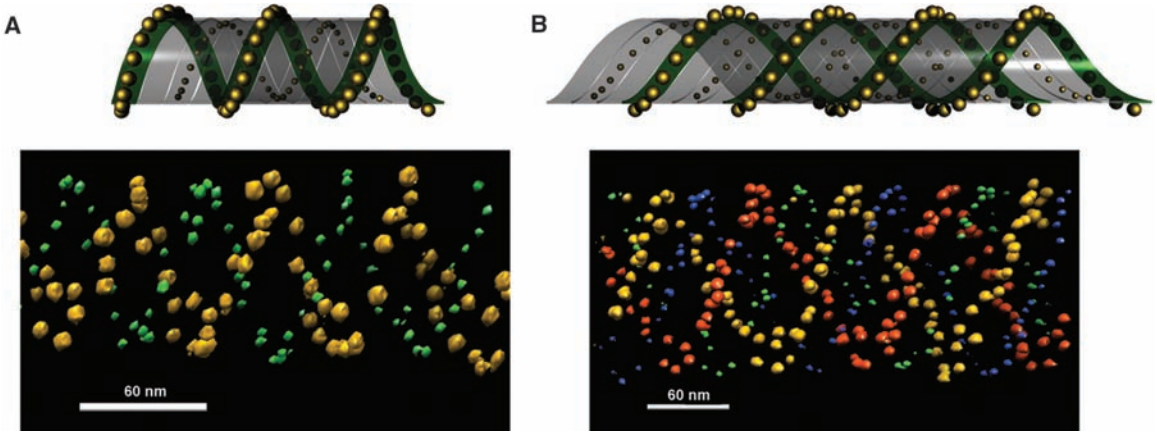

Fig. 4. The tubes formed with 5- and 10-nm AuNPs placed on opposite surfaces of the DNA tile array. (**A** and **B**) The top panels are schematic side and top views of the binary particle tube architectures; the bottom panels are corresponding representative electron tomographic images clearly showing the 3D architectures. Movies of electron tomographic images corresponding to these structures are available in (*28*).

demonstrate such dual-labeled AuNP tube architectures. The image shown in Fig. 4A contains a single spiral of 5-nm AuNPs wrapped around a single spiral of 10-nm AuNPs (an architecture resembling a double helix). The image shown in Fig. 4B contains a double spiral of 5-nm AuNPs wrapped around a double spiral of 10 nm AuNPs (an architecture resembling a quadruplex). From the design, it is expected that the steric repulsion force among the 10-nm particles is greater than that among the 5-nm AuNPs so that the tubes would tend to have the 5-nm AuNPs wrapped inside and the 10-nm AuNPs displayed outside. However, when these tube samples were imaged by cryo-EM (Fig. 4, A and B) in which the native conformations of the tubes were preserved, the two AuNP sizes seemed to stay at about the same layer. It is possible that the 5-nm AuNPs repel one another sufficiently that they are squeezed outward through the gaps between the arms of the two DNA crossovers.

These types of AuNP superstructures and 3D complexities reflect the kind of complex architectures that naturally existing systems display (for example, diatoms) but with artificial control of precision at nanometer scales. By further engineering the tile structures, it should be possible to place different sizes or types of nanoparticles in or outside of the tubes. For example, self-assembled nanoinductors could be constructed when magnetic nanoparticles are placed inside of spiral wires made of metallic nanoparticles, which might represent a substantial advancement in small-scale device applications.

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28. Materials and methods are available as supporting material on *Science* Online.

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Supporting Online Material

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Material and Methods
Fig. S1 to S21
Table S1
Movies S1 to S7

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Declining Coral Calcification on the Great Barrier Reef

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Reef-building corals are under increasing physiological stress from a changing climate and ocean absorption of increasing atmospheric carbon dioxide. We investigated 328 colonies of massive *Porites* corals from 69 reefs of the Great Barrier Reef (GBR) in Australia. Their skeletal records show that throughout the GBR, calcification has declined by 14.2% since 1990, predominantly because extension (linear growth) has declined by 13.3%. The data suggest that such a severe and sudden decline in calcification is unprecedented in at least the past 400 years. Calcification increases linearly with increasing large-scale sea surface temperature but responds nonlinearly to annual temperature anomalies. The causes of the decline remain unknown; however, this study suggests that increasing temperature stress and a declining saturation state of seawater aragonite may be diminishing the ability of GBR corals to deposit calcium carbonate.

There is little doubt that coral reefs are under unprecedented pressure worldwide because of climate change, changes in water quality from terrestrial runoff, and overexploitation (*1*). Recently, declining pH of the upper seawater layers due to the absorption of increasing atmospheric CO_2 [termed ocean acidification (*2*)] has been added to the list of potential threats to coral reefs, because laboratory studies show that coral calcification decreases with declining pH (*3*–*6*). Coral calcification is an important determinant of the health of reef ecosystems, because tens of thousands of species associated with reefs depend on the structural complexity provided by the calcareous coral skeletons. Several studies have documented globally declining coral cover (*7*) and reduced coral diversity (*8*). However, few field studies have so far investigated long-term changes in the physiology of living corals as indicated by coral calcification.

We investigated annual calcification rates derived from samples from 328 colonies of massive *Porites* corals [from the Coral Core Archive of the Australian Institute of Marine Science (*9*, *10*)] from 69 reefs ranging from coastal to oceanic locations and covering most of the >2000-km length of the Great Barrier Reef (GBR, latitude 11.5° to 23° south; Fig. 1, A and B) in Australia. Like other corals, *Porites* grow by precipitating aragonite onto an organic matrix within the narrow space between their tissue and the previously deposited skeletal surface. Massive *Porites* are commonly used for sclerochronological studies because they contain annual density bands (*11*), are widely distributed, and can grow for several centuries. Numerous studies have established that changes in environmental conditions are recorded in their skeletons (*12*).

Annual data for three growth parameters [skeletal density (grams per cubic centimeter), annual extension (linear growth) rate (centimeters per year), and calcification rate (the

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product of skeletal density and annual extension; grams per square centimeter per year)] were obtained from each colony with the use of standard x-ray and gamma densitometry techniques (*10*, *13*). Mean annual sea surface temperature (SST) records were obtained from the HadISST1 global SST compilation (1°-square resolution) for the period 1900–2006 (*14*, *15*) (Fig. 1, A and C). The composite data set contains 16,472 annual records, with corals ranging from 10 to 436 years in age, most of which were collected in two periods covering 1983–1992 and 2002–2005 (Fig. 1B).

Preliminary exploratory analysis of the data showed strong declines in calcification for the period 1990–2005, based on growth records of 189 colonies from 13 reefs. Despite high variation of calcification between both reefs and colonies, the linear component of the decline was consistent across both reefs and colonies for 1990–2005. Of the 13 reefs, 12 (92.3%) showed negative linear trends in calcification rate, with an average decline of 1.44% year^{-1} (SE = 0.31%), and of the 189 colonies, 137 (72.5%) showed negative linear trends, with an average decline of 1.70% year^{-1} (SE = 0.28%). To determine whether this decline was an ontogenetic artifact, we compared these findings with similar analyses of the past 15 years of calcification for each of the remaining 139 colonies sampled before 1990. In this group, linear increases and declines were approximately equal in number, with 29 of the 56 reefs (51.7%) declining at an average rate of 0.11% year^{-1} (SE = 0.18%), and 68 of the 139 colonies (48.9%) declining at 0.16% year^{-1} (SE = 0.21%). This strongly suggests that the 1990–2005 decline in calcification was specific to that period, rather than reflecting ontogenetic properties of the outermost annual growth bands in coral skeletons.

Because of the imbalance of sampling intensity over years and the desire to focus on time scales varying from a few years to centuries, the records were broken into two data sets for further analyses. The 1900–2005 data set contained all 328 colonies, whereas the 1572–2001 data set focused only on long-term change and contained 10 long cores from colonies that covered all or most of that period.

The dependencies of calcification, extension, and density of annual growth bands on year (the year the band was laid down), location (the relative distance of the reef across the shelf and along the GBR), and SST were assessed with linear mixed-effects models (*16*) [supporting online material (SOM)]. SST covaries strongly with space and year (Fig. 1, A and C), and to understand the confounding of these effects and to assist in the interpretation of temperature effects, SST was partitioned into three components: the large-scale spatial trend (SST-SPAT; predominantly latitudinal), the long-term temporal trend (SST-TEMP), and the anomalies (SST-ANOM). The latter represent annual deviations from the large-scale and long-term trends in SST. The models included fixed effects in year, SST components, and location, and random effects in reef, colony, and year (SOM). Fixed effects were represented as smooth splines, with the degree of smoothness being determined by cross-validation (*17*). Results are illustrated through partial-effects plots. Three sets of analyses were conducted; the first and second focused solely on temporal change and used the 1900–2005 and 1572–2001 data sets, whereas the third used only the 1900–2005 data set but also included the three SST components and the relative distance across the shelf and along the GBR.

The temporal models of calcification, extension, and density from the 1900–2005 data showed strong patterns of change (Fig. 2, A to C). The rate of calcification increased from ~1.67 g cm^{-2} year^{-1} in the period 1900–1930 to a maximum of ~1.76 g cm^{-2} year^{-1} in 1970, but since 1990 has declined from 1.76 to 1.51 g cm^{-2} year^{-1}, an overall decline of 14.2% (SE = 2.3%). The rate of this decline has increased from 0.3% year^{-1} in 1990 to 1.5% year^{-1} in 2005. The decline in calcification was largely due to the decline in extension from 1.43 to 1.24 cm year^{-1} (13.3%, SE = 2.1%). Density varied nonlinearly from 1.24 to 1.22 g cm^{-3} (1.7%; SE = 1.9%) over the period 1990–2005.

The 1572–2001 data showed that calcification increased in the 10 colonies from ~1.62 g cm^{-2} year^{-1} before 1700 to ~1.76 g cm^{-2} year^{-1} in ~1850, after which it remained relatively constant before a decline from ~1960 (Fig. 2D). However, this finding should be treated with caution because of the small sample size (7 reefs and 10 colonies).

Smooth terms in the three SST components and two spatial predictors (across and along the reef) were then added to the temporal models of the 1900–2005 data. SST-TEMP and distance

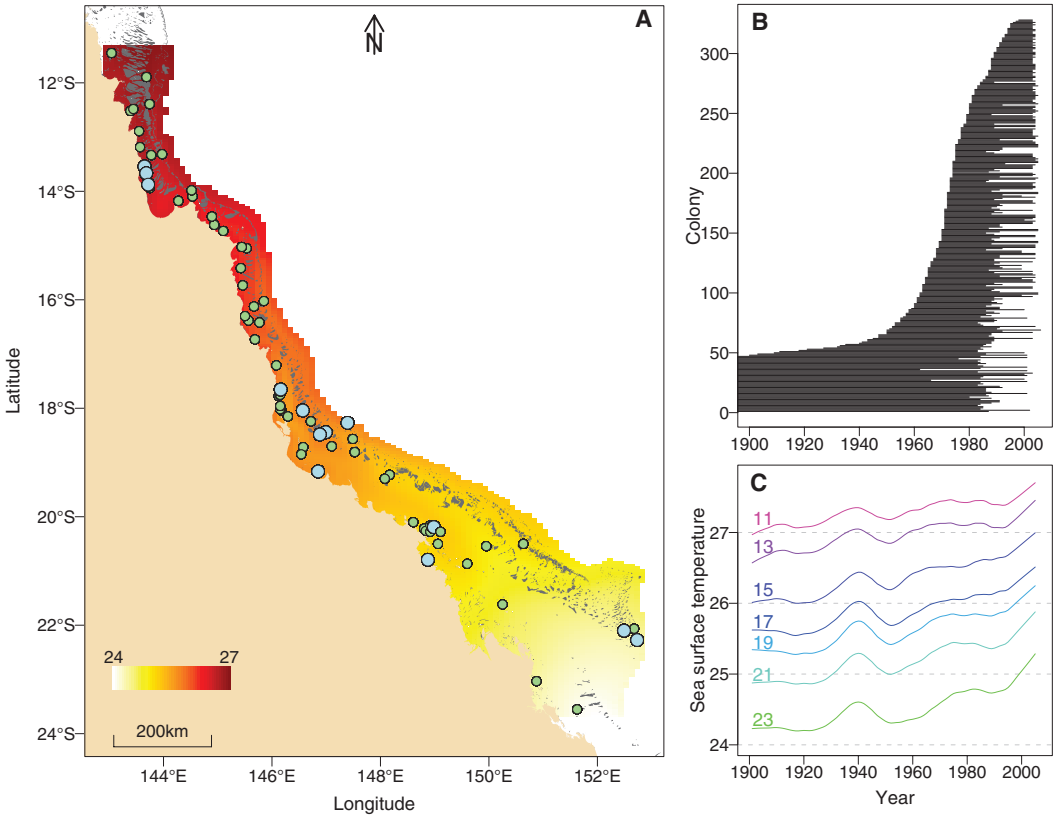

Fig. 1. (**A**) Map of the GBR, showing locations of the sampled reefs and large-scale spatial patterns in SST averaged over the past 105 years (color ramp, in °C). Blue circles indicate the 13 reefs with *Porites* cores sampled in 2005, and green circles indicate the 56 reefs sampled before 1993. (**B**) Distribution of age ranges of the 328 *Porites* colonies. Truncated records extend back in time as far as 1572. (**C**) Temporal trends in annual SST for 2° latitudinal bands (labeled with colored numbers, in °S) in the GBR.

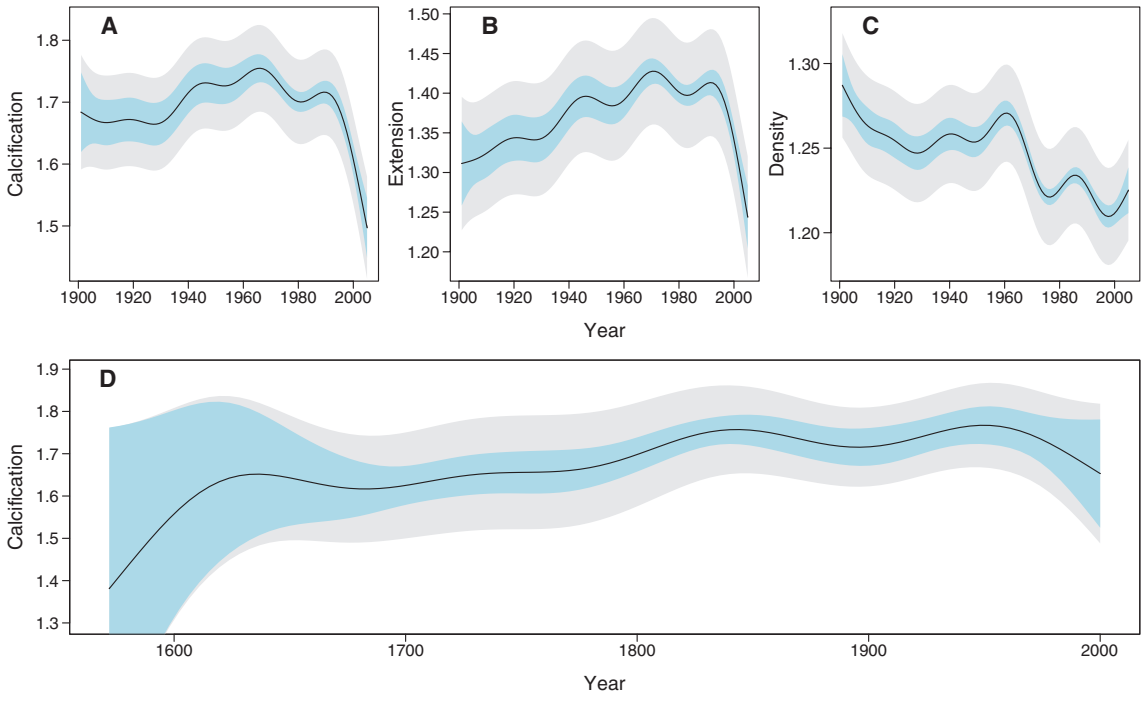

Fig. 2. Partial-effects plots showing the variation of calcification (grams per square centimeter per year), linear extension (centimeters per year), and density (grams per cubic centimeter) in *Porites* over time. Plots (A) to (C) are based on 1900–2005 data for all colonies, and plot (D) on data for the 10 long cores. Light blue bands indicate 95% confidence intervals for comparison between years, and gray bands indicate 95% confidence intervals for the predicted value for any given year. Calcification declines by 14.2% from 1990–2005 (**A**), primarily due to declining extension (**B**). Density declines from 1900 onward (**C**). The 1572–2001 data show that calcification increased weakly from ~1.62 before 1700 to ~1.76 in ~1850, after which it remained relatively constant (**D**) before a weak decline since ~1960.

across and along the GBR were nonsignificant and were omitted from all models (SOM); and year, SST-SPAT, and SST-ANOM were retained (Fig. 3). The models showed that calcification varied little between 1900–1990, followed by a decline since 1990 from 1.76 to 1.51 g cm^{-2} $year^{-1}$, or 14.2% (SE = 2.3%). As with the purely temporal model, this was largely due to a decline in extension rate from 1.43 to 1.24 cm $year^{-1}$ (13.3%, SE = 2.1%). The variation in density over this period was indistinguishable from the purely temporal model. Calcification also increased linearly with SST-SPAT at a rate of 0.122 g cm^{-2} $year^{-1}$ $°C^{-1}$ (SE = 0.041), corresponding to an increase of 0.36 g cm^{-2} $year^{-1}$ from south to north of the GBR due to the 3°C mean temperature difference. Calcification also decreased with negative SST-ANOM values but was highly variable for positive SST-ANOM.

The causes for the GBR-wide decline in coral calcification of massive *Porites* remain unknown, but this study shows that the causes are probably large-scale in extent and that the observed changes are unprecedented within the past 400 years. Cooper *et al.* (*18*) previously demonstrated a 21% decline (1988–2002) in the calcification rate of 38 small *Porites* colonies; however, the study was limited to two GBR inshore locations; comprised short time series, thereby precluding comparison with earlier periods; and local environmental effects such as coastal influences could not be excluded. Factors known to determine coral growth and calcification include competition for space, water quality, salinity, diseases, irradiance, currents, large-scale and long-term oceanographic oscillations, SST, temperature stress, and carbonate saturation state (6, *10*, *19*).

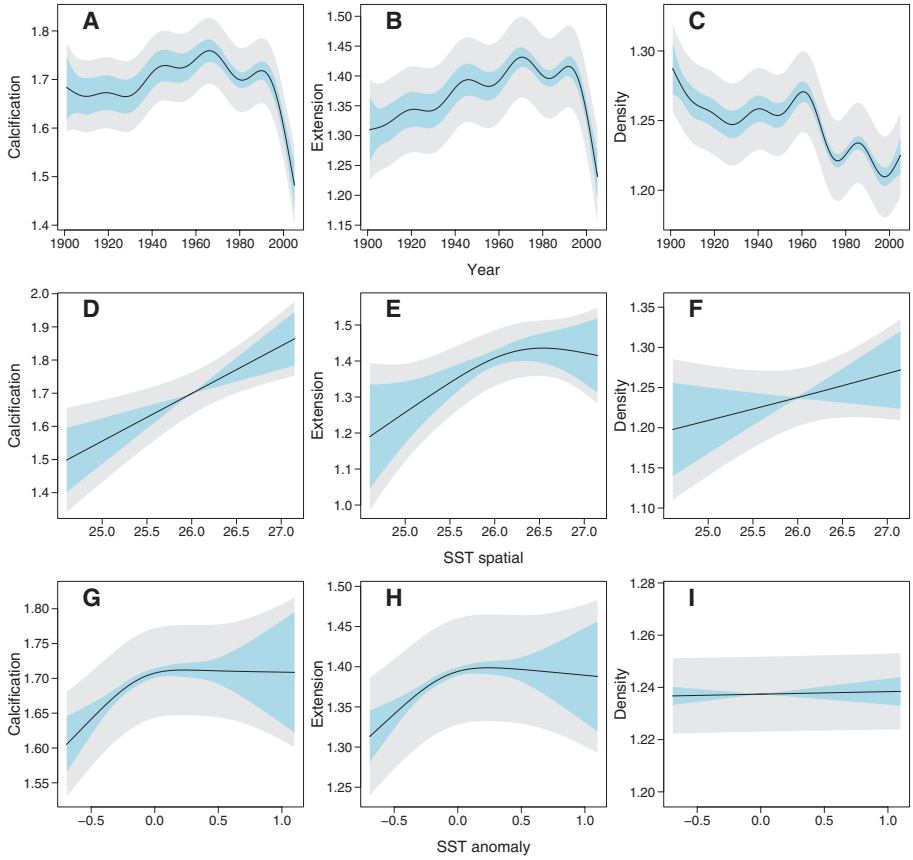

Fig. 3. Partial-effects plots showing the variation of calcification (grams per square centimeter per year), linear extension (centimeters per year), and density (grams per cubic centimeter) over time (**A** to **C**) and with SST (**D** to **I**). The effects of SST are partitioned into large-scale spatial (predominantly latitudinal) patterns in SST and SST-ANOM that represent annual deviations from the spatial-temporal trends. The models are based on all 328 *Porites* colonies from 1900–2005. Light blue bands indicate 95% confidence intervals for comparison between years or SST, and gray bands indicate 95% confidence intervals for the predicted value at a chosen level of the predictor.

Competition with neighboring corals is unlikely to have intensified during a period when coral cover has either remained similar or declined on most GBR reefs (*7*). Terrestrial runoff and salinity, although potentially affecting inshore reefs (*20*), are also unlikely causes because calcification declines at similar rates on offshore reefs away from flood plumes. Diseases can also be excluded because only visibly healthy colonies were sampled. Benthic irradiance depends on turbidity and cloud cover, but there are no data suggesting they have recently changed at GBR-wide scales. The Interdecadal Pacific Oscillation has been associated with changing currents and pH in a lagoon (*21*); however, these and other large-scale long-term oceanographic oscillations can be excluded because they would have affected *Porites* calcification not only after 1990 but throughout the observation period. Hence, by excluding these potential alternatives, we suggest that SST and carbonate saturation state are the two most likely factors to have affected *Porites* calcification at a GBR-wide scale.

SST is an important environmental driver of coral growth. Our data confirmed previous studies (*10*, *22*) that coral calcification increases linearly with large-scale mean annual SST. However, studies addressing shorter time periods show declining calcification at both high and low SST (*18*, *23, 24*) and that thermally stressed corals show reduced calcification for up to 2 years (*19*). In our study, calcification was likewise reduced during cooler-than-average years (negative SST-ANOM). However, during warmer years it was highly variable, suggesting increasing calcification in some warm years but declines in others. This is possibly due to the year-averaged SST-ANOM inconsistently reflecting short hot periods that reduce calcification during warm years. The recent increase in heat stress episodes (*25*) is likely to have contributed to declining coral calcification in the period 1990–2005.

The supersaturation of tropical sea surface waters with the calcium carbonate mineral forms calcite and aragonite is considered a prerequisite for biotic calcification, with saturation state being a function of pH and temperature. Since industrialization, global average atmospheric CO_2 has increased by ~36% (from 280 to 387 parts per thousand), the concentration of hydrogen ions in ocean surface waters has increased by ~30% (a 0.1 change in pH), and the aragonite saturation state (Ω_{arag}) has decreased by ~16% (*6*, *26*). Studies based on meso- or microcosm experiments show that reduced Ω_{arag}, due to the doubling of CO_2 as compared with preindustrial levels, reduces the growth of reef-building corals by 9 to 56% (*6*), with most of these experiments suggesting a linear relationship between calcification and Ω_{arag}.

Ω_{arag} data from the GBR or adjacent waters are sparse, but estimates of a global decline in Ω_{arag} of 16% since the beginning of global industrialization are similar in magnitude to our finding of a 14.2% decline in calcification in massive *Porites*. However, the decline in calcification observed in this study began later than expected, based on the model of proportional absorption of atmospheric CO_2 by the oceans' surface waters (*26*). Thus, our results may suggest that, after a period of a slight increase in extension and prolonged decline in density, a tipping point was reached in the late 20th century. The nonlinear and delayed responses may reflect synergistic effects of several forms of environmental stress, such as more frequent stress from higher temperatures and declining Ω_{arag}. Laboratory experiments have provided the first evidence documenting strong synergistic effects on corals (*27*), but clearly more studies are needed to better understand this key issue.

Laboratory experiments and models have predicted negative impacts of rising atmospheric CO_2 on the future of calcifying organisms (*5*, *6*). Our data show that growth and calcification of massive *Porites* in the GBR are already declining and are doing so at a rate unprecedented in coral records reaching back 400 years. If *Porites* calcification is representative of that in other reef-building corals, then maintenance of the calcium carbonate structure that is the foundation of the GBR will be severely compromised. Verification of the causes of this decline should be made a high priority. Additionally, if temperature and carbonate saturation are responsible for the observed changes, then similar changes are likely to be detected in the growth records from other regions and from other calcifying organisms. These organisms are central to the formation and function of ecosystems and food webs, and precipitous changes in the biodiversity and productivity of the world's oceans may be imminent (*28*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5910/116/DC1
Materials and Methods
References

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Stretching the Envelope of Past Surface Environments: Neoproterozoic Glacial Lakes from Svalbard

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The oxygen isotope composition of terrestrial sulfate is affected measurably by many Earth-surface processes. During the Neoproterozoic, severe "snowball" glaciations would have had an extreme impact on the biosphere and the atmosphere. Here, we report that sulfate extracted from carbonate lenses within a Neoproterozoic glacial diamictite suite from Svalbard, with an age of ~635 million years ago, falls well outside the currently known natural range of triple oxygen isotope compositions and indicates that the atmosphere had either an exceptionally high atmospheric carbon dioxide concentration or an utterly unfamiliar oxygen cycle during deposition of the diamictites.

Terrestrial sulfate (SO_4^{-2}) has diverse origins and participates in many important physicochemical and biological processes that can be inferred from large ranges in stable sulfur and oxygen isotope compositions (*1*). The $\delta^{18}O$ (*2*) of sulfate ranges from ~+8 to ~+27 per mil (‰) [Vienna standard mean ocean water (VSMOW)] (*3*) for marine sulfate

of different geological ages (*4*) and down to ~−18 ‰ for sulfate formed in continental Antarctica (*5*). In recent years, the sulfate oxygen isotope composition has been found to vary along another dimension, the $\Delta^{17}O$ [$\equiv \ln(\delta^{17}O+1) - 0.52\ln(\delta^{18}O+1)$]. The $\Delta^{17}O$ is close to zero for most samples but positive (up to ~+5.94 ‰) for those of atmospheric origin (*6*, *7*). Most recently, small but negative $\Delta^{17}O$ values have been reported for sulfate derived from oxidative weathering by atmospheric oxygen, with a conspicuous spike (down to −0.70 ‰) in the immediate aftermath of a Marinoan glaciation at ~635 million years ago (*8*). Here we report that these ranges are surpassed dramatically by carbonate-associated sulfate (CAS) extracted from a Neoproterozoic Marinoan (*9*, *10*) carbonate unit from Svalbard, Arctic Ocean. This carbonate member (W2) is from the Wilsonbreen Formation (Polarisbreen Group), a formation dominated by diamictites representing a continental-scale glaciation (*9*, *11*). W2 contains both limestones and primary dolostones with exceptionally preserved geochemistry (*12*). We have greatly extended the previous geochemical database, including isotope compositions of CAS (*13*) (fig. S1 and table S1). Note that the "cap carbonate," the lower Dracoisen Formation, is ~80 m above W2 and is not the subject of this study.

Two features of the data set are exceptional in terms of what we know about sulfate on Earth (Fig. 1): (i) The $\Delta^{17}O_{CAS}$ reaches as low as −1.64 ‰, the most negative anomaly ever reported for terrestrial (versus extraterrestrial) minerals, and (ii) the $\delta^{18}O_{CAS}$ reaches as high as ~+37.7 ‰, the most positive value ever reported for natural sulfate oxygen. Additional features are also intriguing. For example, (iii) CAS with a distinct ^{17}O anomaly is invariably from a limestone phase, whereas CAS from the dolostone phase does not have such a distinct ^{17}O anomaly (Fig. 1). (iv) Among CAS from the limestones, there is a strong positive correlation in $\Delta^{17}O$–$\delta^{34}S$ space, and the trend line connects with CAS from the immediately preglacial dolostone (member E4) (Fig. 1B). (v) The positively correlated $\delta^{18}O$ and $\delta^{13}C$ of dolomite range from ~−11 to +15 ‰ [Vienna Pee Dee belemnite (VPDB)] (*3*) and from ~−2 to +5 ‰ (VPDB), respectively. This $\delta^{18}O_{CO3}$ is the most positive value ever reported (Fig. 2A). (vi) Excluding preglacial samples, a slope of ~1 links two clusters of data in $\delta^{18}O_{CAS}$–$\delta^{18}O_{CO3}$ space (Fig. 3). (vii) Lastly, those calcite samples with negative $\Delta^{17}O_{CAS}$ have a $\Delta^{17}O_{CO3}$ value close to zero, indicating that the evaporated water itself did not bear an ^{17}O anomaly (table S1).

We propose the following model to account for this unusual carbonate paragenesis. The environment consisted of lacustrine oases within a continental ice sheet. The lakes were dominantly suboxic and, where local meltwater inflow was low, they and their near-surface porewaters were driven to high salinities by extreme evaporation accompanied by intense microbial sulfate redox reactions. Sulfate with negative $\Delta^{17}O$ values was produced by oxidative weathering involving atmospheric O_2 on the land surface and was washed into the lakes, along with pre-existing sulfate in rocks undergoing weathering. This mixed sulfate was reduced to sulfides (H_2S or HS^-) by bacteria and subsequently reoxidized to sulfate by a different microbial community in an oxic-to-suboxic condition. The re-oxidation was highly efficient, thus causing sulfur isotope mass balance and resulting in a change in the $\delta^{34}S_{SO4}$ much smaller than in the corresponding $\delta^{18}O_{SO4}$ or $\delta^{18}O_{CO3}$ value (which should be heavily influenced by the changing $\delta^{18}O_{H2O}$ due to variable degrees of evaporation). No atmospheric O_2 signal was incorporated into the newly regenerated sulfate in the water column, as abundant Mn and Fe (table S1) would shuttle electrons between sulfite and dissolved O_2 in ambient solution (*14*–*16*) without direct contact between reduced sulfur species and O_2. Such redox cycling effectively eliminated the initial sulfate ^{17}O–anomalous oxygen and replaced it with ^{17}O–normal oxygen from the ambient water during the precipitation of dolostones. The sulfate in limestones, however, appears not to have been subjected to such intense redox cycling, thus enabling it to retain its ^{17}O-anomalous signature. Intensive evaporation resulted in highly positive $\delta^{18}O$ values for the remaining water in a restricted lake or basin. Therefore, carbonate formed in the water would also carry extremely positive $\delta^{18}O$ values. Both the dolomite mineralogy and the ranges of $\delta^{13}C_{CO3}$ and $\delta^{18}O_{CO3}$ (displaying heavier-than-marine values) are characteristic of restricted evaporitic settings (*17*).

The evaporative dolomite-precipitating environments are evidence of microbial activity because they contain microbial laminites (*12*) and because of the intense redox cycling required to explain both the extremely high $\delta^{18}O_{CAS}$ values and the disappearance of the negative ^{17}O anomalies in these dolomites. As $\delta^{18}O_{CAS}$ increases at the same magnitude as $\delta^{18}O_{CO3}$ (Fig. 3), this suggests that almost all of the oxygen in the sulfate was replaced by oxygen from ambient water after microbially mediated sulfur redox cycling. The highly positive $\delta^{18}O_{SO4}$ (up to +37.7 ‰) should therefore be correlated with the highly positive $\delta^{18}O_{H2O}$ in the lakes. The $\delta^{18}O$ of evaporated H_2O can be estimated from the highly positive $\delta^{18}O_{dolomite}$, which is unlikely to be the result of late alteration. Taking into account the uncertainties in precipitation temperature, precipitation kinetics, and diagenetic imprint, we estimate (from Fig. 3) a difference between $\delta^{18}O_{CAS}$ and $\delta^{18}O_{H2O}$ ranging from ~20 to 30 ‰, which is similar to the ~25 to 30 ‰ recently obtained from an experimental study of sulfate reduction (*18*). We know of no other environments where the microbial sulfate redox cycling has reached a complete steady state with highly evaporitic ambient water. The closest analog is the modern hypersaline lagoons near Rio de Janeiro, Brazil, where the $\delta^{18}O_{SO4}$ reaches +21.3 ‰

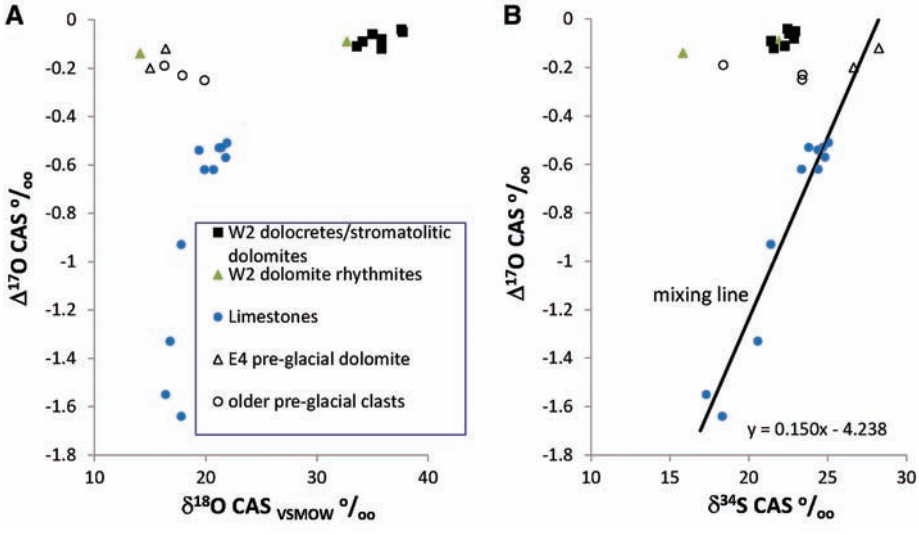

Fig. 1. (**A**) $\Delta^{17}O_{CAS}$ versus $\delta^{18}O_{CAS}$ and $\Delta^{17}O_{CAS}$ versus $\delta^{34}S_{CAS}$ from member W2. Dolomites with both high and low $\delta^{18}O_{CAS}$ display no $\Delta^{17}O_{CAS}$ anomaly, whereas a negative anomaly is variably developed in limestones. (**B**) A clear colinearity of $\Delta^{17}O_{CAS}$ versus $\delta^{34}S_{CAS}$ for limestones and immediately preglacial dolomites, interpreted as a mixing line between normal marine sulfate (high $\delta^{34}S$ and zero $\Delta^{17}O$) and sulfate from continental weathering (low $\delta^{34}S$ and negative $\Delta^{17}O$). Error bars for data are smaller than the symbols.

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in pore waters and +20.8 ‰ in surface brines with an apparent $\Delta\delta^{18}O_{SO4-H2O}$ at ~+20 ‰ (*19*).

The most negative value of $\Delta^{17}O_{SO4}$ (−1.64 ‰) greatly extends the magnitude and geographic occurrence of the negative $\Delta^{17}O_{SO4}$ associated with the Marinoan glaciation, as first reported by Bao *et al*. (*8*). Bao *et al*. proposed that the negative $\Delta^{17}O$ was derived from that of atmospheric O_2 involved in the oxidative weathering of sulfur compounds on the surface of Earth (*8*). Preexisting ocean sulfate may not have a distinct negative $\Delta^{17}O$ value, because continuous microbial sulfur redox cycling tends to replace sulfate oxygen with a water oxygen signal. Thus, sulfate in a restricted continental basin could have the most negative $\Delta^{17}O_{SO4}$ value, where a large portion of the sulfate was derived from nonmarine sources. The value −1.64 ‰ is more than twice the magnitude of the −0.70 ‰ reported for barites from Marinoan cap carbonates from South China (*8*). This finding is consistent with W2's setting, where much of the drainage could have been internal.

We interpret the strong correlation between $\Delta^{17}O_{CAS}$ and $\delta^{34}S_{CAS}$ in limestones (data feature 4, Fig. 1B) as a mixing line between two sulfate endmembers—a pre-existing marine sulfate and a sulfate newly supplied from continental weathering—rather than as an evaporative trend for an already mixed sulfate in the lakes. This can be justified because of the absence of evaporative characteristics in the limestones and their much narrower scatter of $\delta^{18}O$ value (from −13 to −4 ‰ VPDB) (Figs. 2B and 3) compared with that of the evaporative dolomites (from −11 to +15 ‰ VPDB) (Fig. 2A). The mixing scenario is also supported by the isotope compositions of CAS in the preglacial carbonates, which fall close to the $\delta^{34}S$ high end of the line (Fig. 1B). Because sulfate evaporite relics are known to occur in the underlying E4 tidal-flat carbonates (*20*), it is likely that weathering of coeval nodular or bedded sulfate from lateral equivalents of E4 helped to stabilize the position of the mixing line.

If sulfate derived from oxidative weathering of sulfides in glacial rock flour had a global average crustal $\delta^{34}S$ value of ~0 ‰, the mixing line (Fig. 1B) would point to a $\Delta^{17}O$ value of ~−4.2 ‰ for the nonmarine sulfate endmember. This would imply an atmospheric $\Delta^{17}O(O_2)$ at ~−42 ‰ if only 10% of the oxygen in sulfate came from atmospheric O_2 (*8*). This estimate contains two large uncertainties—the end-member $\delta^{34}S_{sulfides}$ value and the fraction of sulfate oxygen derived from atmospheric O_2—and is probably the most negative bound. If the nonmarine sulfate endmember had a $\delta^{34}S$ value of ~+18 ‰, as seen near the low end of the data array (Fig. 1B), the atmospheric $\Delta^{17}O(O_2)$ value would be ~−6.6 ‰, assuming 1/4 of the oxygen in sulfate (a probable maximum) came from atmospheric O_2 during surface sulfide oxidation (*16*, *21*). A realistic $\Delta^{17}O(O_2)$ value probably lies somewhere between −42 and −6.6 ‰ at the time of W2 deposition. Note that the modern $\Delta^{17}O(O_2)$ value is only ~−0.10 to −0.20 ‰, depending on the reference slope value ($\ln\alpha^{17}/\ln\alpha^{18}$) used for calculation (*22*, *23*).

There may be alternative scenarios where a large negative $\Delta^{17}O(O_2)$ could occur mathematically, although geologically there are far fewer scenarios that are viable. The magnitude of the negative $\Delta^{17}O$ value of atmospheric O_2 is determined by parameters such as stratosphere O_3-CO_2-O_2 reactions, the size of the atmospheric O_2 and CO_2 reservoirs, stratosphere-troposphere flux, and troposphere O_2 fluxes (*8*). The controlling parameters are often hard to determine, especially for an unfamiliar Earth system, but some constraints exist. Other things being equal, higher CO_2 concentrations lead to a more negative $\Delta^{17}O(O_2)$ value as the CO_2 develops a positive anomaly by exchange with oxygen. Assuming a steady-state O_2 reservoir and modern gas fluxes, the one-dimensional model in Bao *et al*. (*8*) would predict an atmospheric CO_2 concentration (pCO_2) of ~12,500 to ~80,000 parts per million during W2 deposition, consistent with Earth having gone through a prolonged, ice-covered period (a "snowball" Earth) (*24*). It should be noted that a much lower pO_2 and a moderately high pCO_2 could also produce a similarly negative $\Delta^{17}O(O_2)$, but only if the corresponding O_2 residence time (i.e., pO_2/flux) was disproportionally long. Prolongation of the residence time while keeping oxygen concentrations low implies much lower rates of both O_2 removal by respiration/decomposition and input by photosynthesis, a balance that is consistent with near-global glaciations. Other non–steady state scenarios can also be imagined where bizarre combinations of changes in reservoirs and fluxes of atmospheric O_2 and CO_2 could have resulted in the observed large negative $\Delta^{17}O(O_2)$ in certain time windows. These scenarios could be ex-

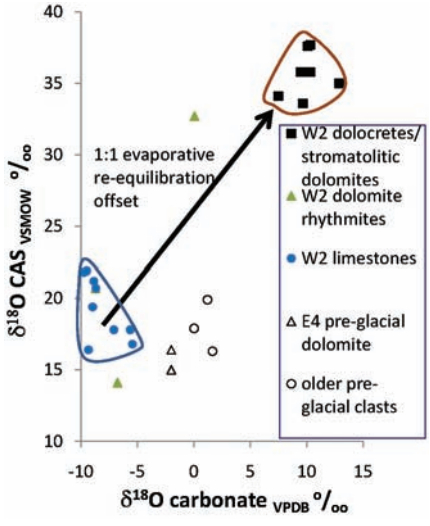

Fig. 3. $\delta^{18}O$ of CAS versus $\delta^{18}O$ of host carbonate from member W2. The offset of evaporative dolomites from less evaporative limestones has a 1:1 trend pointing to an isotopic steady state between sulfate and water permitted by intense redox recycling.

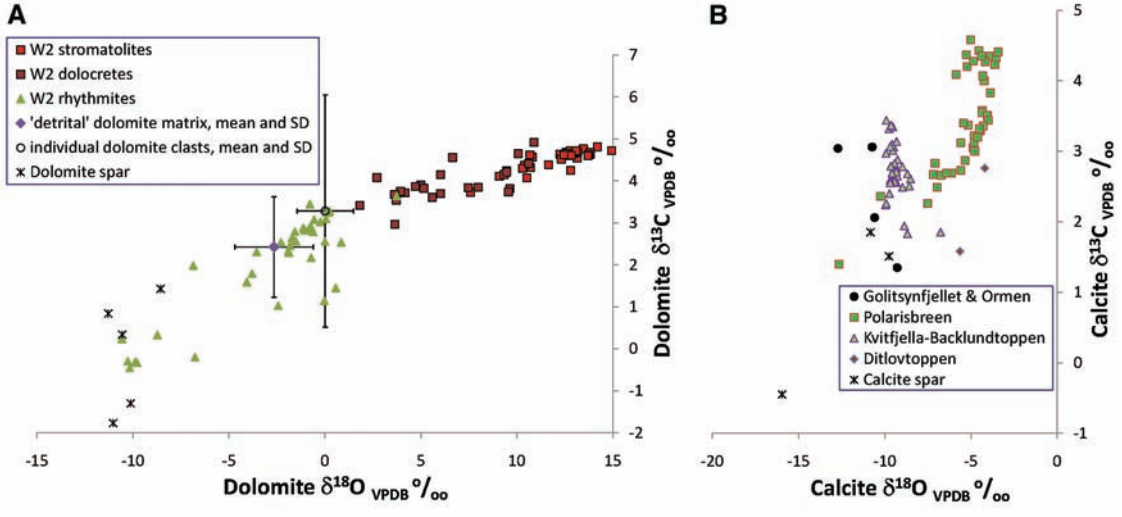

Fig. 2. (**A**) $\delta^{13}C$ versus $\delta^{18}O$ for different types of dolomites from different locations of member W2. Dolomite rhythmite precipitates have mostly similar compositions to detritus but define a clear evaporative trend to high-$\delta^{18}O$ samples. (**B**) $\delta^{13}C$ versus $\delta^{18}O$ for limestones from different locations of member W2. Limestones show variation in $\delta^{18}O$ value that may be primary or reflect variable timing of stabilization from Mg-calcite. For all data points, the error bars are smaller than the symbols, except for those displayed.

plored by combining models and further empirical data, but geology offers a stronger constraint because circumstances under which sulfate can be preserved in terrestrial sedimentary records are uncommon.

Although various aspects of Neoproterozoic glaciations are intensely disputed (*25*), our results confirm a profound difference from Phanerozoic ice ages. A near-global distribution of glaciated continents during the Marinoan phase ending ~635 million years ago is supported by evidence of low palaeomagnetic latitudes (*26*). The snowball Earth model (*27*) predicts a progressive accumulation of volcanic volatiles in the atmosphere that are not removed by weathering until the rapid demise of the ice age as the ice-albedo feedback reverses. If sulfate with large negative $\Delta^{17}O$ signals derived from oxidative weathering could only be generated in a large quantity after melting of the "snowball" and exposure of continents, then the diamictites above W2 had to be deposited during final glacial retreat, a hypothesis that should prompt a re-examination of their sedimentology. The alternative "slushball" model, in which parts of the ocean area are ice-free (*28*), would also permit accumulation of sulfate from prolonged oxidative weathering in certain continental "oases" where arid but cold conditions prevailed. This study provides an effective way to study the dynamics of sedimentation and atmospheric-hydrosphere-biosphere interactions during a global glaciation and highlights the need for further stratigraphically constrained $\Delta^{17}O_{SO4}$ data on continental carbonate precipitates to ground-truth flux-balance models.

References and Notes

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3. Reference units for stable isotope compositions: VSMOW for sulfate $\delta^{18}O$, $\delta^{17}O$, and $\Delta^{17}O$; VPDB for carbonate $\delta^{13}C$ and $\delta^{18}O$; and Vienna Canyon Diablo Troilite for sulfate $\delta^{34}S$.
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29. H.B. and I.J.F. designed research and led the writing of the manuscript; H.B. performed CAS extraction and triple oxygen isotope measurements; I.J.F secured samples from field expeditions and conducted sedimentological, petrographic, mineralogical and elemental studies; P.M.W. conducted preliminary CAS extraction and performed $\delta^{34}S_{CAS}$ analysis; and C.S. carried out $\delta^{13}C$ and $\delta^{18}O$ analysis of host carbonates. We thank G. Halverson for discussion and Y. Peng for analytical assistance. Financial and facility supports were provided by Louisiana State University, NSF, and Chinese Academy of Science (H.B.), Natural Environment Research Council (NERC) standard grant (GR3/C511805/1) and NERC inductively coupled plasma mass spectrometry facilities (I.J.F.), and Austrian Science Funds (C.S.). The authors declare no competing financial interests.

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Why Peer Discussion Improves Student Performance on In-Class Concept Questions

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When students answer an in-class conceptual question individually using clickers, discuss it with their neighbors, and then revote on the same question, the percentage of correct answers typically increases. This outcome could result from gains in understanding during discussion, or simply from peer influence of knowledgeable students on their neighbors. To distinguish between these alternatives in an undergraduate genetics course, we followed the above exercise with a second, similar (isomorphic) question on the same concept that students answered individually. Our results indicate that peer discussion enhances understanding, even when none of the students in a discussion group originally knows the correct answer.

In undergraduate science courses, conceptual questions that students answer using personal response systems or "clickers" are promoted as a means to increase student learning [e.g. (*1*, *2*)], often through peer instruction (PI) (*3*). Instructors using this approach break up their lectures with multiple-choice questions to test understanding of the concepts being presented. When PI is used, students are first asked to answer a question individually, and then a histogram of their responses may be displayed to the class. If there is substantial disagreement among responses, students are invited to discuss questions briefly with their neighbors and then revote before the correct answer is revealed. The instructor then displays the new histogram and explains the reasoning behind the correct answer. Most instructors report that the percentage of correct answers, as well as students' confidence in their answers, almost always increases after peer discussion (*2*–*4*).

It is generally assumed that active engagement of students during discussion with peers, some of whom know the correct answer, leads to increased conceptual understanding, resulting in improved performance after PI. However, there is an alternative explanation: that students do not in fact learn from the discussion, but simply choose the answer most strongly supported by neighbors they perceive to be knowledgeable. We sought to distinguish between these alternatives, using an additional, similar clicker question that students answered individually to test for gains in understanding. Our results indicate that peer discussion enhances understanding, even when none of the students in a discussion group originally knows the correct answer.

In an undergraduate introductory genetics course for biology majors at the University of Colorado–Boulder (additional demographic in-

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formation in table S1), we asked an average of five clicker questions per 50-min class throughout the semester and encouraged students to discuss questions with their neighbors. Students were given participation points for answering clicker questions, regardless of whether their answers were correct. Exam questions were similar to the clicker questions, so that students had an incentive to take clicker questions seriously.

Sixteen times during the semester we assessed how much students learned from peer discussion by using a paired set of similar (isomorphic) clicker questions. Isomorphic questions have different "cover stories," but require application of the same principles or concepts for solution (*5*, *6*). Sample isomorphic question pairs are shown in fig. S1. In class, students were first asked to answer one question of the pair individually (Q1). Then they were invited to discuss the question with their neighbors and revote on the same question ($Q1_{ad}$ for "Q1 after discussion"). Finally, students were asked to answer the second isomorphic question, again individually (Q2). Neither the answers to the two questions (Q1/$Q1_{ad}$ and Q2) nor the histograms of student answers were revealed until after the voting on Q2, so that there was minimal instructor or whole-course peer influence on the Q2 responses. The isomorphic questions were randomly assigned as Q1/$Q1_{ad}$ or Q2 after both questions were written. Data analysis was limited to students who answered all three questions of an isomorphic pair with a total of 350 students participating in the study (*7*) (see supporting online text).

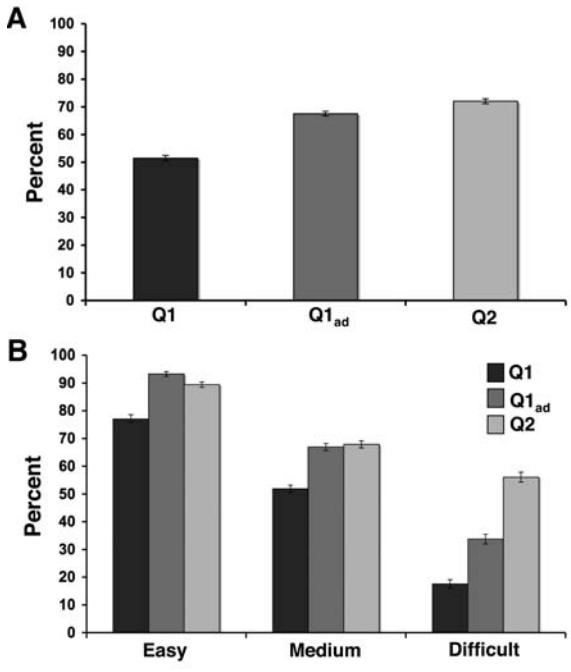

Fig. 1. The percentage of students who can correctly answer a question as individuals increases after peer discussion of a similar (isomorphic) question. Q1: One question of an isomorphic pair was voted on individually; $Q1_{ad}$: the same question was voted on again after peer discussion; Q2: the second isomorphic question was voted on individually. (**A**) Results for all 16 question pairs were averaged for each individual (n = 350 students), and the class averages of these scores are shown. (**B**) The 16 paired questions were grouped according to difficulty based on the percentage of correct answers for Q1 (five easy questions, seven medium questions, and four difficult questions), and performance results were again averaged for each individual (n = 343 students for easy, 344 for medium, and 337 for difficult) before computing the averages shown. Error bars show the SEM.

Table 1. Mean differences between Q1, $Q1_{ad}$, and Q2. The SEM is in parentheses.

Question category	$Q1_{ad}$ − Q1* (%)	Q2 − Q1* (%)	Q2 − $Q1_{ad}$* (%)
All questions	16(1)	21(1)	5(1)
Easy questions	16(1)	12(2)	−4(1)†
Medium questions	15(1)	16(2)	1(1)†
Difficult questions	16(2)	38(2)	22(2)

*Mean values are the averages of the differences between $Q1_{ad}$-Q1, Q2-Q1, and Q2-$Q1_{ad}$ for each student. †No significant improvement between these questions.

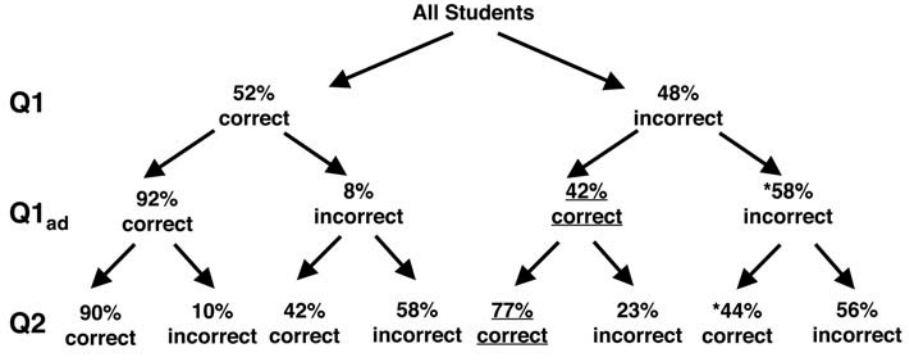

Fig. 2. Breakdown of student responses for the pool of 16 Q1, $Q1_{ad}$, and Q2 questions. Percentages of the category are connected by arrows from the preceding line. Underlined entries represent students who initially did not answer Q1 correctly but did so after group discussion; entries with an asterisk represent students who did not answer either Q1 or $Q1_{ad}$ correctly, but nevertheless were able to correctly answer the isomorphic question Q2. Of the 32 questions in our 16 question pairs, 7 had 5 answer choices, 5 had 4 choices, 3 had 3 choices, and 1 had 2 choices.

Two results indicate that most students learned from the discussion of Q1. First, using data pooled from individual mean scores on Q1, $Q1_{ad}$, and Q2 for all 16 question pairs, the average percentage correct for Q2 was significantly higher than for Q1 and $Q1_{ad}$ (Fig. 1A and Table 1). Second, of the students who answered Q1 incorrectly and $Q1_{ad}$ correctly, 77% answered Q2 correctly (Fig. 2). This result suggests that most students who initially did not understand a concept were able to apply information they learned during the group discussion and correctly answer an isomorphic question. In contrast, almost all students who answered Q1 correctly, presumably because they understood the concept initially, did not change their votes on $Q1_{ad}$ and went on to answer Q2 correctly (Fig. 2).

In addition, students who answered both Q1 and $Q1_{ad}$ incorrectly still appeared to learn from discussions with peers and answering a second question on the same topic. Of these students, 44% answered Q2 correctly, significantly better than expected from random guessing (Fig. 2; on average, the questions in our 16 isomorphic pairs had four answer choices each). This result was unexpected because when students answered Q2, they had not been told the correct answer to Q1/$Q1_{ad}$, had not seen histograms of student responses, and had not discussed Q2 with their peers. We speculate that when this group of students discussed Q1, they were making sense of the information, but were unable to apply their new knowledge until presented with a fresh question on the same concept (Q2). There may also be a learning benefit to considering successive clicker questions on the same topic (*8*).

Although the difficulty of the question pairs varied, as judged by the percentage of correct answers on Q1 (see supporting online text), students performed significantly better on $Q1_{ad}$ and Q2 compared to Q1 for each difficulty level (Fig. 1B and Table 1). On the most difficult questions there was another significant increase between $Q1_{ad}$ and Q2, suggesting that there was an additional delayed benefit to the group discussions.

Our results suggest that peer discussion can be effective for understanding difficult concepts even when no one in the group initially knows the correct answer. In a postsemester survey (n = 98 responding), students reported an average of three participants in their peer discussion groups. If students who knew the answer to Q1 were randomly distributed throughout the classroom, then on the difficult questions (Fig. 1B), more than half of the 84 groups would have included no one who knew the correct answer to Q1 (naïve groups). Statistical analysis (see supporting online text) shows that some students who answered Q2 correctly must have come from naïve groups.

Student opinion supported the view that having someone in the group who knows the correct answer is unnecessary. On an end-of-year survey (n = 328 responding), 47% of students disagreed with the statement: "When I discuss clicker questions with my neighbors, having someone in the group who knows the correct answer is necessary in order to make the discussion productive." Representative comments from these students included the following: "Often when talking through the questions, the group can figure out the questions without originally knowing the answer, and the answer almost sticks better that way because we talked through it instead of just hearing the answer." "Discussion is productive when people do not know the answers because you explore all the options and eliminate the ones you know can't be correct."

This study supports the substantial value of student peer discussion as an effective means of active learning in a lecture class. Our findings are consistent with earlier demonstrations of social learning, including the value of discussion with peers (*9*–*13*). The significant increases in performance between Q1 and $Q1_{ad}$ confirm results from earlier classroom studies (*2*–*4*). In addition, we have presented new evidence showing that these increases result primarily from student gains in conceptual understanding rather than simply from peer influence.

Previous explanations for the value of PI have maintained the "transmissionist" view (*14*) that during discussion, students who know the right answer are explaining the correct reasoning to their less knowledgeable peers, who consequently improve their performance on the revote (*3*, *4*). Our finding that even students in naïve groups improve their performance after discussion suggests a more constructivist explanation: that these students are arriving at conceptual understanding on their own, through the process of group discussion and debate.

Some instructors who use clicker questions skip peer discussion entirely, believing that instructor explanation of the correct reasoning will be more clear and accurate than an explanation by peers, and will therefore lead to more student learning. Although our current work does not directly compare the benefits of instructor versus peer explanation, research in physics has shown that instructor explanations often fail to produce gains in conceptual understanding (*15*). We have shown that peer discussion can effectively promote such understanding. Furthermore, justifying an explanation to a fellow student and skeptically examining the explanation of a peer provide valuable opportunities for students to develop the communicative and metacognitive skills that are crucial components of disciplinary expertise.

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Supporting Online Material

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Regulation of Neuronal Survival Factor MEF2D by Chaperone-Mediated Autophagy

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Chaperone-mediated autophagy controls the degradation of selective cytosolic proteins and may protect neurons against degeneration. In a neuronal cell line, we found that chaperone-mediated autophagy regulated the activity of myocyte enhancer factor 2D (MEF2D), a transcription factor required for neuronal survival. MEF2D was observed to continuously shuttle to the cytoplasm, interact with the chaperone Hsc70, and undergo degradation. Inhibition of chaperone-mediated autophagy caused accumulation of inactive MEF2D in the cytoplasm. MEF2D levels were increased in the brains of α-synuclein transgenic mice and patients with Parkinson's disease. Wild-type α-synuclein and a Parkinson's disease–associated mutant disrupted the MEF2D-Hsc70 binding and led to neuronal death. Thus, chaperone-mediated autophagy modulates the neuronal survival machinery, and dysregulation of this pathway is associated with Parkinson's disease.

In neurodegenerative diseases, certain populations of adult neurons are gradually lost because of toxic stress. The four myocyte enhancer factor 2 (MEF2) transcription factors, MEF2A to MEF2D, have been shown to play an important role in the survival of several types of neurons, and a genetic polymorphism of the MEF2A gene has been linked to the risk of late onset of Alzheimer's disease (*1*–*3*). In cellular models, inhibition of MEF2s contributes to neuronal death. Enhancing MEF2 activity protects neurons from death in vitro and in the substantia nigra pars compacta in a mouse model of Parkinson's disease (PD) (*4*). Neurotoxic insults cause MEF2 degradation in part by a caspase-dependent mechanism (*5*), but how MEF2 is regulated under basal conditions without overt toxicity is unknown. Autophagy refers to the degradation of intracellular components by lysosomes. Relative to macro- and microautophagy, chaperone-mediated autophagy (CMA) selectively degrades cytosolic proteins (*6*). This process involves binding of heat shock protein Hsc70 to substrate proteins via a KFERQ-like motif and their subsequent targeting to lysosomes via the lysosomal membrane receptor Lamp2a. Dysregulation of autophagy plays a role in neurodegeneration (*7*–*9*). However, the direct mechanism by which CMA modulates neuronal survival or death is unclear.

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Because the level of MEF2 protein is critical to neuronal survival (*5*), we investigated the role of autophagy in the degradation of MEF2D protein by blocking lysosomal proteolysis with ammonium chloride (NH_4Cl) in a mouse midbrain dopaminergic progenitor cell line, SN4741 (*10*). NH_4Cl caused a dose-dependent increase in the level of MEF2D (Fig. 1A). Exposing primary cortical and cerebellar granule neurons to NH_4Cl also resulted in accumulation of MEF2D (fig. S1). In mice lacking the lysosomal hydrolase cathepsin D (*11*), MEF2D levels were increased in the substantia nigra and cortex (Fig. 1B and fig. S1). Thus, the lysosomal system controls MEF2D levels in neurons. The SN4741 neuronal cell line is useful for studying the neuronal stress response (*12*) and so was used as the primary model for the rest of the study.

Lysosomes regulate substrate proteins in the cytoplasmic compartment (*6*), but MEF2D has been reported only in the neuronal nucleus (*6*). Our findings suggested translocation of MEF2D to the cytoplasm for direct modulation by autophagy. To test this idea, we fractionated SN4741 cells and found a fraction of MEF2D present in the cytoplasm (Fig. 1C) under normal conditions. Leptomycin B (LMB), a specific inhibitor of receptor CRM-1–dependent nuclear export, reduced the level of cytoplasmic MEF2D while concomitantly increasing nuclear MEF2D (Fig. 1C). Inhibition of lysosomal function consistently reduced nuclear MEF2D but caused its concurrent accumulation in the cytoplasm (Fig. 1D). LMB attenuated the NH_4Cl-induced accumulation of MEF2D in the cytoplasm (Fig. 1E), which suggests that MEF2D is constantly exported to the cytoplasm under basal conditions for lysosomal degradation. To study the degradation of MEF2D by autophagy, we activated CMA by serum removal (*13*, *14*), which led to a marked reduction in MEF2D levels and metabolically labeled MEF2D (Fig. 1F and fig. S2A). No change was detected in the levels of *mef2d* transcript after serum withdrawal (fig. S2B). Similarly, when CMA was stimulated with 6-aminonicotinamide (6-AN) (*15*), MEF2D levels were reduced (Fig. 1G). In contrast, the macroautophagy inhibitor 3-methyladenine had only a small effect on MEF2D levels (fig. S3) (*16*). Thus, our results support CMA as the major mode of autophagy that controls MEF2D degradation.

The CMA pathway involves two key regulators, Hsc70 and Lamp2a. Reducing the level of Hsc70 by overexpression of antisense RNA in SN4741 cells increased MEF2D levels (Fig. 2A). To probe the interaction between MEF2D and Hsc70, we incubated N- and C-terminal fragments of purified GST-MEF2D (glutathione *S*-transferase fused to MEF2D) with cellular lysates and then performed immunoblotting to detect bound Hsc70. Hsc70 interacted with GST-MEF2D (amino acid residues 1 to 86; MEF2D 1-86), but not with the C terminus (residues 87 to 507) of MEF2D or with GST alone (Fig. 2B). Reducing Lamp2a protein by overexpression of antisense RNA also increased MEF2D levels (Fig. 2C). Conversely, overexpression of Lamp2a markedly reduced MEF2D levels (Fig. 2D). To show that lysosomes directly regulate MEF2D, we performed lysosomal binding and uptake assays. Purified lysosomes isolated from rat liver (*13*) readily took up purified CMA substrate ribonuclease A (fig. S4), confirming the integrity of our lysosomal preparations. GST-MEF2D fragments were incubated with lysosomes and detected by anti-GST immunoblotting. MEF2D 1-86 readily bound to lysosomes (Fig. 2E). For uptake studies, we incubated lysosomes and substrates in the presence of hydrolase inhibitors, digested proteins outside of lysosomes with proteinase K, and showed that MEF2D 1-86 was present inside lysosomes (Fig. 2E).

Hsc70 interacts with a conserved KFERQ-like motif in substrate proteins (*17*). Analysis of the N terminus of MEF2D revealed the presence of several imperfect CMA recognition sequences (fig. S5). We tested whether they may serve as Hsc70 interacting sequences by incubating purified GST-Hsc70 with cellular lysates containing overexpressed Flag-MEF2D. Mutation of these motifs individually did not disrupt the binding between MEF2D and GST-Hsc70, as exemplified by mutant QR10-11 (fig. S5). Deletion of the 18 amino acids expanding several overlapping motifs (Flag-MEF2DΔN18) abolished its binding to GST-Hsc70 (Fig. 2F) or to endogenous Hsc70 (Fig. 2G). Moreover, MEF2DΔN18 was poorly taken up by lysosomes (Fig. 2H), and its level was not significantly reduced by serum deprivation (Fig. 2I). Thus, multiple motifs at the MEF2D N terminus mediate its interaction with Hsc70 and degradation by CMA.

We determined the effect of α-synuclein, itself a CMA substrate and PD risk factor (*18*, *19*), on MEF2D in SN4741 cells. Both wild-type and disease-causing mutant α-synuclein (Ala^{53} → Thr, A53T) attenuated degradation of MEF2D that had been induced by serum deprivation and 6-NA (Fig. 3A and fig. S6A). Furthermore, overexpression of wild-type α-synuclein or the A53T mutant reduced the interaction of endogenous MEF2D and GST-Hsc70 in a pull-down assay (Fig. 3B) and the binding of Flag-MEF2D to endogenous Hsc70 in coprecipitation experiments (fig. S6B). Increasing the level of wild-type or A53T α-synuclein inhibited the uptake of MEF2D by lysosomes (Fig. 3C). Moreover, cytoplasmic MEF2D levels in the cortex of A53T α-synuclein transgenic mice (*20*) were significantly higher than in wild-type control mice (Fig. 3D). MEF2D levels were significantly higher in the brains of PD patients than in controls (Fig. 3E) with a substantial portion of MEF2D in neuronal cytoplasm, correlating with high levels of α-synuclein in the brains of PD patients (fig. S7).

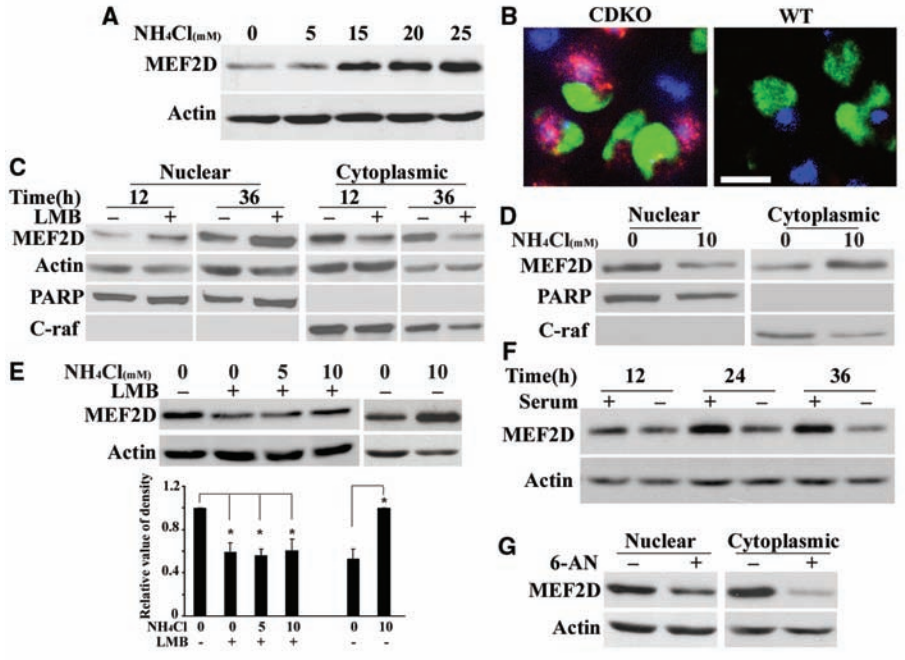

Fig. 1. Degradation of MEF2D by an autophagy pathway. (**A**) Inhibition of MEF2D degradation by blocking autophagy. NH_4Cl causes MEF2D accumulation in SN4741 cells. (**B**) Increased MEF2D immunoreactivity in substantia nigra in brains of cathepsin D–deficient (CDKO) versus wild-type (WT) mice. Green, tyrosine hydroxylase; red, MEF2D; blue, 4′,6′-diamidino-2-phenylindole. Scale bar, 10 μm. (**C**) Translocation of MEF2D to the cytoplasm. LMB (50 ng/ml) caused accumulation of MEF2D in the nucleus ($n = 4$ independent experiments). (**D**) Increased cytoplasmic MEF2D induced by NH_4Cl accompanies the reduction of nuclear MEF2D ($n = 3$). (**E**) Reduction of NH_4Cl-induced accumulation of MEF2D in the cytoplasm by LMB ($n = 3$, $^*P < 0.05$). (**F**) Degradation of cytoplasmic MEF2D by serum deprivation ($n = 3$). (**G**) Effect of CMA on MEF2D degradation. Levels of cytoplasmic MEF2D from SN4741 cells treated with 6-AN (5 mM, 24 hours) were determined by immunoblotting ($n = 4$).

To assess whether the accumulated MEF2D is functional, we determined MEF2D DNA binding activity by electrophoretic mobility shift assay (EMSA). For this study, we treated cells with NH_4Cl, confirmed the increase of MEF2D (fig. S8A), and incubated lysates with a labeled MEF2 DNA probe. MEF2D in the control cell lysates bound specifically to the DNA probe. The accumulated MEF2D in NH_4Cl-treated samples or resulting from a reduction of Hsc70 bound to the DNA probe at a far lower rate than did controls (Fig. 4A and fig. S8B). Phosphatase treatment did not affect MEF2D DNA binding (fig. S8, B and C) (*21*). NH_4Cl markedly reduced MEF2-dependent reporter activity in SN4741 cells (fig. S9). To test whether maintaining MEF2D in the nucleus might preserve its function, we showed that the nuclear level of MEF2D-VP16, a fusion protein lacking the putative nuclear export signals at the C terminus of MEF2D (*22*), was not significantly altered by 6-AN (Fig. 4B). NH_4Cl repressed endogenous MEF2D- or Flag-MEF2D–dependent but not MEF2D-VP16–induced reporter activity (Fig. 4B). MEF2D-VP16–attenuated NH_4Cl induced death of SN4741 cells (fig. S9). Similar to NH_4Cl, α-synuclein also inhibited MEF2D function (Fig. 4C) and caused a 40% loss

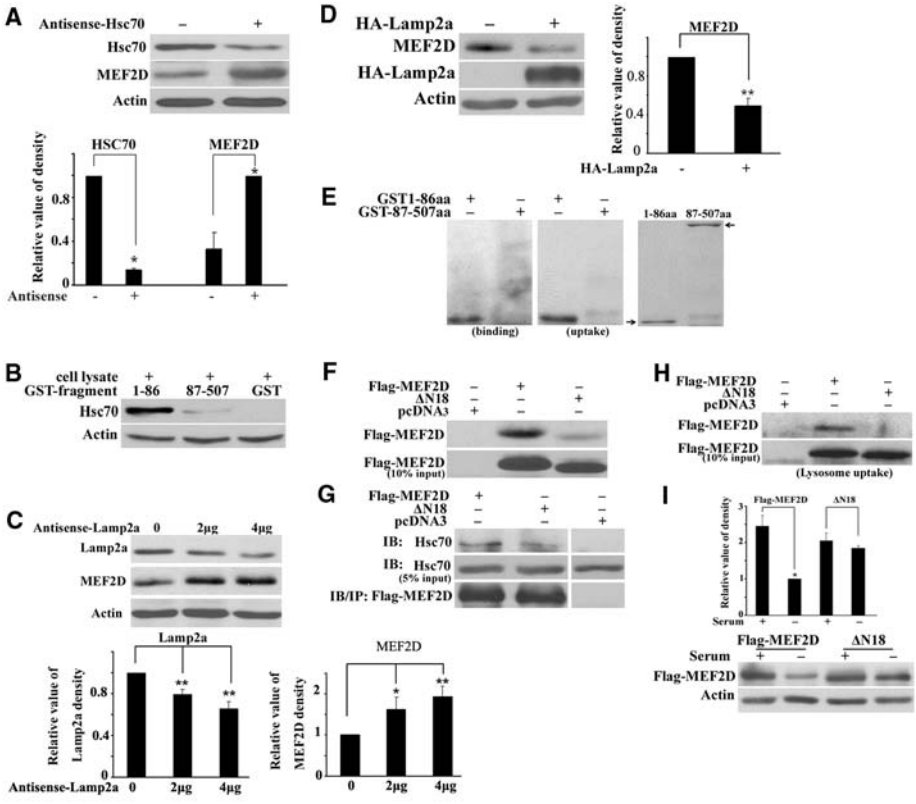

Fig. 2. Interactions of MEF2D with key CMA regulators. (**A**) Effect of reducing Hsc70 on MEF2D. The level of cytoplasmic MEF2D in SN4741 cells was determined after transfection of control plasmid or plasmid encoding antisense Hsc70 (36 hours) (n = 3, $^*P < 0.05$). (**B**) Interaction of N-terminal MEF2D with Hsc70. GST pull-down assay was carried out by incubating GST or GST-MEF2D fragments with cell lysates. Bound Hsc70 was detected by immunoblotting. (**C**) Effect of reducing Lamp2a on MEF2D. The experiments were carried out as described in (A) with plasmid encoding antisense Lamp2a (n = 4; $^*P < 0.05$, $^{**}P < 0.01$). (**D**) Effect of increasing Lamp2a on MEF2D. The experiment was carried out as described in (C) with plasmid encoding Lamp2a (n = 3, $^{**}P < 0.01$). HA, hemagglutinin. (**E**) Lysosomal binding and uptake of MEF2D. The presence of purified GST-MEF2D fusion proteins was determined after incubation with purified lysosomes by anti-GST immunoblotting (right panel shows the positions of GST-MEF2D proteins by Coomassie stain; n = 4). (**F** and **G**) Effect of deleting the N-terminal 18 amino acid residues on MEF2D binding to Hsc70. Wild-type and mutated (ΔN18) MEF2D expressed in HEK293 cells was detected by GST-Hsc70 pull-down assay (n = 3) (F) or by coimmunoprecipitation (G). (**H**) Uptake of MEF2DΔN18 by lysosomes. The assay was carried out as described in (E) with the use of lysates containing overexpressed MEF2D. (**I**) Degradation of MEF2DΔN18 by serum deprivation. SN4741 cells transfected with indicated plasmids were assayed as described in Fig. 1F (n = 3, $^*P < 0.05$).

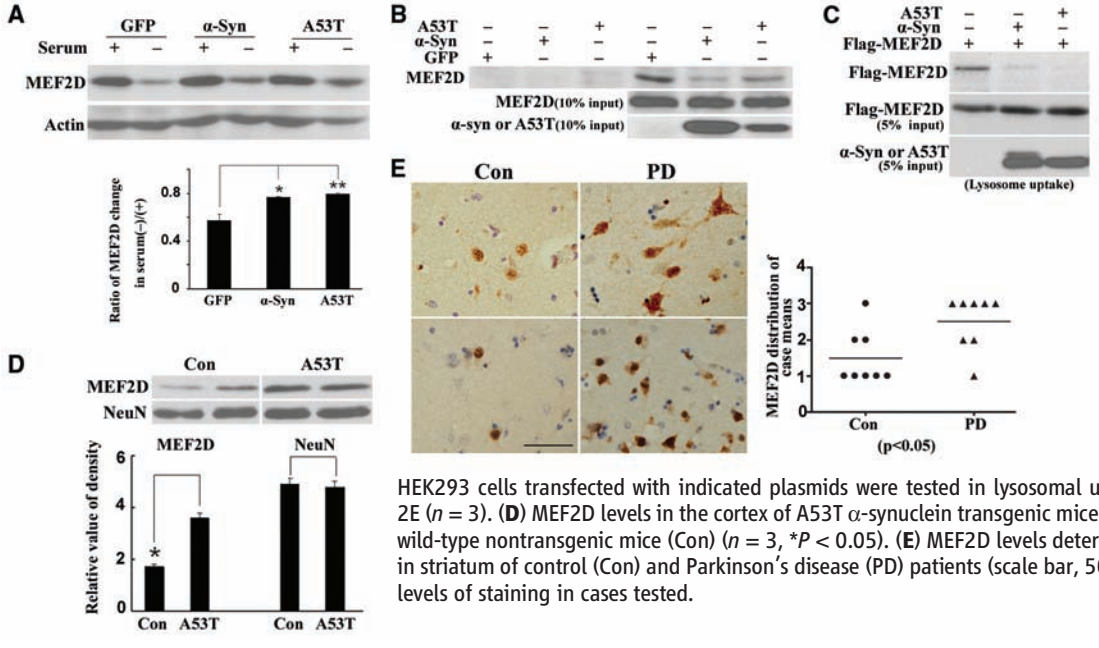

Fig. 3. Effect of α-synuclein on CMA-mediated degradation of MEF2D. (**A**) Inhibition of CMA-mediated degradation of MEF2D by α-synuclein. SN4741 cells transfected with indicated plasmids were assayed as described in Fig. 2I (n = 4; $^*P < 0.05$, $^{**}P < 0.01$). (**B**) Inhibition of binding of endogenous MEF2D to GST-Hsc70 by α-synuclein. The experiments were carried out as described in Fig. 2F after expression of α-synuclein in SN4741 cells (n = 3). (**C**) Inhibition of lysosomal uptake of MEF2D by α-synuclein. Lysates of HEK293 cells transfected with indicated plasmids were tested in lysosomal uptake assay as described in Fig. 2E (n = 3). (**D**) MEF2D levels in the cortex of A53T α-synuclein transgenic mice (A53T) were compared to that of wild-type nontransgenic mice (Con) (n = 3, $^*P < 0.05$). (**E**) MEF2D levels determined by immunohistochemistry in striatum of control (Con) and Parkinson's disease (PD) patients (scale bar, 50 μm). Graph depicts the relative levels of staining in cases tested.

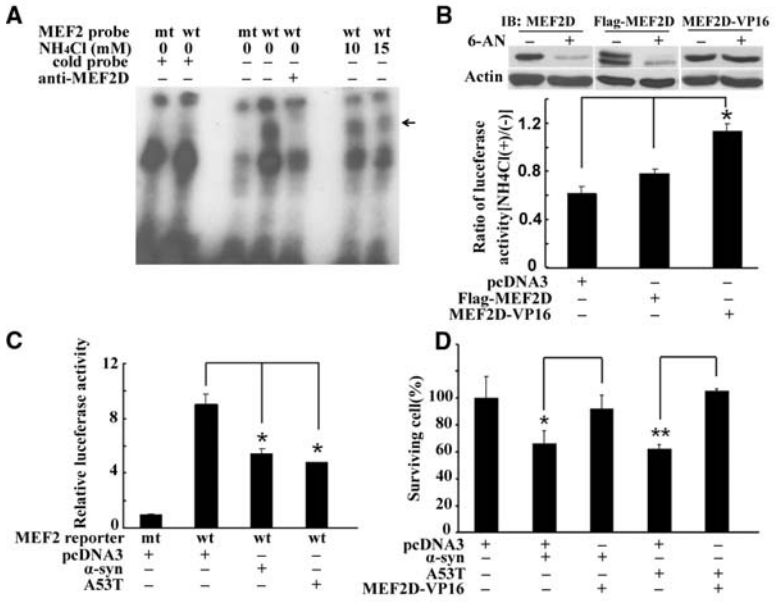

Fig. 4. Impairment of MEF2 function and neuronal survival after blockade of CMA. (**A**) Inhibition of MEF2D DNA binding activity by NH_4Cl. MEF2D DNA binding activity in SN4741 cells was assessed by EMSA after NH_4Cl treatment (arrow indicates the specific MEF2D-probe complex). (**B**) Effect of enhanced nuclear MEF2D on NH_4Cl-mediated inhibition. Levels of endogenous and transfected MEF2D in the nucleus (top panel) and MEF2 reporter activities (lower graph) in SN4741 cells were determined after 6-AN or NH_4Cl treatment, respectively ($n = 3$, $^*P < 0.05$). (**C**) Inhibition of MEF2 transactivation activity by α-synuclein. MEF2 reporter gene expression was measured after 36 hours of overexpression of wild-type or A53T α-synuclein in SN4741 cells ($n = 4$, $^*P < 0.05$). (**D**) Effect of increasing nuclear MEF2D function on α-synuclein–induced neuronal death. The viability of SN4741 cells was determined by WST assay after overexpression of indicated proteins (mean ± SEM, $n = 4$; $^*P < 0.05$, $^{**}P < 0.01$).

in neuronal viability (Fig. 4D). Coexpression of MEF2D-VP16 protected the cells against α-synuclein toxicity.

Our studies link CMA directly to the nuclear survival machinery. Because only α-synuclein mutants block substrate uptake in CMA (*18*), it has been unclear why an increase in the level of wild-type α-synuclein causes PD (*23*). Our findings that α-synuclein disrupts CMA-mediated degradation of MEF2D at a step prior to substrate uptake explain the toxic effects of both wild-type and mutant α-synuclein. Expression of Hsc70 suppresses α-synuclein toxicity in a *Drosophila* model of PD (*24*), consistent with our finding that maintenance of MEF2 function attenuates α-synuclein–induced neuronal death. Blocking CMA is accompanied by a clear decline of MEF2 function. Because the accumulated MEF2D binds poorly to DNA, the finding that the accumulated MEF2D binds poorly to DNA suggests important mechanisms in addition to nuclear export for the control of MEF2 activity. MEF2s play diverse roles in non-neuronal systems under physiological and pathological conditions (*25*). Our findings raise the possibility that degradation of MEF2s by CMA may function in other processes.

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Supporting Online Material

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Signal Sequences Activate the Catalytic Switch of SRP RNA

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The signal recognition particle (SRP) recognizes polypeptide chains bearing a signal sequence as they emerge from the ribosome, and then binds its membrane-associated receptor (SR), thereby delivering the ribosome–nascent chain complex to the endoplasmic reticulum in eukaryotic cells and the plasma membrane in prokaryotic cells. SRP RNA catalytically accelerates the interaction of SRP and SR, which stimulates their guanosine triphosphatase (GTPase) activities, leading to dissociation of the complex. We found that although the catalytic activity of SRP RNA appeared to be constitutive, SRP RNA accelerated complex formation only when SRP was bound to a signal sequence. This crucial control step was obscured because a detergent commonly included in the reaction buffer acted as a signal peptide mimic. Thus, SRP RNA is a molecular switch that renders the SRP-SR GTPase engine responsive to signal peptide recruitment, coupling GTP hydrolysis to productive protein targeting.

Secretory and transmembrane proteins are delivered to the membrane cotranslationally by the signal recognition particle (SRP) and its membrane-associated receptor (SR) (*1*). SRP recognizes signal sequences as they emerge from the ribosome (*2*) and then associates with SR at the membrane where the ribosome is transferred to the translocon. The guanosine triphos-

phatase (GTPase) domains of SRP and SR mediate this interaction cycle (*3*). Interaction of SRP with SR leads to the reciprocal stimulation of their GTPase activities, and GTP hydrolysis dissociates the complex (*4*, *5*). In *Escherichia coli*, SR is a single protein, FtsY, and SRP consists of 4.5*S* RNA and a single protein, Ffh (*6*). 4.5*S* RNA catalyzes the interaction of Ffh and FtsY, accelerating both on and off rates by a factor of more than 100 (*7*).

If the energy of GTP hydrolysis is to be harnessed for protein targeting, recruitment of targeting substrates by SRP should be coupled to the SRP-SR interaction cycle. Both signal sequences and 4.5*S* RNA bind to the M domain of Ffh, which suggests that the catalytic activity of 4.5*S* RNA could be responsive to signal sequence binding (*8*). However, under typical assay conditions, 4.5*S* RNA is constitutively active, negating this role for the RNA (*4*, *7*, *9*, *10*). A small amount of the nonionic detergent octaethyleneglycol dodecylether ($C_{12}E_8$) has been used in assays for SRP function, including kinetic characterization of the Ffh-FtsY interaction (*4*, *7*, *9*–*11*). We found that $C_{12}E_8$ was required for the stimulation of Ffh-FtsY binding rate caused by 4.5*S* RNA (Fig. 1A and table S1).

Assembly of the Ffh-FtsY complex can be measured by tryptophan fluorescence (*7*, *9*). In the presence of 4.5*S* RNA, $C_{12}E_8$ stimulated the rate of Ffh-FtsY association by a factor of 70 (Fig. 1A). Likewise, the stimulation of Ffh-FtsY disassembly caused by 4.5*S* RNA required $C_{12}E_8$ (faster with $C_{12}E_8$ than without by a factor of 23; Fig. 1B and table S1). $C_{12}E_8$ had no effect on the assembly or disassembly reactions in the absence of 4.5*S* RNA (Fig. 1, A and B). Thus, $C_{12}E_8$ is not a passive stabilizing additive but "activates" 4.5*S* RNA to accelerate Ffh-FtsY complex formation. Moreover, as most previous studies characterizing 4.5*S* RNA catalysis of the Ffh-FtsY interaction were carried out with detergent, they monitored this activated state.

The molecular properties of $C_{12}E_8$ that are important for activating 4.5*S* RNA suggested that it acts as a signal peptide mimic. We tested E_8, the nonionic head group of $C_{12}E_8$, and the detergents cetyltrimethylammonium bromide (CTABr) and sodium dodecyl sulfate (SDS), which share a long carbon chain with $C_{12}E_8$ but are positively and negatively charged, respectively (Fig. 1, C and D). CTABr stimulated binding similarly to $C_{12}E_8$, whereas SDS and E_8 did not (Fig. 1D). Thus, the long carbon chain of $C_{12}E_8$ with a neutral or positively charged head group is sufficient to activate 4.5*S* RNA. This suggests that $C_{12}E_8$ acts as a signal peptide mimic, because signal peptides generally have a hydrophobic core and positively but not negatively charged amino acids (*12*). Additionally, Ffh was crystallized with detergents (*13*), and density in the signal sequence–binding groove may have been attributable to the detergent. Finally, the Hill coefficient (n = 5.8) for $C_{12}E_8$ stimulation of Ffh–4.5*S* RNA–FtsY complex formation (fig. S1A) suggested that at least six detergent molecules cooperate to activate each Ffh–4.5*S* RNA and corresponded well with the size of the putative signal sequence–binding pocket in Ffh (fig. S1B).

We sought to determine whether signal peptides activate 4.5*S* RNA in the absence of $C_{12}E_8$. Because most signal peptides are insoluble (*14*), we chose the ΔEspP signal peptide (*15*), which is less hydrophobic than most signal peptides. We measured binding of ΔEspP peptide labeled with carboxyfluorescein (ΔEspP-FAM) to Ffh by fluorescence anisotropy. ΔEspP-FAM bound Ffh–4.5*S* RNA with an equilibrium dissociation constant (K_d) of 1.5 ± 0.4 μM (Fig. 2A). The K_d for Ffh alone was 19.6 ± 6.4 μM (Fig. 2A), which confirms that 4.5*S* RNA contributes to the binding of signal peptides as predicted (*8*). The addition of $C_{12}E_8$ weakened ΔEspP-FAM binding to Ffh–4.5*S* RNA (K_d = 5.5 ± 1.5 μM) but not to Ffh alone (21.6 ± 7.9 μM) (Fig. 2B), suggesting that ΔEspP and detergent compete for binding to SRP. ΔEspP-FAM did not bind Ffh lacking its signal sequence–binding M domain (Fig. 2A), binding was reversible (fig. S2A), and ΔEspP-FAM did not impair the solubility of Ffh–4.5*S* RNA (fig. S2, B and C).

To test whether saturating concentrations of ΔEspP stimulate the activity of 4.5*S* RNA, we used ΔEspP with added lysines at the C terminus to improve its solubility [ΔEspP* (*16*)]. Like $C_{12}E_8$, ΔEspP* accelerated Ffh–4.5*S* RNA–FtsY association (by a factor of >40; Fig. 2C and table S1) and dissociation (by a factor of ~10; table S1) but had no effect in the absence of 4.5*S* RNA (Fig. 2C and table S1). In the presence of both $C_{12}E_8$ and ΔEspP*, the rate of Ffh–4.5*S* RNA–FtsY complex formation was not substantially changed relative to individual additions (table

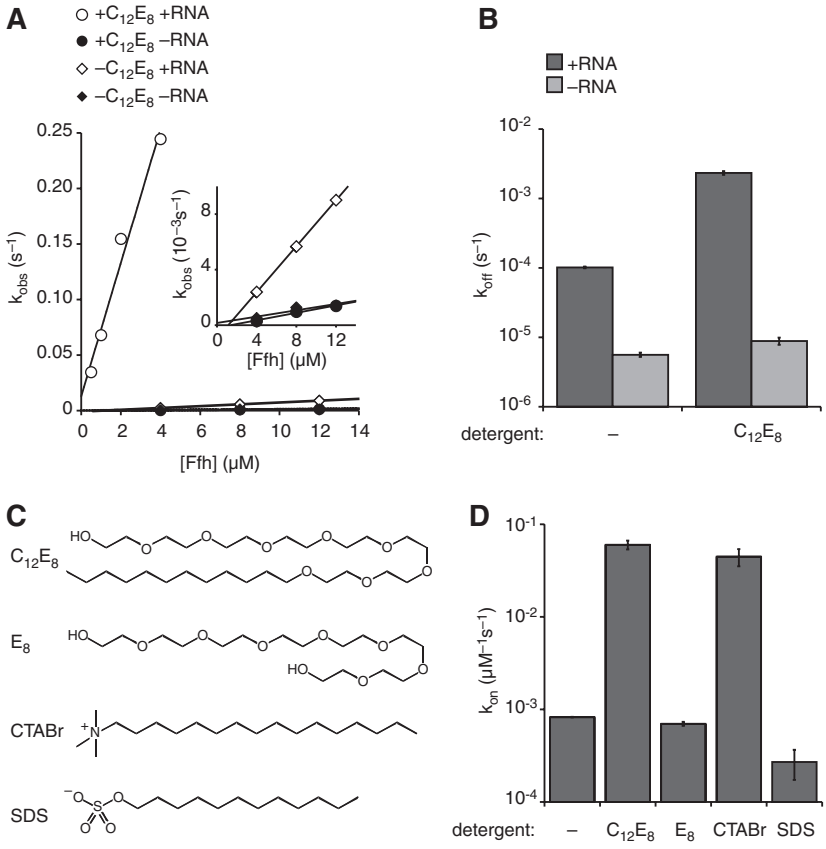

Fig. 1. Detergent activates 4.5*S* RNA to catalyze the Ffh-FtsY interaction. (**A**) $C_{12}E_8$ stimulates the binding of Ffh and FtsY only in the presence of 4.5*S* RNA. Observed binding rates for formation of Ffh-FtsY complexes are plotted as a function of Ffh concentration, [Ffh], in the presence and absence of 4.5*S* RNA and 185 μM $C_{12}E_8$. Lines represent fits to the equation $k_{obs} = k_{off} + k_{on}$[Ffh]. Inset shows the slow reactions on an expanded scale. (**B**) $C_{12}E_8$ activates 4.5*S* RNA stimulation of Ffh-FtsY complex dissociation. Dissociation rate constants are plotted in the absence and presence of $C_{12}E_8$. (**C**) Chemical structures of $C_{12}E_8$, E_8, CTABr, and SDS. (**D**) Association rate constants for Ffh–4.5*S* RNA–FtsY complex formation with no detergent, 185 μM $C_{12}E_8$, 100 μM E_8, 70 μM CTABr, or 100 μM SDS. Error bars in (B) and (D) are SEs of the fits.

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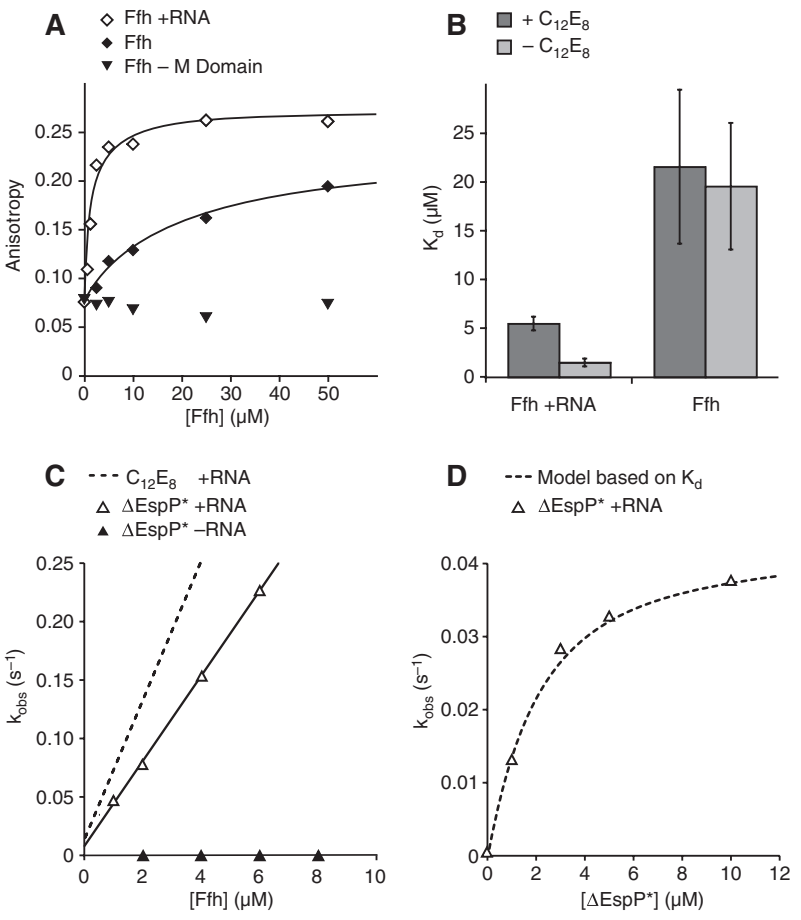


Fig. 2. ΔEspP binds SRP with micromolar affinity and stimulates 4.5*S* RNA catalysis of Ffh-FtsY interaction. (**A**) Fluorescence anisotropy of ΔEspP-FAM is plotted as a function of [Ffh]. Lines represent fits to the equation Anisotropy = $\text{Anisotropy}_{\text{free}}$ + $\text{Anisotropy}_{\text{bound}}$([Ffh]/($K_d$ + [Ffh])). (**B**) $C_{12}E_8$ increased the K_d of ΔEspP for Ffh–4.5*S* RNA. K_d values for ΔEspP binding to Ffh from fluorescence anisotropy in the presence and absence of 4.5*S* RNA are plotted. Dark bars represent K_d in the presence of 185 μM $C_{12}E_8$. Error bars are SEs of the fits. (**C**) In the presence of 4.5*S* RNA, ΔEspP stimulates the association rate for Ffh-FtsY complex formation. Observed rate constants are plotted as a function of [Ffh]. Lines are fits to the equation $k_{obs} = k_{off} + k_{on}$[Ffh]. The dashed line is a reference to the binding rate in the presence of $C_{12}E_8$ from Fig. 1. (**D**) ΔEspP* activates 4.5*S* RNA by binding to SRP. Observed rates for 1 μM Ffh–4.5*S* RNA binding to 1 μM FtsY are plotted as a function of ΔEspP* concentration. The dashed line represents the equation k_{obs} = [(fraction bound)(maximum stimulated rate)] + [(fraction unbound)(unstimulated rate)], where the fraction bound was calculated from the K_d measured in (A); $\chi^2 = 5.4 \times 10^{-6}$.

S1). Thus, the ΔEspP peptide and $C_{12}E_8$ act by the same mechanism.

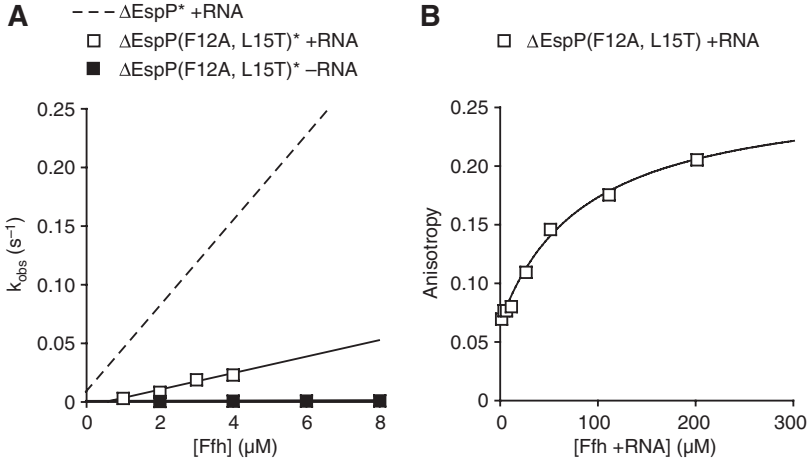
If ΔEspP* activates 4.5*S* RNA by associating with SRP, then the rate of Ffh–4.5*S* RNA–FtsY interaction should correlate with the fraction of ΔEspP*-bound SRP (calculated from the K_d in Fig. 2A). We measured the rate of Ffh–4.5*S* RNA and FtsY interaction as a function of ΔEspP* concentration (Fig. 2D). When we compared the observed Ffh-FtsY binding rates to the rate predicted from the fraction of SRP bound to ΔEspP* (Fig. 2A), the data matched this model exceptionally well (Fig. 2D).

In addition to accelerating Ffh-FtsY association, 4.5*S* RNA increases the rate of GTP hydrolysis by Ffh^{GTP}-$FtsY^{GTP}$ complexes (*4*) (fig. S3). However, neither ΔEspP* nor $C_{12}E_8$ affected this rate (fig. S3). Thus, signal peptides specifically affect the ability of 4.5*S* RNA to accelerate Ffh-FtsY complex formation.

To assess the specificity of 4.5*S* RNA activation, we used a version of ΔEspP* bearing Phe^{12} → Ala and Leu^{15} → Thr mutations [ΔEspP(F12A, L15T)*] that reduce SRP-dependent targeting in vivo (*15*). In the presence of 10 μM ΔEspP(F12A, L15T)*, the 4.5*S* RNA–stimulated association and dissociation of Ffh and FtsY was slower than that measured with "wild-type" ΔEspP* by a factor of ~5 (Fig. 3A and table S1). Similar to ΔEspP*, ΔEspP (F12A, L15T)* had no effect in the absence of 4.5*S* RNA (Fig. 3A). To determine whether this was due to reduced binding of ΔEspP(F12A, L15T)* to SRP, we measured the K_d by fluorescence anisotropy and found that binding was substantially weaker (K_d = 87 ± 18 μM, Fig. 3B). Consistent with this result, increasing concentrations of ΔEspP(F12A, L15T)* increased the observed rate for SRP-FtsY association (fig. S4).

Thus, SRP RNA acts as a switchable regulatory module at the center of the SRP protein-targeting machine to link recruitment of cargo (a signal peptide) to the next step in the targeting reaction (binding to SR). If free SRP and SR interacted efficiently with each other, they would undergo futile cycles of binding and GTP hydrolysis. Cargo-dependent activation of SRP RNA

Fig. 3. Mutations in ΔEspP that impair SRP-mediated targeting show decreased binding to SRP and decreased stimulation of 4.5*S* RNA. (**A**) ΔEspP(F12A, L15T)* stimulates SRP-FtsY complex formation less than does ΔEspP*. The dashed line represents the ΔEspP* + RNA peptide binding rate from Fig. 2C. (**B**) Fluorescence anisotropy of FAM-labeled ΔEspP bearing Phe^{12} → Ala and Leu^{15} → Thr mutations [ΔEspP(F12A, L15T)] is plotted as a function of [Ffh +RNA]. Line is fit as in Fig. 2A.



prevents this, harnessing the energy of GTP hydrolysis for protein targeting.

High-affinity interaction of SRP with ribosomes can occur before SRP interaction with the signal peptide when a short nascent chain is still inside the ribosome, raising the question of how SRP selectively targets signal sequence–containing substrates (*17*). Our results show that the interaction of the signal peptide with SRP accelerates SRP-SR complex formation, thereby providing a mechanism for selective delivery of appropriate substrates to the membrane. This is conceptually analogous to the kinetic mechanism by which translation achieves fidelity, where cognate codon-anticodon pairing accelerates GTP hydrolysis by elongation factor Tu (EF-Tu) (*18*, *19*).

Our results provide an intuitive model for how each step of the targeting process activates the next to achieve productive, directional targeting. Signal peptides bind to SRP's conformationally flexible M domain that forms a continuous surface with SRP RNA (*8*, *13*). Binding induces a conformational change that activates SRP RNA (*20*). Activated SRP RNA facilitates the displacement of the N-terminal helices of SRP and SR that slow their association without SRP RNA (*21*). This commits the ribosome–nascent chain complex to membrane targeting. The kinetic control described here, where substrate recruitment accelerates downstream interactions, provides a generalizable principle for coordination of multistep pathways.

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Floral Iridescence, Produced by Diffractive Optics, Acts As a Cue for Animal Pollinators

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Iridescence, the change in hue of a surface with varying observation angles, is used by insects, birds, fish, and reptiles for species recognition and mate selection. We identified iridescence in flowers of *Hibiscus trionum* and *Tulipa* species and demonstrated that iridescence is generated through diffraction gratings that might be widespread among flowering plants. Although iridescence might be expected to increase attractiveness, it might also compromise target identification because the object's appearance will vary depending on the viewer's perspective. We found that bumblebees (*Bombus terrestris*) learn to disentangle flower iridescence from color and correctly identify iridescent flowers despite their continuously changing appearance. This ability is retained in the absence of cues from polarized light or ultraviolet reflectance associated with diffraction gratings.

Biological iridescence results from various mechanisms, including multilayered materials, crystalline inclusions, and surface diffraction gratings (*1*–*6*). Diffraction gratings, surface striations of particular amplitude and frequency, cause interference, giving rise to an angular color variation (*7*). Although epidermal plant cell shape has been shown to influence the capture of all wavelengths of light by pigments (*8*–*10*), the mechanisms of iridescence have been poorly studied in plants; however, multilayered effects are occasionally observed in leaves (*11*, *12*).

Hibiscus trionum petals are white with a patch of red pigment at the base. This pigmented patch is iridescent, appearing blue, green, and yellow depending on the angle from which it is viewed (Fig. 1, A and B). Scanning electron microscopy (SEM) shows a sharply defined difference between the surface structure overlying the pigment and the rest of the petal (Fig. 1C). This iridescence is visible to the human eye; however, in flowers with similar surface structures, such as many species of *Tulipa* (table S1), the iridescence is only evident to humans when the pigment color and petal surface structure are separated.

When the surface structure of hibiscus and tulip petals was replicated in colorless optical epoxy (*13*), iridescent color was visible independent of pigment (fig. S3A). SEM of these replicas showed that long, ordered, cuticular striations overlay the iridescent epidermal cells. These cuticular striations resemble a diffraction grating. The diffraction grating of compact discs (CDs) has been previously characterized (*7*), so we used SEM to compare an epoxy cast made from the plastic interior of a disassembled CD with a cast of *Tulipa kolpakowskiana* (Fig. 2, A and B). The tulip cast (Fig. 2, C and D) shows a rounded cross-section of the striations (as opposed to the square profile of the CD) and a long wavelength undulation with a periodicity of 29 ± 2 μm, reflecting the surface of the epidermal cells.

We further investigated the tulip casts with optical spectroscopy in the 300-to-900-nm wavelength range [near-ultraviolet (near-UV) to infrared]. A collimated light beam of ~2 mm in diameter was reflected off the cast at an incidence angle $\theta_I = 30°$, and the reflected and scattered light was detected at angles θ_D varying from 0° to 90° in 1° steps (fig. S1). The angular detection aperture was less than 1° [supporting online material (SOM) text].

The spectrally resolved reflectivity was determined for the tulip cast (Fig. 3, A and B),

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a CD cast (Fig. 3C), and a planar reference sample (Fig. 3D). The reference shows only the specular signal at $\theta_D = 30°$ (sin $\theta_D = 0.5$) for all wavelengths. The CD cast additionally shows first-order interference (Fig. 3C, diagonal lines) and a weak second-order signal (Fig. 3C, bottom left), both of which are quantitatively described by the grating equation $m\lambda = d(\sin\theta_D - \sin\theta_I)$, where λ is the wavelength of light, d is the periodicity of the grating ($d = 1.45$ μm), and m is the diffraction order (*14*).

The optical signature of the tulip cast (Fig. 3A) was more complex. The first-order diffraction signal was clearly visible at large angles (sin $\theta_D > 0.7$). It is broadened as compared with that of the CD because of the surface undulation (that is, the cells) shown in Fig. 2D. The two lines in Fig. 3A, which we calculated with the grating equation, delimit the predicted spectral range of first-order diffraction for such a wavy surface (SOM text). Compared with Fig. 3, C and D, the specular reflection is broadened and shows an intensity decrease toward long wavelengths. This is a combination of the ~30-μm surface undulation and the overall disorder in the pattern of the epidermal cells. Most of the optical intensity is at short wavelengths, coinciding with the high sensitivity of the bee eye in the blue and near-UV (*15*) ranges. UV signals caused by iridescence are known in animals (*3*, *16*, *17*). Furthermore, bees recognize contrasting patterns in the UV range that occur on flowers (*18*). Therefore, the optical signature and its angular dependence, because of its particular strength in the UV range, may be even more meaningful in terms of insect vision than human vision. Although this optical effect may have its origins in pollinator attraction, striations also occur in many cultivated varieties of tulip (table S1), which may have resulted from additional human selection for the luster that iridescence lends the flower.

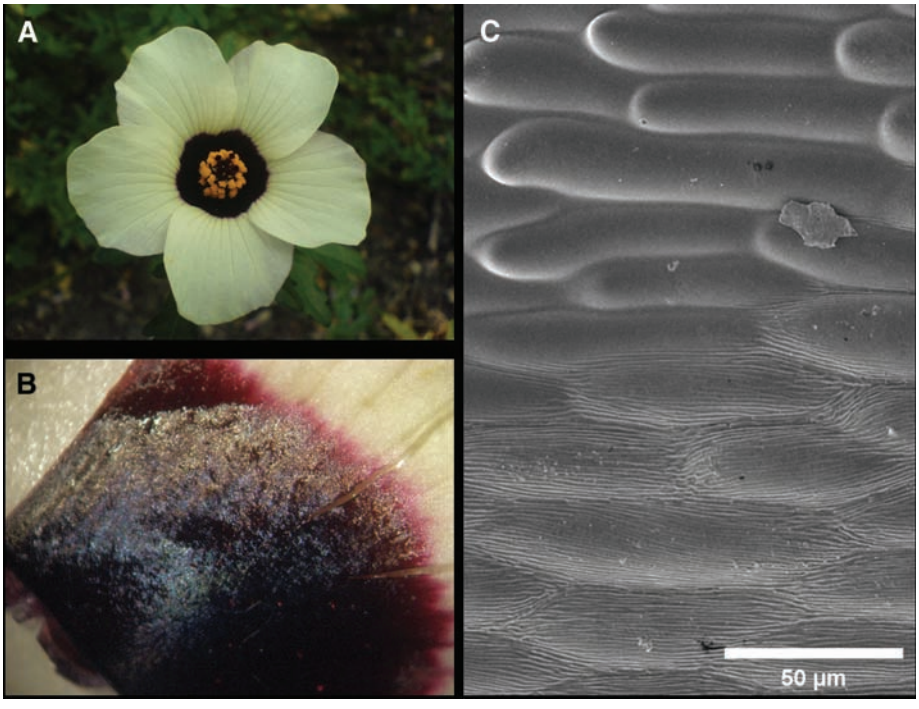

Fig. 1. (**A**) *H. trionum* flower. (**B**) Base of *H. trionum* petal, showing iridescence overlying red pigment. (**C**) SEM of *H. trionum* petal, the upper half of the picture spanning the white (smooth cells) and the lower half spanning the pigmented (heavily striated longitudinally toward the petal base) epidermis.

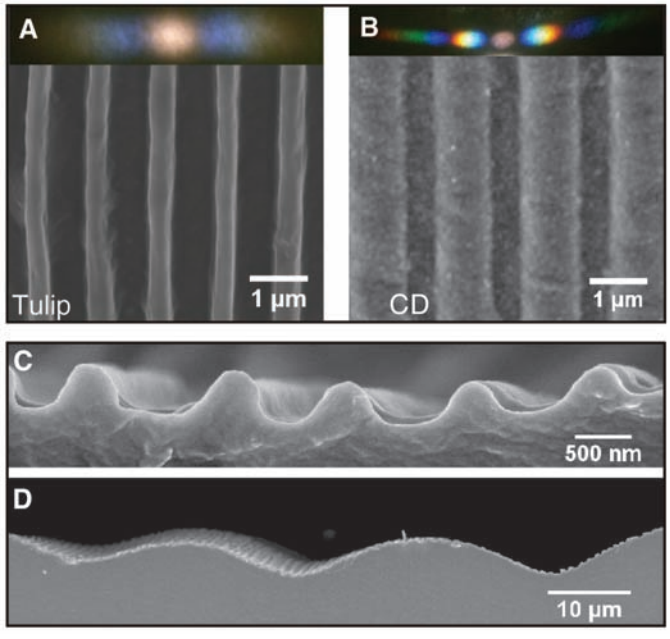

Fig. 2. SEM images of tulip epoxy casts. (**A**) Top view showing striations on the petal surface of *T. kolpakowskiana*, resembling a line grating with a periodicity of 1.2 ± 0.3 μm. (**B**) Top view of an epoxy cast of a disassembled CD, showing a grating periodicity of 1.45 ± 0.05 μm. (**C**) Side view of the structure from (A), showing the rounded cross-section of the striation. The lower magnification image in (**D**) shows undulations with a periodicity of 29 ± 2 μm, reflecting the epidermal cells themselves. The insets in (A) and (B) show the optical appearance of the two gratings in transmission.

Animals use iridescence for species recognition and mate selection (*1*–*4*), and iridescence is under selective pressure in some species—for example, arising from intraspecific competition between male butterflies in their attractiveness to females (*4*). Floral iridescence, in contrast, is presumably a signal to pollinating animals. Previous discussions about flower fluorescence show that a floral optical phenomenon, however intricate, must be demonstrated to have a biological signaling function (*19*–*21*). To test whether iridescence, as displayed by *H. trionum*, is distinguished by pollinators, we measured the variation in hue shown by the iridescent patch with spectroscopy both across the striations (measuring maximum iridescence) and along the striations (measuring minimum iridescence). The color loci of these two measurements were calculated in a bee hexagon color space [a representation of color perception designed using information about receptor sensitivity and color-opponent coding, so that distances between points generated by two objects indicate the degree to which the two are distinguishable (*22*)] (Fig. 4A). This indicated that bees will perceive a change in flower color from different angles. We observed that the difference in color between the two measurements, when calculated as the Euclidean distance between color loci, was 0.217. As a color distance of 0.15 is distinguishable by bees with above 90% accuracy, and even a distance of 0.05 can be distinguishable by trained bees (*23*), the variation in hue demonstrated by a single *H. trionum* flower viewed from different angles is sufficient for ready visual discrimination by bees.

We tested the ability of flower-naïve bumblebees to discriminate between iridescent and noniridescent disks (cast, respectively, on the plastic diffraction grating removed from the outer edge of a disassembled CD and on smooth molded plastic) by using differential conditioning (*24*). Colored disks were generated by adding 50 μg of

pigment (ultramarine blue, chrome yellow, manganese violet, or quinacridone red) to every 10 g of epoxy resin (fig. S4A).

We trained bees that iridescent disks containing yellow, blue, or violet pigment offered a sucrose reward, whereas identically pigmented noniridescent disks offered a bitter quinine hemisulphate salt solution (*24*). After 80 visits, bees visited iridescent disks more frequently than after their immediate introduction to the arena [first 10 visits = 4.7 ± 0.5 (mean ± SE); last 10 visits = 8.1 ± 0.4; Student's *t* test, $t(9) = 4.96$, $P < 0.001$] (Fig. 4B). Preference for iridescence did not differ according to pigment color (analysis of variance, $F_{2,16} = 1.57$, $P = 0.238$). For each bee, these disks were then removed and replaced with five noniridescent and five iridescent red disks. When shown this previously unseen color, bees continued to visit iridescent flowers with 83.4 ± 3.4% accuracy [$n = 10$ bees; as compared with random choosing, $t(9) = 5.72$, $P < 0.001$] during their first foraging bout, demonstrating their ability to use iridescence as a cue to distinguish between rewarding and nonrewarding substrates irrespective of pigment-based reflectance. This suggests that although pollinators are typically thought to identify rewarding flowers by their pigment-based reflectance, in our studies they were able to discriminate between the disks having a weak superposed angular-dependent color signal arising from grating interference.

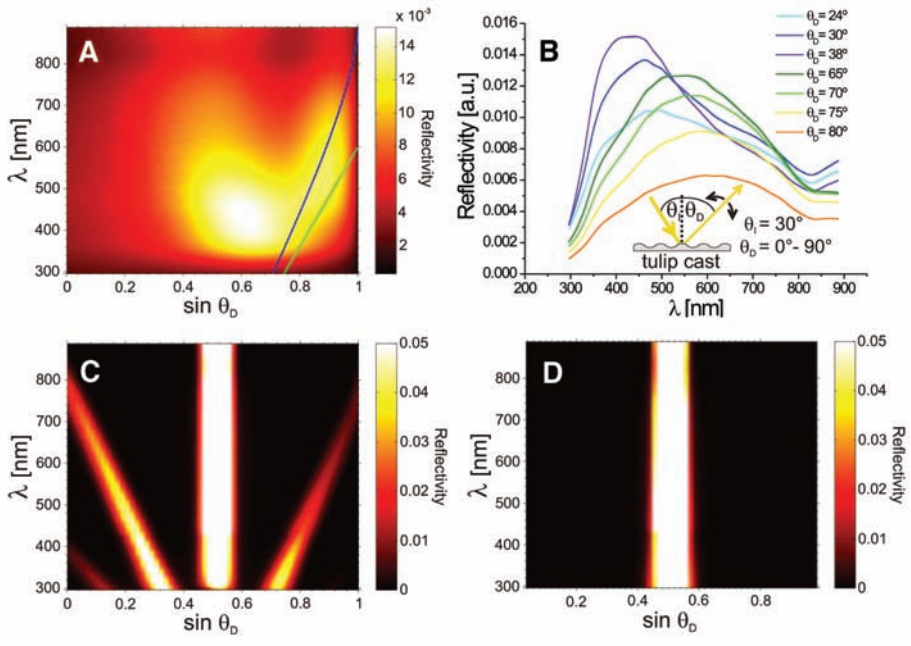

Fig. 3. Spectrally resolved reflection of the structures in Fig. 2. (**A**) Reflected intensity of the tulip cast [different representation in (**B**); a.u., arbitrary units] from Fig. 2A for $\theta_i = 30°$ as a function of sin θ_D and λ in comparison with (**C**) a CD cast (Fig. 2B) and (**D**) an unstructured surface. The central stripe in (C) and (D) is the signature of specular reflection. The two diagonal stripes in (C) are the first-order interference of the CD grating, with a weak second-order signal at the bottom left. The tulip cast in (A) shows the clear optical signature of an interference grating with a broadened first-order diffraction for sin $\theta_D > 0.7$. The two lines are the delimiting predictions of the grating equation for a ~30-μm surface undulation leading to an inclination of the surface normal by −18° (blue) and 0° (green).

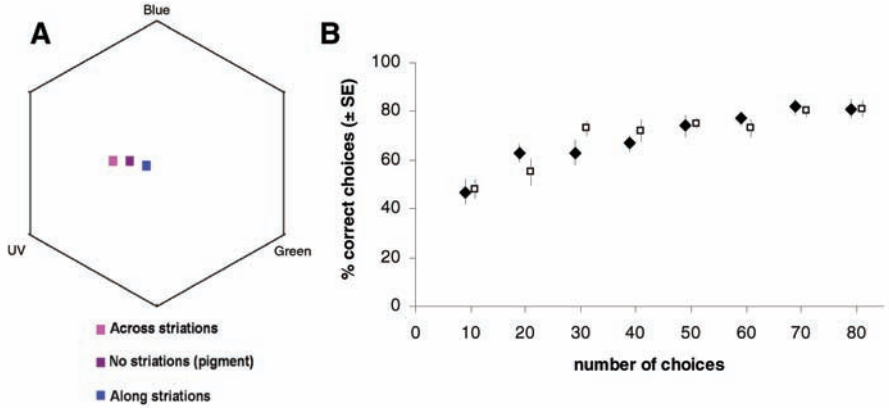

Fig. 4. Bee recognition of iridescent epoxy surfaces. (**A**) Loci of *H. trionum* in the bee color hexagon. (**B**) Two learning curves, each of 10 bees (with SE), choosing between rewarding iridescent flowers and nonrewarding noniridescent flowers. Filled diamonds indicate bees that were offered casts of CDs; clear squares indicate bees that were offered casts of tulip petals.

The polarization of light reflected by iridescent colors on female *Heliconius* butterflies is recognized by males, but the changing colors are not (*2*). To assess if the visual cue used by the bumblebees was independent of a polarization effect, we repeated the discrimination experiment with only violet pigment disks and depolarizing Mylar over each disk. This removed polarization signals but left the color intact (*2*). Bees were again more likely to visit iridescent flowers as time progressed [mean visits ± SE; visits 1 to 10 = 4.3 ± 0.50; visits 71 to 80 = 9.2 ± 0.34; $t(9) = 8.97$, $P < 0.001$]. We repeated the experiment again with a polycarbonate filter opaque to wavelengths below 400 nm (*25*) blocking any UV signal, to ensure that the UV component of the diffraction grating was not acting as a specific cue. After 80 visits, bees visited iridescent disks more frequently than after their immediate introduction to the arena [first 10 visits = 4.9 ± 0.41; last 10 visits = 8.2 ± 0.25; $t(9) = 8.10$, $P < 0.001$] (learning curves shown in fig. S4B). We conclude that iridescence generated by diffraction gratings can be used as a pollination cue by bumblebees independent of underlying pigment, UV signals, or polarization effects.

To confirm that bees could discriminate the less regular iridescence of a real flower, yellow-pigmented epoxy casts were made from *T. kolpakowskiana* petals. Casts with floral iridescence were taken from the adaxial petal surface, which has striations, and casts without iridescence were taken from the abaxial petal surface, which lacks striations. Overall epidermal cell size and shape was similar on both surfaces. Our results show that bees were able to use the floral iridescence as accurately and effectively as they used the CD iridescence as a pollination cue. After 80 visits, bees visited iridescent disks more frequently than after their immediate introduction to the arena [first 10 visits = 4.8 ± 3.89 (mean ± SE), last 10 visits = 8.1 ± 3.14; $t(9) = 5.75$, $P < 0.001$] (Fig. 4B).

Over 50% of angiosperm species produce a striated cuticle over their petals (*26*), and although the degree to which such striations are ordered will strongly influence their visual effect, it is nonetheless probable that many flowers produce iridescence. We have so far identified 10 angiosperm families containing species with petal iridescence generated by diffraction gratings (table S2). Such striations may also influence pollinators through their tactile effects (*27*). As demonstrated by *H. trionum*, structures causing iridescence may occur in an overlying pattern to those caused by pigment color. This floral patterning is known to be important in pollinator attraction (*18*, *28*). It has

previously been shown in both birds and butterflies that structural color can enhance pigment color either by an additive or a contrast effect (*8*, *16*, *29*, *30*). This interplay of structure and pigment may therefore also add to the diversity of pollination cues utilized by the flowers of many angiosperm species.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5910/130/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 and S2
References

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Real-Time DNA Sequencing from Single Polymerase Molecules

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We present single-molecule, real-time sequencing data obtained from a DNA polymerase performing uninterrupted template-directed synthesis using four distinguishable fluorescently labeled deoxyribonucleoside triphosphates (dNTPs). We detected the temporal order of their enzymatic incorporation into a growing DNA strand with zero-mode waveguide nanostructure arrays, which provide optical observation volume confinement and enable parallel, simultaneous detection of thousands of single-molecule sequencing reactions. Conjugation of fluorophores to the terminal phosphate moiety of the dNTPs allows continuous observation of DNA synthesis over thousands of bases without steric hindrance. The data report directly on polymerase dynamics, revealing distinct polymerization states and pause sites corresponding to DNA secondary structure. Sequence data were aligned with the known reference sequence to assay biophysical parameters of polymerization for each template position. Consensus sequences were generated from the single-molecule reads at 15-fold coverage, showing a median accuracy of 99.3%, with no systematic error beyond fluorophore-dependent error rates.

The Sanger method for DNA sequencing (*1*) uses DNA polymerase to incorporate the 3′-dideoxynucleotide that terminates the synthesis of a DNA copy. This method relies on the low error rate of DNA polymerases, but exploits neither their potential for high catalytic rates nor high processivity (*2*–*4*). Increasing the speed and length of individual sequencing reads beyond the current Sanger technology limit will shorten cycle times, accelerate sequence assembly, reduce cost, enable accurate sequencing analysis of repeat-rich areas of the genome, and reveal large-scale genomic complexity (*5*, *6*). Alternative approaches that increase sequencing performance have been reported [(*7*–*10*), reviewed in (*11*, *12*)]. Several of these methods have been deployed as commercial sequencing systems (*13*–*16*), which have greatly increased overall throughput, enabling many applications that were previously unfeasible. However, because these methods all gate enzymatic activity, using various termination approaches, they have not yielded longer sequence reads (limited to ~400 nucleotides), nor do they exploit the high intrinsic rates of polymerase-catalyzed DNA synthesis.

The use of DNA polymerase as a real-time sequencing engine—that is, direct observation of processive DNA polymerization with base-pair resolution—has long been proposed but has been difficult to realize (*7*, *8*, *17*–*22*). To fully harness the intrinsic speed, fidelity, and processivity of these enzymes, several technical challenges must be met simultaneously. First, the speed at which each polymerase synthesizes DNA exhibits stochastic fluctuation, so polymerase molecules would need to be observed individually while they undergo template-directed synthesis. Because of the high nucleotide concentrations required by DNA polymerases (*20*), a reduction in the observation volume beyond what is afforded by conventional methods, such as confocal or total internal reflection microscopy, directly improves single-molecule detection. Second, deoxyribonucleoside triphosphate (dNTP) substrates must carry detection labels that do not inhibit DNA polymerization even when 100% of the native nucleotides are replaced with their labeled counterparts. Third, a surface chemistry is required that retains activity of DNA polymerase molecules and inhibits nonspecific adsorption of labeled dNTPs. Finally, an instrument is required that can faithfully detect and distinguish incorporation of four different labeled dNTPs. Here, we provide proof-of-concept for an approach to highly

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multiplexed single-molecule, real-time DNA sequencing based on the observation of the temporal order of fluorescently labeled nucleotide incorporations during unhindered DNA synthesis by a polymerase molecule.

For the observation of incorporation events, we used a nanophotonic structure, the zero-mode waveguide (ZMW), which can reduce the volume of observation by more than three orders of magnitude relative to confocal fluorescence microscopy (*20*). This level of confinement enables single-fluorophore detection despite the relatively high labeled dNTP concentrations—between 0.1 and 10 μM—required by DNA polymerase for fast, accurate, and processive synthesis. This range produces average molecular occupancies between ~0.01 and 1 molecules for a ZMW 100 nm in diameter (*20*, *23*), compared with ~3 to 300 molecules for total internal reflection microscopy (*24*–*26*). The ZMW fabrication process was recently improved, resulting in a higher yield of devices suitable for single-molecule sequencing (*23*).

Other DNA sequencing approaches have used base-linked fluorescent nucleotides (*7*, *8*, *14*, *17*, *20*, *27*, *28*). These cannot be used in real-time sequencing because they are poorly incorporated in consecutive positions by DNA polymerase. In contrast, when a fluorophore is linked to the terminal phosphate moiety (phospholinked), phosphodiester bond formation catalyzed by the DNA polymerase results in release of the fluorophore from the incorporated nucleotide, thus generating natural, unmodified DNA (*21*, *29*–*31*). Φ29 DNA polymerase was selected for these studies because it is a stable, single-subunit enzyme with high speed, accuracy, and processivity that efficiently uses phospholinked dNTPs (*32*). It is capable of strand-displacement DNA synthesis and has been used in whole-genome amplification, showing minimal sequencing context bias (*33*). We introduced site-specific mutations in the enzyme and devised a linkage chemistry that allows 100% replacement of native nucleotides with four distinct phospholinked dNTPs while retaining near wild-type polymerase kinetics (*32*).

Recently, we reported a surface chemistry that enables selective immobilization of DNA polymerase molecules in the detection zone of ZMW nanostructures with high yield (*34*). Binding of polymerase molecules to the side walls is inhibited through the use of an alumina-specific polyphosphonate passivation layer. Here, an additional biotinylated polyethylene glycol layer was used to orient the polymerase and to prevent direct protein contact with the silica floor of the ZMW (*26*).

Extensions in the state-of-the-art of single-molecule detection were required to enable continuous, high-fidelity detection and discrimination of four spectrally distinct fluorophores simultaneously in large numbers of ZMWs. We reported a high-multiplex confocal fluorescence detection system (*35*) that uses targeted, uniform multilaser illumination of 3000 ZMWs through holographic phase masks. The instrument uses a confocal pinhole array to reject out-of-focus background, and a prism dispersive element for wavelength discrimination that provides flexibility in the choice of fluorescent dyes used while transmitting >99% of the incident light.

The architecture of our method is shown in Fig. 1A. DNA sequence is determined by detecting fluorescence from binding of correctly base-paired (cognate) phospholinked dNTPs in the active site of the polymerase (Fig. 1B). A fluorescence pulse is produced by the polymerase retaining the cognate nucleotide with its color-coded fluorophore in the detection region of the ZMW. It lasts for a period governed principally by the rate of catalysis, and ends upon cleavage of the dye-linker-pyrophosphate group, which quickly diffuses from the ZMW detection region. The duration of the fluorophore retention is much longer than the time scales associated with diffusion (2 to 10 μs) or noncognate sampling (<1 ms), which manifest as a low and constant background signal. Translocation prepares the polymerase active site for binding of the subsequent cognate phospholinked dNTP, which marks the beginning of the next pulse. Thus, the interpulse duration is a combination of the translocation and subsequent nucleotide binding times. The sequence of fluorescence pulses recorded in the plot of intensity versus time is referred to as a read.

To illustrate the principle of our approach to DNA sequencing, we used a synthetic, linear, single-stranded DNA template with a two-base artificial sequence pattern (Fig. 2A). Alternating template sections that omitted either cytosine or guanine were interrogated with their complementary phospholinked dNTPs, A555-dCTP and A647-dGTP. Reactions were initiated by addition of catalytically essential metal ions while collecting movies of fluorescence emissions simultaneously from an array containing 3000 ZMWs (movie S1). Time-resolved fluorescence spectra are presented for an example ZMW in Fig. 2B. For each movie frame, these spectra were reduced by dye-weighted summation to two values, representing the emission rate from each of the two phospholinked dNTPs as a function of time (Fig. 2C).

Single-molecule events corresponding to phospholinked dNTP incorporations manifested as fluorescent pulses whose variable duration reflected the enzyme kinetics and exhibited stochastic fluctuations in intensity (because of counting statistics and dye photophysics). The reads contained pulses with the expected pattern: alternating blocks of like-colored pulses corresponding to the alternating blocks in the template. Furthermore, we observed the hallmarks of single-molecule fluorescent events: single-frame rise and fall times at the start and end of the pulse, respectively (<<10 ms), which facilitate

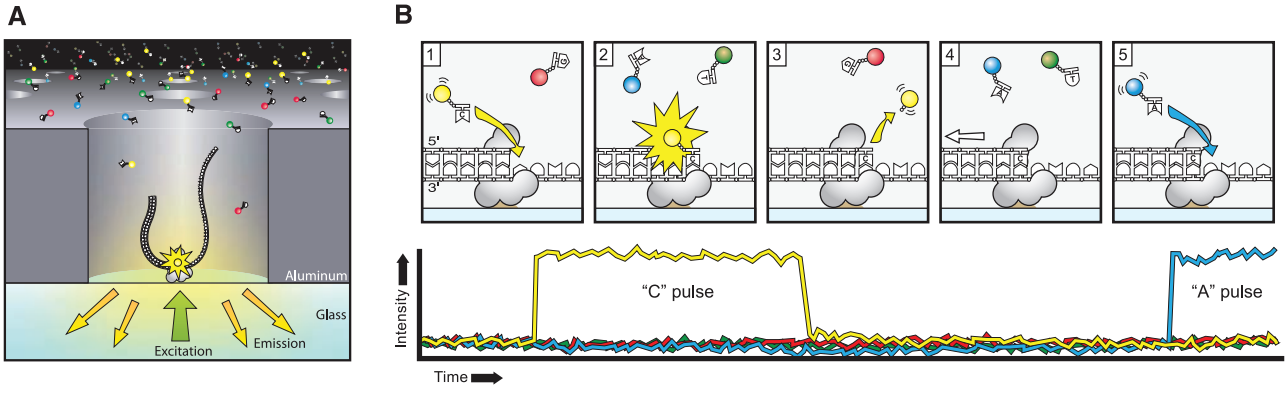

Fig. 1. Principle of single-molecule, real-time DNA sequencing. (**A**) Experimental geometry. A single molecule of DNA template-bound Φ29 DNA polymerase is immobilized at the bottom of a ZMW, which is illuminated from below by laser light. The ZMW nanostructure provides excitation confinement in the zeptoliter (10^{-21} liter) regime, enabling detection of individual phospholinked nucleotide substrates against the bulk solution background as they are incorporated into the DNA strand by the polymerase. (**B**) Schematic event sequence of the phospholinked dNTP incorporation cycle, with a corresponding expected time trace of detected fluorescence intensity from the ZMW. (1) A phospholinked nucleotide forms a cognate association with the template in the polymerase active site, (2) causing an elevation of the fluorescence output on the corresponding color channel. (3) Phosphodiester bond formation liberates the dye-linker-pyrophosphate product, which diffuses out of the ZMW, thus ending the fluorescence pulse. (4) The polymerase translocates to the next position, and (5) the next cognate nucleotide binds the active site beginning the subsequent pulse.

pulse detection and base calling even when pulses are close together (see, e.g., Fig. 2C, bottom right). Pulse activity ceased after completion of the 123 total nucleotide incorporations needed to reach the end of the linear template.

Read parameters for 10 representative molecules as well as an entire data set from a ZMW array are shown in table S1. The average DNA synthesis rate for 740 single-molecule reads was 4.7 ± 1.7 bases/s. Median and standard error values for the fluorescence intensity were 7353 ± 2970 (A555-dCTP) and 8408 ± 3381 (A647-dGTP) detected photons per second, yielding signal-to-noise ratios of 24 ± 10 and 25 ± 10, respectively. The observed pulse brightness showed uniformity within individual traces consistent with a stationary single-molecule process (modal coefficient of variation was 25%). When essential reaction components were withheld (e.g., DNA polymerase, primer/template, one or more phospholinked dNTPs or metal ions), <0.01 pulses ZMW^{-1} s^{-1} were observed, versus >1 pulses ZMW^{-1} s^{-1} for DNA polymerization conditions, additionally confirming that fluorescence pulse activity corresponded to DNA polymerase catalytic activity.

Immobilized enzymes in ZMWs maintained high activity. In the current implementation, polymerase molecules are randomly distributed among the ZMWs, leading to a Poisson distribution of occupancy (*34*). At optimal loading, the distribution is 36.8% empty ZMWs, 36.8% with just one polymerase, and 26.4% with two or more. In this experiment, ~35% of the ZMWs produced traces indicative of single DNA polymerase occupancy, of which 82% produced full-length reads. The yield limitations inherent to random polymerase immobilization could be exceeded using molecular self-assembly to place a single polymerase in each ZMW.

The real-time nature of the collected data allows the kinetics of the enzyme to be directly observed (fig. S1 and table S2). Single-polymerase kinetic parameters from these two-color block traces responded as expected when reaction conditions were modified. Reduction of the pH from 7.1 to 6.5 increased median pulse widths, consistent with a decrease of the rate of phosphodiester bond formation (*36*). Increasing the phospholinked dNTP concentration from 100 to 250 nM increased the median DNA synthesis rate, consistent with rate-limited dNTP binding at low concentration.

To investigate the potential of this method for long-read DNA sequencing, we performed a similar two-base signature sequence pattern experiment using a single-stranded 72-base circular DNA template (Fig. 3A). The template was designed such that cytosines were present on only half of the circle, and guanines on the other half. Φ29 DNA polymerase is highly processive (>70,000 bases) without cofactors in bulk reactions (*2*). It will carry out multiple laps of DNA strand-displacement synthesis around the circular template and has been shown to retain this activity in ZMWs (*34*).

A representative read (Fig. 3B) showed the expected continuous signature sequence pattern of alternating periods of A555-dCTP and A647-dGTP pulses. Pulse characteristics were similar to those described for Fig. 2 and remained uniform throughout a read. DNA polymerization activity lasted for thousands of seconds, allowing observation of several kilobases of DNA synthesis (top axis, Fig. 3B). Occasional pauses in DNA polymerization activity are visible as gaps in the trace. The total synthesized DNA length as a function of time (Fig. 3C) shows periods of different persistent polymerization rates during these long reads. Two characteristic polymerization rates of ~2 bases/s and ~4 bases/s were determined, suggesting the existence of different long-lived polymerase modes that occasionally and suddenly interconvert. No spatial correlation in the polymerase speed was observed across a ZMW array. Pulse characteristics underlying these two states were statistically identical, with the exception of a decreased interpulse duration for the faster state (fig. S2). Similar behavior was also observed using different combinations of the fluorophores and bases and for templates with different sequences (fig. S3), which implies that these states are specific neither to the phospholinked dNTPs used nor to the sequence context. Several prolonged observations were made in these experiments, showing continuous polymerase activity for more than 1 hour and >4000 bases

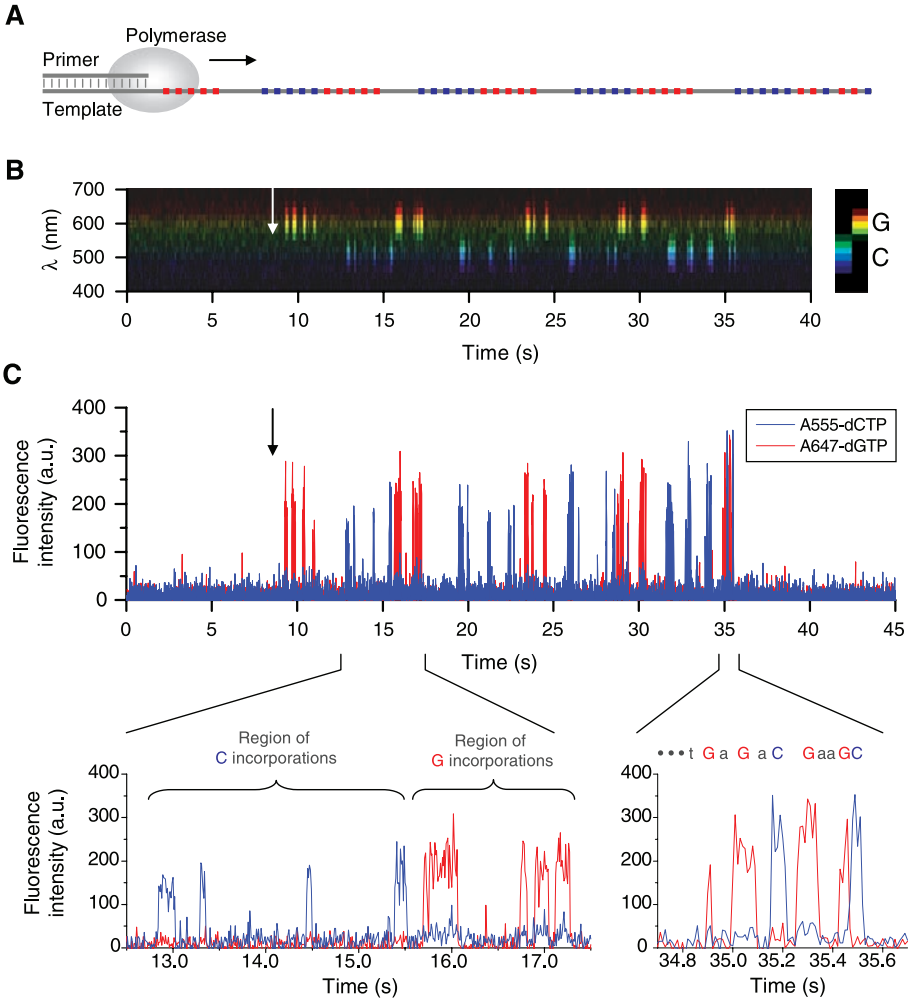

Fig. 2. Real-time detection of single-molecule DNA polymerase activity. (**A**) DNA template design for two-base sequence pattern detection. The sequence of a linear, single-stranded DNA template was designed to yield incorporation of alternating blocks of two phospholinked nucleotides (A555-dCTP and A647-dGTP), interspersed with the other two, unmodified dNTPs. (**B**) Time-resolved fluorescence intensity spectrum from a ZMW. Data from a 15 × 5 pixel area from each movie frame were spatially collapsed to a 15-pixel spectrum, which is shown as a function of time. The expected fluorescence emission profiles for the two labeled nucleotides are shown at the right. The arrow denotes addition of the catalytic metal ion that initiated the polymerization reaction. The complete data set from which the time trace was extracted, containing 3000 ZMWs measured simultaneously, is shown in movie S1. (**C**) Corresponding fluorescence time trace after spectral processing. Two regions are magnified and annotated with the expected nucleotide incorporation sequence. Pulse heights show level setting with a coefficient of variation of 27% and a maximal excursion of 61%.

synthesized. The mean number of pulses per block was uniform over at least 1000 bases of incorporation (Fig. 3D); hence, this sequencing approach maintains accuracy irrespective of read length.

The measurements described above were extended to four-color DNA sequencing. All four native nucleotides were fully replaced with the following set of phospholinked dNTPs: A555-dATP, A568-dTTP, A647-dGTP, and A660-dCTP (fig. S4). Two lasers were used for the excitation of the four fluorophores. Fluorescence pulses were identified by a threshold detection algorithm (*37*) based on dye-weighted summation as above. The base identities of pulses were automatically assigned by least-squares fitting of the four phospholinked dNTP reference spectra to the measured spectra (fig. S4) (*26*). The read extracted from the measured pulses matched well with the underlying sequence of the nascent DNA strand, spanning the entire length of the 150-base linear template (Fig. 4, A and B). Of the 158 total bases in the alignment, 131 were correctly identified by the automated base caller. The 27 errors consisted of 12 deletions, eight insertions, and seven mismatches.

Sequencing performance analysis was extended to a set of 449 reads that showed pulse trains consistent with single polymerase occupancy (table S3). In these data, errors are dominated by deletions, which stem from incorporation events or intervals between them that are too short to be reliably detected. Unlabeled nucleotide contamination (dark nucleotides) can be a source of deletion errors in single-molecule sequencing systems. Here, this was not the case because the initial phospholinked dNTP composition was >99.5% pure (fig. S5) and, unlike with base-linked nucleotides, the polymerase showed no preference for unlabeled versus labeled substrates (*32*). Additionally, a comparison of our observed deletion error rate with a deletion rate predicted solely from pulse width distributions shows that dark nucleotides need not be invoked as a source of error. For example, fig. S6 shows the pulse width distribution for A555-dATP and the projected probability of pulse detection for that nucleotide as a function of pulse width. From these data, the deletion rate is estimated to be 7.8%, consistent with the observed 7.4% deletion rate for this nucleotide. This error type can be addressed by engineering the enzyme to reduce the fraction of short incorporation events, increasing fluorophore brightness, and improving efficiency of light collection.

The majority of insertion errors were caused by dissociation of a cognate nucleotide from the active site before phosphodiester bond formation can occur, resulting in the erroneous duplication of a pulse. This error type can be addressed by modifying the enzyme to decrease the free energy of the enzyme-substrate bound state, thus decreasing the dissociation rate before catalysis. Mismatches in the reads were mainly caused by spectral misassignments of the A647 and A660 dyes (accounting for ~60% of the mismatch error), which show the least spectral separation amongst the four dyes (table S3). The remainder of the mismatches involved misassignments between the A555 and A568 dyes (other factors were below the sensitivity of the assay). Finding compatible dye sets with larger spectral separations, as well as increasing the brightness of the dyes and collection efficiency of the instrument, will reduce the frequency of these errors.

To survey possible sequence context dependencies of these error types, we quantitated the two most important kinetic parameters—pulse width and interpulse duration—as a function of sequence position over the 150-base template. To extract these parameters for each template location, we associated individual pulses from the 449 reads with their sequence positions using a Smith-Waterman alignment algorithm (*38*). Pulse widths and interpulse durations are displayed as a function of sequence position in Fig. 4, C and D, respectively. The average pulse widths depend weakly on dNTP identity and show statistically significant but only moderate variation across template position. The average interpulse durations were typically between 200 and 700 ms, except for a few instances with much higher

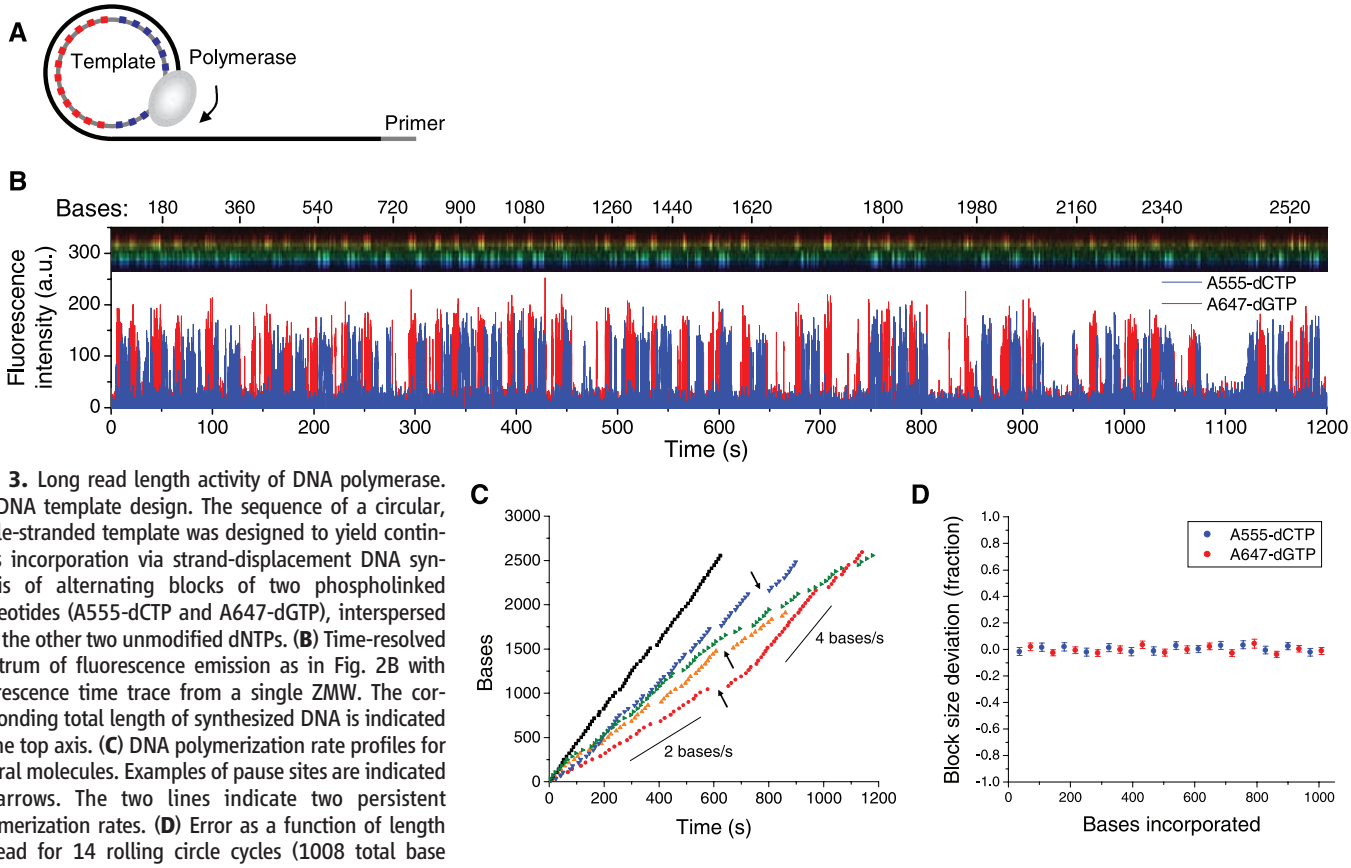

Fig. 3. Long read length activity of DNA polymerase. (**A**) DNA template design. The sequence of a circular, single-stranded template was designed to yield continuous incorporation via strand-displacement DNA synthesis of alternating blocks of two phospholinked nucleotides (A555-dCTP and A647-dGTP), interspersed with the other two unmodified dNTPs. (**B**) Time-resolved spectrum of fluorescence emission as in Fig. 2B with fluorescence time trace from a single ZMW. The corresponding total length of synthesized DNA is indicated by the top axis. (**C**) DNA polymerization rate profiles for several molecules. Examples of pause sites are indicated by arrows. The two lines indicate two persistent polymerization rates. (**D**) Error as a function of length of read for 14 rolling circle cycles (1008 total base incorporations; n = 186 reads). The fractional deviation from the average number of pulses per block (12 A555-dCTP and 12 A647-dGTP observed phospholinked dNTP pulses per cycle, respectively), mean ± SE, is plotted as a function of template position. The 95% confidence interval for the slope is −0.027 to +0.036 blocks per 1008 bases of incorporation.

values. These pause sites corresponded to regions with predicted stable secondary structure in the template and matched well with bulk capillary electrophoresis data (fig. S7). The major pause point seen at position 40 did not result in an increased frequency of dissociation events. The enzymatic rate of incorporation increased immediately after passing through the putative hairpin for experiments performed at 100 nM dNTP (from

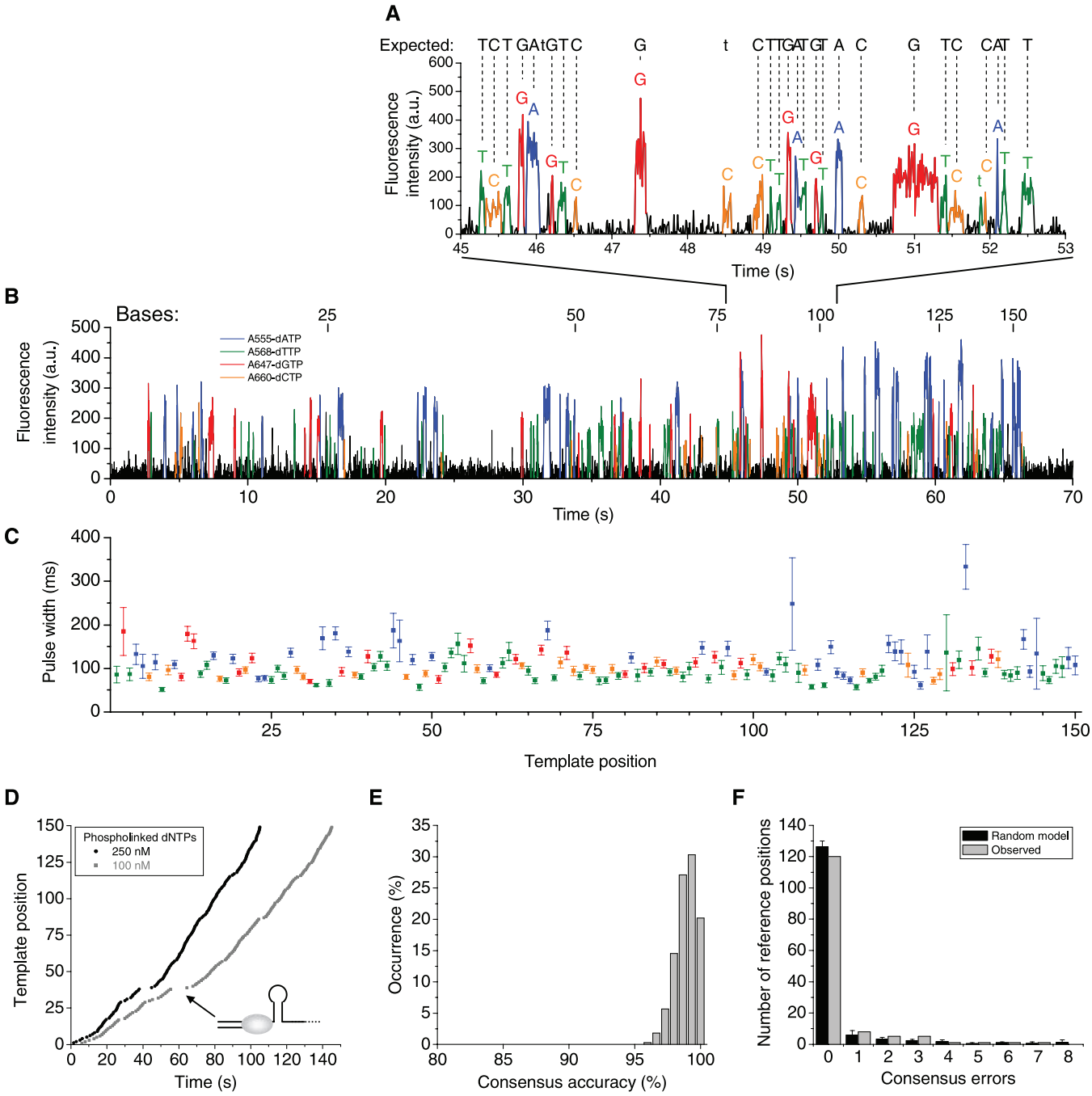

Fig. 4. Single-molecule, real-time, four-color DNA sequencing. (**A**) Total intensity output of all four dye-weighted channels, with pulses colored corresponding to the least-squares fitting decisions of the algorithm. This section of a fluorescence time trace shows 28 bases of incorporations and three errors. The expected template sequence is shown above, with dashed lines corresponding to matches; errors are in lowercase. (**B**) The entire read that proceeds through all 150 bases of the linear template. On average, ~63% of reads proceeded through the entire length of the DNA template. (**C**) Average pulse width as a function of template position (extracted from n = 449 reads). (**D**) Cumulative interpulse duration plotted as a function of template position for two different phospholinked dNTP concentrations (250 nM, n = 449 reads; 100 nM, n = 868 reads). The arrow indicates a pause site observed for both conditions at position 40, corresponding to predicted secondary structure in the template at position 46 (fig. S7), taking into account the enzyme's footprint on the template (*42*). (**E**) Histogram of the sequence accuracy of 100 consensus sequences created by subsampling from 449 single-molecule reads to 15-fold average coverage. The median accuracy of the distribution is 99.3%. (**F**) Observed systematic bias compared with prediction from a random model free of sequence context bias. The error frequencies for observed (gray bars) and bias-free model data (black bars) are plotted in a histogram with the number of errors on the x axis and the number of different reference positions showing this many errors in 100 trials on the y axis. The random model is based on the observed error frequencies (table S3) (*26*).

0.7 to 1.25 bases/s) and at 250 nM dNTP (from 1.1 to 1.5 bases/s). This increased rate resulted from a decrease in interpulse duration; the pulse widths remained nearly constant. It is not surprising that the interpulse durations, which encompass motion of the polymerase relative to the DNA template, would be strongly affected by DNA secondary structure, whereas variations in the pulse widths, which are governed by local chemical processes in the active site, are less affected.

Pulse widths showed only moderate variability with sequence context, and the interpulse durations, although highly dependent on secondary structure, always produced average values above 200 ms. Thus, sequence errors in individual reads should be predominantly uncorrelated and amenable to molecular ensemble averaging. To test this hypothesis, we formed 100 consensus sequences with reads randomly subsampled from the data set to yield 15-molecule coverage, using the center-star algorithm (*39*). The median accuracy over this set of sequences was 99.3%, with a distribution of values shown in Fig. 4E. The consensus accuracy as a function of fold coverage is shown in fig. S8. To explore the possibility of systematic error beyond the fluorophore-dependent error rates (table S3), we analyzed the dependence of consensus error frequency on sequence context via the distribution of the number of times out of the 100 trials that each reference sequence position was reported incorrectly (Fig. 4F) (*26*). This histogram is in agreement with a context bias–free random model, showing that within the sensitivity of this study there were no other biophysical sources of systematic error.

The systematic variations in pulse width and interpulse duration seen in Fig. 4 do not interfere with the development of accurate consensus sequence. In fact, such variations constitute an additional signal that is dependent on DNA primary and secondary structure that can be exploited to increase the accuracy of the consensus. Another appealing feature of this sequencing approach is that, through the strand-displacing capability of the polymerase (demonstrated in Fig. 3), closed circular templates can be sequenced multiple times by a DNA polymerase in a single run. This allows determination of a circular consensus sequence using only one DNA molecule. The resulting insensitivity to sample heterogeneity will greatly improve detection of rare mutations. This single-molecule aspect also enables simplified sample preparation and minimizes reagent consumption because only small amounts of genomic DNA are required.

In addition to the sequence, the real-time aspect of our approach generates unprecedented information about DNA polymerase kinetics that will allow other uses of the technology. Because the system reports the kinetics of every base incorporation through the pulse width and the interpulse duration, the system can be used today to investigate kinetics of DNA polymerization with unprecedented resolution and speed, providing the distribution of kinetic parameters over hundreds of different sequence contexts in a single 5-min experiment. Because polymerase kinetics is sensitive to biological perturbation, our approach would allow investigation of DNA binding proteins, DNA polymerase inhibitors, and the effects of base methylation.

Commercially available high-throughput sequencing systems that rely on stepwise flushing of a solid support with reactants and subsequent scanning to read out a single base currently operate in the regime of ~1 hour per base sequenced (*13*, *14*, *16*). This low rate of sequence production is compensated by high multiplex levels (~10^6 to 10^8). The single-molecule real-time DNA sequencing approach demonstrated here represents an increase in the speed of the underlying sequencing cycle by approximately four orders of magnitude. Stepwise sequencing systems are characterized by relatively short read lengths because of the deleterious effects of interrupting enzyme activity. Exploiting uninterrupted DNA synthesis will enable sequence reads thousands of bases in length.

We have shown that with just 15 molecules, a consensus sequence with 99.3% median accuracy can be formed with no detectable sequence context bias and a uniform error profile within reads. The present level of accuracy can produce alignment and consensus adequate for resequencing applications. However, it would create challenges for de novo assembly or alignment into highly repetitive DNA. The accuracy of the system could be enhanced by improvements in enzyme kinetics. Reducing the free energy of the nucleotide-bound state through polymerase mutation and nucleotide modification would reduce the occurrence of cognate nucleotide dissociation and the attendant insertion errors. Lowering the rate of phosphodiester bond formation would lengthen the pulses, reducing the incidence of deletion errors. Deletions could also be reduced through increases in fluorophore brightness and system optical collection efficiency. Finally, circular consensus sequencing can be used to eliminate stochastic errors in single-molecule sequencing.

The limited experimental multiplex used here could be applied to sequencing small viral and bacterial genomes. Given that each ZMW is capable of producing sequence at a rate greater than 400 kb per day, just 14,000 functioning ZMWs are required to produce a raw read throughput equivalent to 1-fold coverage of a diploid human genome per day. This number is attainable using optics and detector technology available today. Even larger numbers of ZMWs could be simultaneously monitored using multimegapixel charge-coupled device or complementary metal-oxide semiconductor cameras expected within five years (*40*, *41*). As these technologies evolve, it will be possible to provide later generations of this instrument with multiplex commensurate with current stepwise sequencing systems. Combining this level of multiplex with the high intrinsic speed and read length of single-molecule, real-time DNA sequencing will enable low-cost rapid genome sequencing.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1162986/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S3
Movie S1
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Phage-Mediated Intergeneric Transfer of Toxin Genes

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Because bacteriophages generally parasitize only closely related bacteria, it is assumed that phage-mediated genetic exchange occurs primarily within species. Here we report that staphylococcal pathogenenicity islands, containing superantigen genes, and other mobile elements transferred to *Listeria monocytogenes* at the same high frequencies as they transfer within *Staphylococcus aureus.* Several staphylococcal phages transduced *L. monocytogenes* but could not form plaques. In an experiment modeling phage therapy for bovine mastitis, we observed pathogenicity island transfer between *S. aureus* and *L. monocytogenes* in raw milk. Thus, phages may participate in a far more expansive network of genetic information exchange among bacteria of different species than originally thought, with important implications for the evolution of human pathogens.

A major class of bacteriophages, the transducing phages, can package bacterial host DNA and transfer it to new hosts. The frequency of this transfer is dramatically increased for genetic elements that are designed for packaging by phages. At the same time, the ability of a bacterial species to intercept and sample the phage-carried genetic information of foreign species and genera could represent a vast source of new genetic information. However, phages are generally assumed to have narrow host ranges (*1*, *2*), and phage-mediated genetic exchange is considered to be restricted. Given the numbers of extant bacteria and phages, intergeneric phage-mediated genetic exchange is a possibility. Of particular concern is the potential for the phage-mediated transfer of virulence determinants (*3*).

Highly mobile toxin-carrying pathogenicity islands of *Staphylococcus aureus* (SaPIs) are specially adapted to packaging and transfer by particular staphylococcal phages. The SaPIs, which encode toxic shock toxin (TSST-1) and other superantigens, are 14- to 18-kb elements that are inserted at specific chromosomal sites and are induced to excise and replicate by certain temperate phages. After replication, SaPI DNA is packaged into special small infectious particles in numbers similar to those of the plaque-forming phage particles, resulting in extremely high SaPI-specific transfer frequencies (*4*, *5*). Although they are ubiquitous among *S. aureus* strains (*6*), the SaPIs

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Table 1. High-frequency transfer of *S. aureus* pathogenicity islands SaPI1 and SaPIbov1 to *L. monocytogenes.* TRU, TRU/ml; PFU, PFU/ml on RN450. *Sa, S. aureus*. *Lm*, *L. monocytogenes.* JCSA109, SaPI1 Δ*attC*. Strains were transduced with *S. aureus* helper phages 80α, Φ11, ΦNM2, or ΦNM4 carrying SaPI1, SaPIbov1, or pRN0870 (pT181 replicon). There were no TRU observed for cell- or phage-only controls. The results are represented as the ratios of TRU to PFU/ml. %PA, relative phage adsorption, where 0% = no phage bound. Values are means ± SD (n = 3 independent samples). NO, none obtained at the highest phage titer.

			Phage 80α (TRU/PFU)	Phage Φ11 (TRU/PFU)	Phage ΦNM2 (TRU/PFU)	Phage ΦNM4 (TRU/PFU)	Phage 80α (TRU/PFU)	Phage 80α (%PA)
Species	**Serotype**	**Strain**	**SaPI1**	**SaPIbov1**	**SaPI1**	**SaPI1**	**pRN0870**	**–**
Sa	–	RN6734	$8.1 \times 10^{-1} \pm 4.4 \times 10^{-2}$	$1.4 \times 10^{-1} \pm 1.2 \times 10^{-2}$	$3.9 \times 10^{0} \pm 1.7 \times 10^{-1}$	$5.7 \times 10^{0} \pm 8.5 \times 10^{-1}$	$4.2 \times 10^{-2} \pm 3.7 \times 10^{-3}$	77.1 ± 1.3
		JCSA109	$1.6 \times 10^{-1} \pm 1.7 \times 10^{-2}$	$1.9 \times 10^{-1} \pm 2.4 \times 10^{-2}$	$6.6 \times 10^{-1} \pm 1.1 \times 10^{-1}$	$1.1 \times 10^{0} \pm 1.3 \times 10^{-1}$	$3.9 \times 10^{-2} \pm 1.8 \times 10^{-3}$	78.0 ± 1.3
Lm	1/2a	EGDe	$2.8 \times 10^{-1} \pm 1.8 \times 10^{-2}$	$1.1 \times 10^{-2} \pm 2.6 \times 10^{-3}$	$1.6 \times 10^{-1} \pm 1.9 \times 10^{-2}$	$2.0 \times 10^{-1} \pm 3.1 \times 10^{-2}$	$9.3 \times 10^{-3} \pm 3.1 \times 10^{-3}$	54.9 ± 1.7
		SLCC5764	$2.1 \times 10^{-1} \pm 2.0 \times 10^{-2}$	$3.3 \times 10^{-2} \pm 2.1 \times 10^{-3}$	$7.9 \times 10^{-1} \pm 1.8 \times 10^{-1}$	$2.1 \times 10^{-1} \pm 1.0 \times 10^{-2}$	$8.5 \times 10^{-3} \pm 2.3 \times 10^{-3}$	53.1 ± 1.4
	1/2b	F8964	$3.9 \times 10^{-3} \pm 1.7 \times 10^{-4}$	$5.6 \times 10^{-3} \pm 4.4 \times 10^{-3}$	$6.1 \times 10^{-2} \pm 2.5 \times 10^{-3}$	$6.1 \times 10^{-2} \pm 1.0 \times 10^{-2}$	$1.4 \times 10^{-4} \pm 3.2 \times 10^{-5}$	50.0 ± 2.7
		SK1429	$6.2 \times 10^{-4} \pm 1.4 \times 10^{-5}$	$1.4 \times 10^{-3} \pm 1.6 \times 10^{-4}$	$1.3 \times 10^{-2} \pm 2.3 \times 10^{-3}$	$1.1 \times 10^{-2} \pm 1.2 \times 10^{-3}$	$2.8 \times 10^{-5} \pm 1.8 \times 10^{-6}$	42.8 ± 3.1
	1/2c	LO28	$9.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$	$8.1 \times 10^{-3} \pm 7.5 \times 10^{-4}$	$7.8 \times 10^{-3} \pm 2.0 \times 10^{-3}$	$4.5 \times 10^{-3} \pm 9.7 \times 10^{-4}$	$2.3 \times 10^{-4} \pm 3.0 \times 10^{-5}$	54.3 ± 1.7
		WSLC1001	$1.2 \times 10^{-2} \pm 2.0 \times 10^{-3}$	$2.2 \times 10^{-2} \pm 1.1 \times 10^{-2}$	$4.1 \times 10^{-2} \pm 6.9 \times 10^{-3}$	$4.1 \times 10^{-2} \pm 8.6 \times 10^{-3}$	$1.1 \times 10^{-3} \pm 1.8 \times 10^{-4}$	55.3 ± 3.3
	3a	WSLC1002	$1.2 \times 10^{-4} \pm 2.5 \times 10^{-5}$	$1.4 \times 10^{-3} \pm 4.3 \times 10^{-4}$	$2.9 \times 10^{-5} \pm 7.8 \times 10^{-6}$	$4.3 \times 10^{-4} \pm 1.6 \times 10^{-5}$	$5.1 \times 10^{-5} \pm 6.7 \times 10^{-6}$	72.1 ± 2.4
		WSLC1153	$2.4 \times 10^{-4} \pm 1.4 \times 10^{-5}$	$7.6 \times 10^{-3} \pm 2.0 \times 10^{-4}$	$8.2 \times 10^{-5} \pm 1.7 \times 10^{-5}$	$6.3 \times 10^{-4} \pm 7.3 \times 10^{-5}$	$1.3 \times 10^{-6} \pm 2.7 \times 10^{-7}$	72.3 ± 1.2
	3b	SK1351	$1.0 \times 10^{-2} \pm 3.0 \times 10^{-3}$	$7.7 \times 10^{-2} \pm 2.4 \times 10^{-2}$	$1.9 \times 10^{-1} \pm 3.5 \times 10^{-2}$	$1.3 \times 10^{-1} \pm 2.4 \times 10^{-2}$	$3.3 \times 10^{-3} \pm 6.2 \times 10^{-4}$	96.9 ± 1.8
		SK1442	$5.1 \times 10^{-1} \pm 3.1 \times 10^{-2}$	$2.9 \times 10^{-1} \pm 2.4 \times 10^{-2}$	$8.1 \times 10^{0} \pm 1.4 \times 10^{0}$	$5.4 \times 10^{0} \pm 5.5 \times 10^{-1}$	$1.7 \times 10^{-2} \pm 1.8 \times 10^{-3}$	97.8 ± 0.5
	3c	WSLC1032	$4.7 \times 10^{-4} \pm 3.1 \times 10^{-5}$	$6.4 \times 10^{-2} \pm 2.6 \times 10^{-3}$	$4.6 \times 10^{-5} \pm 6.5 \times 10^{-6}$	$5.3 \times 10^{-2} \pm 4.9 \times 10^{-3}$	$1.9 \times 10^{-5} \pm 3.5 \times 10^{-6}$	62.8 ± 2.8
		WSLC1175	$7.6 \times 10^{-5} \pm 1.3 \times 10^{-6}$	$1.1 \times 10^{-3} \pm 6.1 \times 10^{-4}$	$1.7 \times 10^{-6} \pm 7.6 \times 10^{-7}$	$2.9 \times 10^{-3} \pm 4.3 \times 10^{-4}$	$1.8 \times 10^{-6} \pm 2.1 \times 10^{-7}$	58.1 ± 2.2
	4b	F2379	NO	NO	NO	NO	NO	48.3 ± 1.5
		Scott A	NO	NO	NO	NO	NO	41.2 ± 2.2

Chromosome	J_L	SaPI1	J_R	Chromosome	Organism	ORF
TGAAAAATACAATGGTGCAG	**TTATTCCTGCTAAATAA**	TATC…CTAA	**TTATTCCTGCTAAATAA**	ATCATATTAATGGCTCAGTA	*S. aureus*	SAOUHSC_00844*
ACAGTGATAGGTACTAAAAT	TAATGCCTGCTAAATAA	TATC…CTAA	TTATTCTGTCGAAATAA	CTGCTGGATGACCTAATGCT	*S. aureus*	SAOUHSC_00216
TTCTTTAAAGCCATTGAGCT	TCACTCCTGCTAAATAA	TATC…CTAA	TTATTCCTACATAATAA	TATTGTATTCATCATATCAT	*S. aureus*	SAOUHSC_00562
ACAACTAATGATAAGGACTT	TTATTCCTGCTAAATAA	TATC…CTAA	TTATTCCTCTCTAACAA	TTATGTATAAACGTTAATAA	*S. aureus*	SAOUHSC_02482
TTGTCTTTATATTTATTTAC	TTGATCCTGCTAAATAA	TATC…CTAA	TTATTCTGCAGTAATAA	CTGCGTCATCTACTGATTCA	*S. aureus*	SAOUHSC_00927
TGCCGAAATAGAACCTGCAG	TAAATCCTGCTAAATAA	TATC…CTAA	TTATTCCAGCTAAACCA	TCATTTTTCCACCGTGTTAG	*S. aureus*	SAOUHSC_00599
TCTCGCAATATGAGGTGCAA	TTAATCCTGCTAAATAA	TATC…CTAA	TTATTCCTAAAAAGCTA	ATACCACCGACAACTGAAAT	*S. aureus*	SAOUHSC_02427
GTAATTTTATTATTAAACAT	TTCATCCTGCTAAATAA	TATC…CTAA	TTATTCCGCAATATTAA	TATTCGCATCATATGTTCCT	*S. aureus*	SAOUHSC_02963
GTATTTTCATTTGTGCTCTG	TTTTACCTGCTAAATAA	TATC…CTAA	TTATTCCTGTTGAATAA	TTTCTTGCACTTCAGATTCA	*L.monocytogenes*	LMO_0146
GAATATAATGGTTCCTGCAG	TTATTCCTGCTAAATAA	TATC…CTAA	TTATTCCTGACGATATA	CCTGCGACGGAGCCAAATAT	*L.monocytogenes*	LMO_0195
GCGCTACCTTCAAAAACGAG	CTTTTCCTGCTAAATAA	TATC…CTAA	TTATTCCTCGTCAATAA	TTCCGATATTCGTAAAACTT	*L.monocytogenes*	LMO_0439
TGAAAGAAGAAAATGTAAAA	TTATTCCTGCTAAATAA	TATC…CTAA	TTATTCCTTCTATGTAA	TCCACAAAATCCTGGTGGAC	*L.monocytogenes*	LMO_2370
CGAAAGAATTTGAGTTATTA	TTATTCCTGCTAAATAA	TATC…CTAA	TTATTCCTGCCGAATCA	TCGTGGCAAAGTTTTCTCTA	*L.monocytogenes*	LMO_2500
GCAAGTCAACCAAATTGAAG	TTAATCCTGCTAAATAA	TATC…CTAA	TTATTCCTTTCCAACAA	CAGACAGCGAATCTAGCTAT	*L.monocytogenes*	LMO_2592
CTACCCTTAACTATTTTCGA	TTATTCCCCCTAAATAA	TATC…CTAA	TTATTCCTGCTAGGTAC	AGTATCTTATTTGTAATTAA	*L.monocytogenes*	LMO_2773

Fig. 1. Alternative SaPI1 insertions in *S. aureus* and *L. monocytogenes.* The chromosomal locations of SaPI1 insertions are taken from the sequenced genomes of *S. aureus* 8325 and *L. monocytogenes* EGDe. The wild-type primary SaPI1 *attC* is precisely duplicated upon integration and is indicated in bold. The left and right insertion junctions (J_L and J_R) are underlined and they indicate the hybrid SaPI1 attachment sites created from integration into a secondary attachment site. Red boxes indicate mismatches with the wild-type primary SaPI1 *attC*. Gray boxes indicate mismatches with the flanking chromosomal regions. *S. aureus* SAOUHSC_00844* is the chromosomal location of the wild-type primary SaPI1 *attC*. ORF, open reading frame.

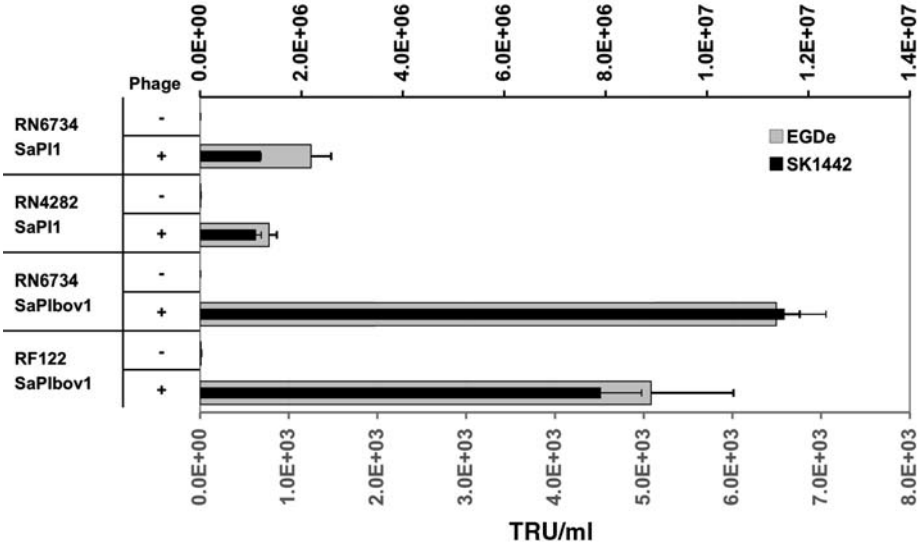

Fig. 2. Phage treatment of *S. aureus* promotes transfer of SaPI to *L. monocytogenes* in milk. *S. aureus* wild-type RN6734 carrying SaPI1 or SaPIbov1, clinical isolate RN4282 (RN6938) carrying SaPI1, or clinical isolate RF122 carrying SaPIbov1 (RN9749) were co-cultured with streptomycin-resistant variants of *L. monocytogenes* strains EGDe and SK1442 and phage 80α [10^7 plaque-forming units (PFU)/ml] in milk for 3 hours at 30°C. The samples were adjusted to 100 mM sodium citrate and plated for SaPI transductants, with selection against the donor strains. The cryptic phages of RN4282 and RF122 do not confer resistance to phage 80α. TRU, transduction units. "+" indicates the addition of phage. The results are represented as TRU/ml. There were no TRU observed for "–" RN6734 samples and phage-only controls. The "–" RN4282 and RF122 samples were below 100 TRU/ml. Values are means ± SD (n = 3 independent samples). Results and units for EGDe-S and SK1442-S are in gray and black, respectively.

are extremely rare in non-*aureus* staphylococci and have yet to be described in other bacterial genera (*7*), despite their great mobility.

We analyzed the sequence specificity of the SaPI1 insertion site by making an *attC* deletion, which was then used as the recipient for the transduction of a detoxified derivative of SaPI1 marked with tetracycline resistance (*tetM*). Although we expected SaPI1 transfer to the Δ*attC* mutant to be rare, we found that phage 80α (*4*) transferred SaPI1 to the Δ*attC* strain at nearly the same frequency as to the parental strain, with an intact *attC* site (Table 1). Southern blots of the Δ*attC* transductants indicated single integrations in a variety of secondary sites (fig. S1). Sequencing of SaPI1 insertion junctions (Fig. 1) confirmed that all the secondary integrations involved single crossovers with the SaPI1 element *attS* site, indicating that the secondary insertions were integrase-mediated events. An SaPI1 mutant lacking integrase could not be transduced either to the $attC^+$ strain (*8*) or to the Δ*attC* strain (table S1).

Alignment of the secondary sites revealed that they bore only a modest resemblance to the primary *attC* site; however, a cytosine pair at the putative recombination crossover point was always present (fig. S2). These results implied that SaPI1 integrase had much lower sequence specificity than other typical integrases, such as that of coliphage lambda, where transduction frequencies are reduced by more than 200-fold in the absence of the primary attachment site (*9*). The frequency and variability of secondary sites in *S. aureus* increase the probability that suitable insertion sites are present in many bacterial species, and any bacterium that can adsorb SaPI helper phage is a potential recipient.

We tested a diverse set of Gram-positive bacterial species as recipients for transduction with a detoxified derivative of SaPI1 (SaPI1-*tst::tetM*). Rare SaPI transduction to *S. epidermidis* and *S. xylosus* had been reported previously (*7*) and was confirmed for *S. xylosus* (table S2). With the striking exception of *Listeria monocytogenes*, transfer to other genera was not detected (table S3). Not only was SaPI1 transferred to *L. monocytogenes* efficiently, but also at the high frequencies observed with *S. aureus* (Table 1). Of the representative strains of *L. monocytogenes* serotypes that we tested, only those of serotype 4b were not transduced. Because the sequenced genomes of *L. monocytogenes* do not appear to have *attC* sites of SaPI1 or SaPIbov1 or even close variants, integration in secondary attachment sites was most likely. Sequencing of SaPI1 insertion joints in transductants of strain EGDe identified chromosomal *L. monocytogenes* DNA flanking integrated SaPI1 at several sites (Fig. 1 and fig. S3), confirming that the alternative SaPI attachment sites in *L. monocytogenes* are similar to those in *S. aureus* and that the SaPI1 integrase catalyzes these insertions [supporting online material (SOM) text].

To evaluate the generality of SaPI transfer to *L. monocytogenes*, we tested other mobile genetic elements and found that several were transferred efficiently. An additional SaPI, the genotypically distant SaPIbov1 (*10*, *11*), and a detoxified derivative (SaPIbov1-*tst::tetM*) (*10*) gave results similar to those with SaPI1 (Table 1). Furthermore, SaPI mobilizing (helper) phages φ11 (*12*), φNM2, and φNM4 (*13*) transferred SaPI elements as efficiently as 80α (Table 1).

Standard phage susceptibility tests are based on plaque formation, but none of the four phages

formed plaques on any of the seven serotypes of *L. monocytogenes* tested (table S4). Thus, the true overall host range of a phage may be much wider if it includes infection without plaque formation, which can be assessed only by gene transfer or phage DNA delivery. To distinguish whether the variation in transduction frequency was due to phage adsorption or host DNA restriction, we measured helper phage adsorption by *L. monocytogenes*. Serotypes 1/2a and 3b exhibited phage adsorption frequencies comparable to that of the staphylococcal control and were the strains most amenable to transduction, indicating there was a correlation between phage adsorption and transfer. This also implied that DNA restriction was not an important determinant of transduction frequency for these serotypes.

We then tested for the transfer of a virulence determinant unrelated to the SaPIs and used a detoxified derivative of phage φSLT (*14*) containing a tetracycline-resistance marker (*tetM*) inserted into the Panton-Valentine leukocidin (PVL) locus (*15*). Although ΦSLT generates lysates with relatively low titers, its transfer to *L. monocytogenes* was demonstrated by selection for the *tetM* marker (table S5). In contrast to SaPI transfer, this transduction requires lysogenization because PVL is carried in the φSLT genome. Similarly to the four SaPI helper phages, ΦSLT did not form plaques on *L. monocytogenes* (table S4), and converted strains did not liberate detectable plaque-forming phage particles upon mitomycin C induction. The potential for environmental ΦSLT transduction to *L. monocytogenes* is disconcerting, considering that PVL has been implicated in staphylococcal diseases (*15*). All the staphylococcal phages we tested mediated genetic transfer to *L. monocytogenes.*

We predicted the occurrence of phage-mediated SaPI transfer in an environment in which *S. aureus* and *L. monocytogenes* occur together. These species are common causes of bovine mastitis (*16*–*18*), and we analyzed cow's milk as a medium for spontaneous prophage induction and SaPI1 transduction. When detoxified *S. aureus* derivatives of laboratory and clinical isolates were co-cultured with *L. monocytogenes* strains in raw milk, we detected spontaneous prophage induction and transfer of SaPI1 and SaPIbov1 to *L. monocytogenes* (SOM text and table S6).

Bovine mastitis costs the world's dairy industries billions in revenue each year; roughly 11% of total production is lost annually (*19*). Of the mastitis pathogens, *S. aureus* is of particular concern because of the low cure rate with antibiotic treatment and the rapid rise of antibiotic-resistant strains (*20*). A promising alternative to antibiotic treatment of *S. aureus* infections is phage therapy, which is currently the focus of several clinical trials for bovine mastitis (*21*–*24*). To determine whether this strategy could promote SaPI transfer, we co-cultured *S. aureus* strains carrying detoxified SaPIs with streptomycin-resistant derivatives of *L. monocytogenes* strains EGDe and SK1442 in raw milk, adding SaPI-less phage 80α (propagated on RN450). As expected, high titers of phage 80α were efficient at clearing the *S. aureus* strains (table S7); however, the phage particles resulting from lysis also promoted the transfer of SaPI1 and SaPIbov1 to *L. monocytogenes* (Fig. 2).

Although superantigen-producing *L. monocytogenes* strains have not yet been reported, it is certainly true that environmental isolates of *S. aureus* carrying SaPIs are ubiquitous. Thus, the widespread use of anti-*aureus* phages in agriculture may accelerate the spread of staphylococcal toxins to *Listeria* or to any other bacteria to which the phages can adsorb (SOM text).

Phages form the framework for a living network of genetic information, interconnecting the microbes of the biosphere. This study hints that there is a pipeline of silent phage-mediated genetic information transfer among bacteria, indicating that phages are involved in far more numerous microbial connections than previously imagined.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5910/139/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S9
References

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Stable Introduction of a Life-Shortening *Wolbachia* Infection into the Mosquito *Aedes aegypti*

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Most pathogens require a relatively long period of development in their mosquito vector before they can be transmitted to a new human host; hence, only older insects are of epidemiological importance. The successful transfer of a life-shortening strain of the inherited bacterial symbiont, *Wolbachia*, into the major mosquito vector of dengue, *Aedes aegypti*, halved adult life span under laboratory conditions. The association is stable, and the *Wolbachia* strain is maternally inherited at high frequency. It is capable of inducing complete cytoplasmic incompatibility, which should facilitate its invasion into natural field populations and its persistence over time. Our data suggest that targeting mosquito age with inherited *Wolbachia* infections may be a viable strategy to reduce the transmission of pathogens such as dengue viruses.

The control of dengue primarily targets *Aedes aegypti*, a domesticated mosquito that prefers to live in and around human habitation (*1*). With few exceptions, dengue management strategies have been complicated by the inability to completely eradicate *A. aegypti* from urban settings and the ineffective application of long-lasting vector-control programs (*2*). This has led to a worldwide resurgence of dengue and has highlighted the urgent need for novel and sustainable disease-control strategies.

Most pathogens that are transmitted by mosquitoes share a common property; they have to undergo a significant period of development in their insect vector before they can be transmitted to a new host. After a female mosquito ingests an

infectious blood-meal, parasites or arboviruses, such as dengue, penetrate the mosquito's midgut and replicate in various tissues before infecting the salivary glands, where they are transmitted to a new host during subsequent blood-feeding. This time period from pathogen ingestion to potential infectivity is termed the extrinsic incubation period (EIP) and lasts ~2 weeks for both dengue (*3*, *4*) and malaria (*5*).

A female mosquito must survive longer than its initial nonfeeding period (usually less than 2 days) plus the EIP to successfully contribute to pathogen transmission. Mosquito survival is therefore considered a critical component of a vector population's capacity for pathogen transmission (*6*). Interventions that aim to reduce the daily survivorship of adult mosquitoes, such as the spraying of residual insecticides in houses and insecticide-treated bed nets for malaria control, yield large reductions in pathogen transmission rates (*7*, *8*), because of the sensitive relationship between mosquito survival and vectorial capacity (*9*, *10*).

A strain of the obligate intracellular bacterium *Wolbachia pipientis*, *w*MelPop, has been described that reduces adult life span of its natural fruit fly host *Drosophila melanogaster* (*11*). It has been proposed that life-shortening *Wolbachia* strains, such as *w*MelPop, might be used to skew mosquito population age structure toward younger individuals, thereby reducing pathogen transmission without eradicating the mosquito population (*12*, *13*). *Wolbachia* are maternally inherited bacteria that use mechanisms such as cytoplasmic incompatibility (CI), a type of embryonic lethality that results from crosses between infected males with uninfected females, to rapidly spread into insect populations (*14*). Evidence from other *Wolbachia*-insect associations, suggests that CI could allow *Wolbachia* strains, such as *w*MelPop, to invade mosquito populations even if they confer a fitness cost such as increased mortality (*15*). Current models predict that this strategy may result in significant reductions in pathogen transmission (*16*, *17*). However, life-shortening *Wolbachia* strains do not occur in mosquitoes naturally.

To facilitate the transfer of the life-shortening *Wolbachia* strain *w*MelPop that infects *D. melanogaster* (*11*) into the mosquito *A. aegypti*, we adapted the bacteria by continuous serial passage in mosquito cell culture for 3 years (*18*, *19*). A consequence of this culturing was a reduction in growth rates and associated virulence when transferred back into *Drosophila* (*19*). We purified the mosquito cell line–adapted isolate of *w*MelPop and microinjected it into naturally uninfected *A. aegypti* embryos (JCU strain). Surviving adult females were isolated and blood-fed, and then, after egg laying, we assayed them for *Wolbachia* infection using diagnostic PCR (*18*, *19*). Eight independent isofemale lines carrying the *w*MelPop infection were generated. Six of these lines were lost from G_1 to G_3 (supporting online text), and the remaining two lines formed stable associations. These two lines, "PGYP1" and "PGYP2" were chosen for further characterization and, after a period of experimental selection, have remained persistently infected by *w*MelPop (100% infection frequency) until G_{33} and G_{30} respectively, when last assayed (fig. S1).

In *Drosophila* species, *w*MelPop shortens the life span of adult flies by up to 50% (*11*, *20*). We performed several life-span assays in *A. aegypti* for a range of experimental conditions. As *w*MelPop-induced early death in *Drosophila* is temperature-sensitive (*11*, *21*), we compared the life span of the newly generated *w*MelPop-infected PGYP1 line to the naturally uninfected JCU strain at 25°C and 30°C (Fig. 1, A and B).

In contrast to *Drosophila,* where the life-shortening phenotype is weakly expressed at 25°C and strongly at 30°C, rapid mortality of PGYP1 mosquitoes (G_6) relative to the uninfected parental JCU strain was observed at both temperatures. Under laboratory conditions at 25°C and 80% relative humidity (RH) (Fig. 1A), the median adult longevity for PGYP1 females of 27.0 days was significantly different from the JCU control of 61.0 days (log-rank statistic 11.67, $P < 0.0001$). A similar trend was observed for males (Fig. 1A). At a higher temperature of 30°C and 80% RH

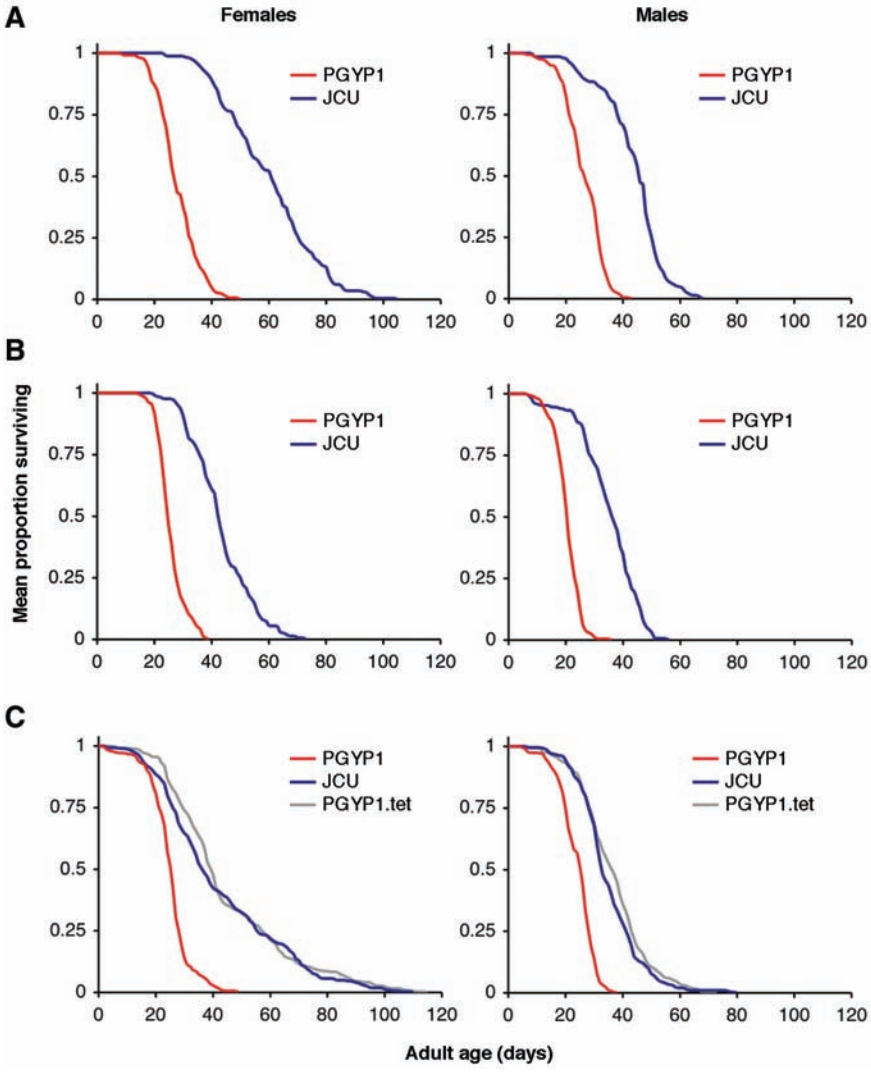

Fig. 1. Survival of *w*MelPop-infected PGYP1 *A. aegypti* (red lines) compared with the naturally uninfected JCU (blue lines) and tetracycline-cleared PGYP1.tet (gray lines) strains. Life-span assays were initially conducted at G_6 post transinfection by the comparison of PGYP1 and JCU strains at 25°C (**A**) and 30°C (**B**). For each strain, six replicate groups of 50 mosquitoes (25 of each sex) were maintained in an incubator at their respective test temperature, and 80% RH. (**C**) Subsequently, after tetracycline treatment at G_{13} post transinfection, survival of PGYP1 was compared with that of PGYP1.tet and JCU strains in larger cages under insectary conditions. For this assay, three replicate 30 by 30 by 30 cm cages of 200 mosquitoes (100 of each sex) were maintained for each strain at 25 ± 1°C, 70 to 90% RH, 12:12 hours light:dark. In all three experiments, mosquitoes were provided with 2% sucrose, and cages were checked daily for mortality.

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(Fig. 1B), the differential effect on median adult longevity was still apparent, although the life span of all the mosquitoes was reduced: females PGYP1, 25.0 days; JCU, 43.0 days (log-rank statistic 11.50, $P < 0.0001$).

To examine the effect of the *w*MelPop infection under more biologically realistic conditions, we exposed a cohort of PGYP1 (G_9) and JCU strains to a fluctuating temperature and humidity regime and provided female mosquitoes with daily access to a human blood meal (fig. S2). Under these conditions, the life span of PGYP1 females was reduced by more than half, relative to JCU females. Median longevity was significantly different between treatments: PGYP1, 21.0 days; JCU, 50.0 days (log rank statistic, 10.13, $P < 0.0001$). A smaller difference in median survival times was observed for males from both strains (PGYP1, 9.0 days; JCU, 10.0 days), although overall PGYP1 males still died at a significantly faster rate than JCU males (log-rank statistic = 3.34, $P = 0.0009$).

To exclude the possibility that observed reductions in life span resulted from genetic drift during the establishment of the PGYP1 strain, we generated an uninfected strain from PGYP1 (PGYP1.tet) by addition of the antibiotic tetracycline to the adult diet (*22*). After antibiotic curing of the *w*MelPop infection (*18*), no significant differences in the rate of mortality were observed between females or males of uninfected PGYP1.tet and JCU strains (e.g., females, log-rank statistic = 1.23, $P = 0.2191$). Both females and males from the PGYP1 (G_{13}) strain had a significantly reduced life span when compared with those from the PGYP1.tet strain (e.g., females, log-rank statistic = 13.70, $P < 0.0001$), indicative of *w*MelPop-induced life-shortening (Fig. 1C). These results were confirmed by using identical assays with the PGYP2 (G_{15}) strain as a biological replicate (fig. S3).

To test for CI, we made crosses between the PGYP1 and wild-type JCU and PGYP1.tet strains and measured egg hatch rates. Consistent with the induction of strong CI in *A. aegypti* (*23*), no eggs hatched from more than 2500 embryos obtained from crosses between male PGYP1 (G_9) and uninfected JCU females (Fig. 2A). Similarly, only 2 eggs hatched from more than 1900 embryos obtained from crosses between male PGYP1 (G_{13}) and the tetracycline-cleared PGYP1.tet females (Fig. 2B). In both assays, PGYP1 females were capable of rescuing CI, as indicated by the high egg hatch seen in PGYP1 × PGYP1 crosses.

In its natural *D. melanogaster* host, *w*MelPop infection induces CI that quickly diminishes with male age (*24*). This effect could slow the invasion of the strain into natural populations. Crosses between uninfected *A. aegypti* females and *w*MelPop-infected males up to 17 days old resulted in a complete absence of egg hatch from more than 9500 embryos (Table 1), which indicated that *w*MelPop infection induced CI that is insensitive to male age.

Overall, no significant differences in fecundity between PGYP1, PGYP1.tet, and JCU strains were observed at G_{13} after transinfection (fig. S4). An evaluation of CI and reproductive fitness in PGYP2 at G_{16} revealed that the *w*MelPop infection induced very strong CI but, unlike PGYP1, had a 19% fecundity cost when compared with its tetracycline-cleared counterpart (fig. S5). In *D. simulans*, fecundity costs associated with the *w*MelPop infection were initially high after transinfection, but subsequently attenuated, whereas the life-shortening effect remained stable (*20*). Further studies are required to determine whether this will be the case for PGYP2, and whether observed differences in reproductive fitness between PGYP1 and PGYP2 are related to *Wolbachia* or host genotypes.

High maternal inheritance of *Wolbachia* from infected females to their progeny is a key parameter for successful population invasion. The maternal transmission rate predicts stable prevalence of the infection once it has invaded a population under the action of CI (*14*). To estimate maternal transmission rates of *w*MelPop over the life span of *A. aegypti*, we used the polymerase chain reaction to determine the proportion of *Wolbachia*-infected progeny derived from the first and third reproductive cycles of PGYP1 females (G_{17}) mated with uninfected wild-type JCU males. Of the 515 larvae screened from 31 females (~17 larvae sampled per female) from the first reproductive cycle (females aged 9 days old), 99.74 ± 0.26% were infected. This estimate of maternal inheritance was not significantly different from that obtained from the third reproductive cycle (females aged 23 days old) in which 527 larvae were screened from five cohorts of 20 females (~105 larvae sampled per cohort) and were 99.45 ± 0.37% infected (Mann-Whitney rank sum test, $P = 0.208$).

In other *Wolbachia*-insect associations, strong CI, high maternal inheritance, and low fecundity costs facilitate the initial spread and subsequent maintenance of the infection at high prevalence in populations (*14*). A comparison of results from this study, with simulations from recent theoretical models that examine the potential of life-shortening *Wolbachia* to modify mosquito population age structure (*16*, *17*), suggest that *w*MelPop should be able to initiate a population invasion of *A. aegypti*. Given the relation between mosquito survival and vectorial capacity (*9*, *10*), if the longevity of adult *A. aegpyti* can be approximately halved under field conditions, as observed in our laboratory experiments, then the introduction of life-shortening *Wolbachia* strains would be predicted to reduce pathogen transmission and the incidence of human disease. Nevertheless, field cage trials conducted

Table 1. Effect of male age on CI. Percent embryo hatch ± SEM and number of replicate crosses (in parentheses) are shown for incompatible crosses between uninfected PGYP1.tet females and aged PGYP1 males, as well as control crosses with aged PGYP1.tet males (minimum 2700 embryos total counted per cross).

Cross (female × male)	Percent embryo hatch for male age of		
	3 days	10 days	17 days
PGYP1.tet × PGYP1	0.00 ± 0.00 (32)	0.00 ± 0.00 (35)	0.00 ± 0.00 (35)
PGYP1.tet × PGYP1.tet	86.86 ± 3.42 (34)	83.67 ± 2.07 (33)	88.30 ± 3.10 (32)

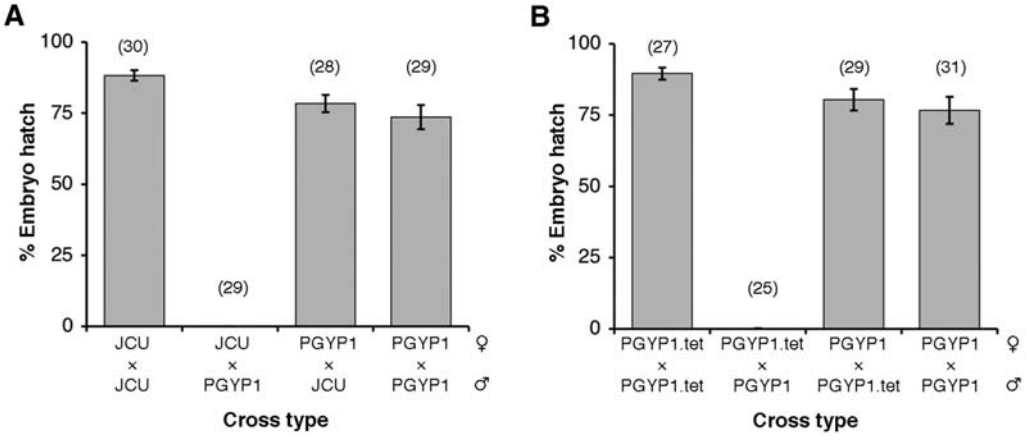

Fig. 2. *Wolbachia*-mediated CI resulting from crosses of the *w*MelPop-infected PGYP1 *A. aegypti* strain with (**A**) the naturally uninfected JCU and (**B**) tetracycline-cleared PGYP1.tet strains. Female parents are listed first in each cross. Results are mean percent embryo hatch ± SEM (minimum 1400 embryos total counted per cross), and number of replicates for each of the four cross types are shown in parentheses. Crosses were conducted as described (*18*).

under seminatural conditions are needed to gain a quantitative estimate of the potential efficacy of this strategy.

Vertically inherited parasites like *Wolbachia* are predicted to evolve toward reduced virulence over time (*25*). Unlike chemical insecticides, biological agents that induce mortality in late life, such as *w*MelPop or entomopathogenic fungi are expected to impose relatively weak selection pressures for the evolution of resistance (*26*). This is because the majority of individuals in the population are able to initiate several reproductive cycles before death, minimizing costs to reproductive output. Moreover, since the initial description of *w*MelPop in *D. melanogaster* over 10 years ago, no signs of resistance to life-shortening have emerged in laboratory stocks.

Hence, the ability of *Wolbachia* to spread into *A. aegypti* populations and persist over time may provide an inexpensive approach to dengue control, particularly in urban areas that are less amenable to conventional vector control strategies. Given the ability of *w*MelPop to induce life-shortening and CI in a range of insect hosts, this strategy may be broadly applicable to reduce pathogen transmission by other insect disease vectors of medical or agricultural importance.

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2009 Annual Meeting

Our Planet and Its Life: Origins and Futures

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Dear Colleagues,

The 2009 AAAS Meeting will bring together an exceptional array of speakers addressing some of the most crucial and timely areas of science, technology, and engineering.

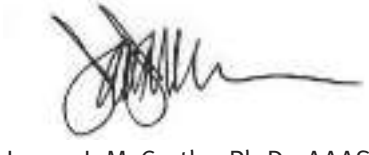
The meeting's theme — Our Planet and Its Life: Origins and Futures — recognizes that 2009 is the 200th anniversary of Charles Darwin's birth and the 150th anniversary of the publication of his book, *On the Origin of Species by Means of Natural Selection*. New understanding of the processes that fascinated Darwin continues to be the focus of intense research 150 years later. Indeed every discipline can demonstrate its own unique evolutionary path and speculate on where it may lead.

Attendees from more than 50 countries will have the opportunity to choose among a broad range of activities, including nearly 175 symposia, seminars, and career development workshops as well as plenary and topical lectures by some of the world's leading scientists and engineers.

Typically the meeting includes thousands of participants and hundreds of members of the international and national media. It offers unparalleled networking opportunities. More details can be found at www.aaas.org/meetings, including how sustainability science, an emerging field, has found a home.

The Annual Meeting reflects tremendous efforts from the AAAS sections, divisions, and committees, which we gratefully acknowledge. I also extend a personal thanks to the members of the Scientific Program Committee who reviewed and assembled the many excellent ideas and proposals into this outstanding meeting.

I urge you to join us in Chicago,



James J. McCarthy, Ph.D., AAAS President and
Alexander Agassiz Professor of
Biological Oceanography, Harvard University

President's Address

James J. McCarthy

AAAS President; Alexander Agassiz Professor of Biological Oceanography, Harvard University, Cambridge, Mass.

McCarthy received his Ph.D. degree from Scripps Institution of Oceanography and B.S. degree in biology from Gonzaga University. He teaches courses in ocean and climate science and oversees Harvard's program in Environmental Science and Public Policy. His research interests relate to marine plankton, biogeochemical cycles, and climate. He has served on and led many national and international groups charged with planning and implementing studies of global change, including chair of the international scientific committee that establishes research priorities and oversees implementation of the International Geosphere–Biosphere Program from 1986 to 1993; founding editor for the American Geophysical Union's *Global Biogeochemical Cycles;* co-chair of the Intergovernmental Panel on Climate Change (IPCC), Working Group II, which had responsibilities for assessing impacts of and vulnerabilities to global climate change for the Third IPCC Assessment (2001); lead author of the Arctic Climate Impact Assessment; and vice-chair of the Northeast Climate Impacts Assessment. He has been elected a fellow of AAAS and the American Academy of Arts and Sciences and a foreign member of the Royal Swedish Academy of Sciences.

President's Reception: Immediately following

Plenary Speakers

Sean B. Carroll

Professor of Molecular Biology and Genetics, University of Wisconsin, Madison

Remarkable Creatures: Epic Adventures in the Search for the Origins of Species

Until recently, scientists studying evolution relied on fossil records and morphology to painstakingly piece together a picture of how animals evolved. Today, scientists are now using DNA evidence collected from modern animals to find new clues. Molecular biologist Sean Carroll focuses on the way new animal forms have evolved, and his studies of a wide variety of animal species have dramatically changed the face of evolutionary biology. Major discoveries from his laboratory have been featured in *Time, US News & World Report, The New York Times, Discover,* and *Natural History.* Carroll is the author of *Endless Forms Most Beautiful* (2005) which was a finalist for the *Los Angeles Times* Book Prize, and *The Making of the Fittest* (2006) which won the Phi Beta Kappa Science Book Award. His most recent book, *Remarkable Creatures: Epic Adventures in the Search for the Origins of Species,* will be published in 2009. He is a member of the National Academy of Sciences and an AAAS Fellow. He received his bachelor's degree at Washington University and his Ph.D. degree in immunology from Tufts University.

Susan W. Kieffer

Center for Advanced Study Professor of Geology and Physics, and Walgreen University Chair, University of Illinois, Urbana-Champaign

Celebrating the Earth: Its Past, Our Present, A Future?

Planetary scientist Susan Kieffer has degrees in math, physics, geology, and planetary science, which is apparent in the interdisciplinary nature of her work. She is internationally renowned and a leading authority on the mechanisms of meteorite impact, geyser dynamics, volcanic eruptions, and river floods. She was the first scientist to describe the physics and chemistry involved in the eruptions on Jupiter's moon Io, the lateral blast associated with the eruption of Mt. St. Helens, the dynamics of Old Faithful as seen by a micro video camera lowered into the geyser between violent eruptions, and the hydraulics of the rapids of the Colorado River. With colleagues, she described the dynamics of the Chixculub meteor impact that caused vaporization of limestone, which resulted in massive amounts of carbon dioxide in the atmosphere and ultimately resulted in a major extinction event 65 million years ago. Kieffer is a member of the National Academy of Sciences, a MacArthur Fellow, and has received numerous awards and honors. She attended Caltech, University of Colorado, Boulder, and Allegheny College.

Svante Pääbo

Director of the Department of Genetics, Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany

A Neanderthal Perspective on Human Origins

A biologist specializing in evolutionary genetics, Svante Pääbo is known as one of the founders of paleogenetics, a discipline that uses the methods of genetics to study early humans and other ancient populations. He is conducting some of the most exacting work ever attempted on the DNA of human and nonhuman primates. His track record of discoveries began in 1985 when he isolated DNA from a 2,400-year-old Egyptian mummy. In 2006, after decoding fragments of DNA from the remains of Neanderthal, he announced plans to reconstruct the entire genome. In 1992, he received the Gottfried Wilhelm Leibniz Prize of the Deutsche Forschungsgemeinschaft, which is the highest honor awarded in German research. Pääbo's department in August 2002 published findings about the evolution of the "language gene," FOXP2, which is lacking or damaged in some individuals with language disabilities. He was born in Stockholm and earned his Ph.D. degree from Uppsala University. He is a member of the National Academy of Sciences.

Topical Lecture Series

Colin F. Camerer
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Ekaterina Dadachova
Sylvia and Robert Olnick Faculty Scholar in Cancer Research, and Associate Professor of Nuclear Medicine and Microbiology and Immunology, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY
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Marjorie I. and Bernard A. Mitchell Professor and Chair of the Department of Human Genetics, University of Chicago, Ill.
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A History of the International Scientific Conference

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Elizabeth Loftus
Distinguished Professor, University of California, Irvine
Illusions and Delusions of Memory

Topical Panel

North-South Scientific International Cooperation—Meeting Global Challenges

Lord Martin Rees, President of the Royal Society, Master of Trinity College, and Professor of Cosmology and Astrophysics, University of Cambridge, U.K.

József Pálinkás, President, Hungarian Academy of Sciences, Budapest *(Invited)*

Jacob Palis, President, Academy of Sciences for the Developing World, Rio de Janeiro, Brazil *(Invited)*

Seminar Tracks

Day-long seminars address topics at the intersection of science and society: assessing and responding to climate change, human evolution, and nanotechnology

Assessing and Responding to Climate Change

Equity, Sustainability, and Governance of Mixed-Use Landscapes
Organized by Ashwini Chhatre, University of Illinois, Urbana-Champaign
Sustainability has emerged as a necessary objective of policy interventions. The future of life on Earth depends on our ability to devise governance systems that guide nature-society interactions toward more sustainable trajectories. Moving beyond the role of institutions in dealing with trade-offs among competing land uses along different outcome dimensions — income generation, biodiversity conservation, ecosystem services provision, greenhouse gas emissions, and carbon sequestration — speakers will discuss the challenge of devising complex multi-level governance systems for mixed-use landscapes.

Risky Business: Assessing and Dealing with Extremes in a Changing Climate
Organized by Claudia Tebaldi, Climate Central, Palo Alto, Calif.
Extreme events are arguably the most crucial aspect of climate change, threatening to have the largest impacts on social and natural systems. They pose tough questions, often with heavy financial and legal implications, about the distinction between natural and human causes. This

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session explores what can and cannot be reliably said about the influence of global warming on several aspects of extreme events: hurricanes, temperature and precipitation extremes over North America, and the attribution of specific historic events to human-caused warming. Speakers will discuss what kind of scientific information can help us better understand past, present, and future patterns of extreme events, while taking steps to protect our resources and adapt to a dynamic climate.

U.S. Cities: Responding to Concerns About Climate Change
Organized by Donald J. Wuebbles, University of Illinois, Urbana-Champaign
Cities cover only 0.4 percent of the Earth's surface but generate the bulk of the world's emissions of carbon dioxide, making urban areas key to alleviating the concerns about global warming. Many cities are already taking action by developing climate adaptation and mitigation strategies for their own communities. Several efforts are already underway to attack the climate issue, for example, by enhancing urban planning, reexamining city policies, improving energy efficiency, and reevaluating local transportation systems. The green roofs in Chicago are one such response. Speakers will discuss the ongoing efforts within U.S. cities toward adaptation and mitigation of climate change.

Human Evolution

The Evolution of Human Diets
Organized by Matt Sponheimer, University of Colorado, Boulder
Recent changes in human diet have been implicated in the etiology of modern diseases including Type II diabetes, arteriosclerosis, and several forms of cancer. As a result, many have argued that our dietary recommendations should be informed by our knowledge of the feeding behavior of human ancestors and our close primate kin. In this session, researchers will examine the evolution of human diets through the lenses of archaeology, morphology, biogeochemistry, ethology, genetics, and energetics. Assembling scientists who address similar questions in different ways will underscore areas of growing consensus and controversy and in so doing should considerably advance our knowledge of hominin dietary adaptations.

The Origin of the Human Species
Organized by Leslie C. Aiello, Wenner-Gren Foundation for Anthropological Research, New York City
In *On the Origin of Species by Means of Natural Selection,* Charles Darwin famously said that "light will be thrown on the origin of man and his history." Although there were no widely accepted human fossils at the time of publication (1859), today there are more than 20 fossil hominin species spanning over 6 million years of prehistory. This session brings together leading international experts to discuss what the extensive fossil and archaeological record can tell us about six major periods in human biological and cultural evolution.

Nanotechnology

Driving Beyond Our Nano-Headlights?
Organized by Joel G. Pounds, Pacific Northwest National Laboratory, Richland, Wash.
Nanotechnology has enormous potential to benefit society and the economy. It also might yield unanticipated, negative environmental change. DDT had long-term ecological side effects that were not understood until organisms failed to adapt. And, commercial development of genetically modified organisms was delayed by the perception that risks from these organisms outweighed their benefits. Speakers will explore where the science of nanotoxicology is heading, the challenges in understanding and predicting long-term effects, approaches to nanotoxicological research, and the policy framework required.

From Donuts to Drugs: Nano-Biotechnology Evolution or Revolution?
Organized by Rodney A. Hill, University of Idaho, Moscow
The foods we eat and the drugs we take in the future could be more revolutionary than evolutionary, if research at the nano-bio interface continues at its current pace. Imagine targeted drugs and guilt-free food; or treatments that make you even better than new. Nanotechnology is driving the development of tools to understand biology better and materials to promote good health. Where is bio-nanoscience heading and how can science and citizens work together to ensure its success? This session will turn to the interface between nanomaterials and humans, and highlight provocative, cutting-edge science.

Special Sessions

2009 Forum for Sustainability Science Programs

One of the biggest challenges that the planet faces is how to balance the needs of human development with the needs of the environment. Policy-makers at all levels of governance increasingly look to scientists and engineers to provide guidance in creating sustainable societies. Universities are increasingly responding by developing academic and research programs in Science and Technology for Sustainable Development or "Sustainability Science" that undertake practical, place-based research to provide decision-support for addressing sustainability challenges.

Since the inaugural Forum at the 2007 AAAS Annual Meeting in San Francisco, the AAAS Center for Science, Technology, and Sustainable Development has convened key university actors in Sustainability Science to dialogue on collaborative approaches to building this emerging field. Though participants from the United States and abroad hail from diverse perspectives and institutions, most are experiencing similar challenges as they develop interdisciplinary programs, which combine both basic and applied research methods.

As a follow-up to previous sessions which identified key challenges and opportunities (2007) and began to identify opportunities to further connect these universities (2008), the 2009 Forum will tackle a number of common concerns for these programs including:

- Curriculum Development
- Sustainability Science and Decision-Making
- Support for Interdisciplinary Sustainability Research

The Forum will include a series of roundtable discussions, led by key actors in the sustainability science community.

2009 Forum for School Science

The quality of science and mathematics education is high on the list of concerns in most countries of the world. Scientists and educators in many countries are developing and testing programs and practices, including a number they have adapted from U.S. initiatives. Many states are visiting programs in other countries and attempting to benchmark those that show high levels of performance on international assessments.

In some cases, economical, cultural, and social differences result in different ideas, strategies, and adaptations. In other countries, where elements of U.S.-developed programs are implemented, lessons can be learned from their results, especially to inform the work of transformation in the United States. The Forum for School Science will offer a series of "global" conversations with U.S. and international presenters. The discussions will include examples of programs and current thinking in each area and reflect on symposia to be offered in the symposium track, Learning and Literacy. Areas to be covered include:

- Rethinking U.S. reforms of the curriculum core (sharing, adapting, and delivering materials)
- Implementing what we know (policy, research, and scaling)
- Restructuring undergraduate and graduate STEM education
- Engaging the public with science and education.

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Symposium Tracks

Brain and Behavior

Beyond the Beagle: Evolutionary Approaches to the Study of Social Behavior
Organized by Jill Mateo, University of Chicago, Ill.

Comparative Cognition: The Science of Mental Evolution
Organized by Edward A. Wasserman, University of Iowa, Iowa City

Embodied Language and Cognition: Brains, Mouths, and Hands
Organized by Philip Rubin, Haskins Laboratories, New Haven, Conn.

Expression of Emotions: Biocultural Perspectives
Organized by Carl A. Maida, University of California, Los Angeles

Languages Without Ancestors
Organized by Karen Emmorey, San Diego State University, Calif.

Post-Traumatic Stress Disorder and the Military
Organized by Stephanie J. Bird, Massachusetts Institute of Technology, Cambridge, Mass.

Post-Traumatic Stress Disorder: The New Battle for Veterans
Organized by Virginia R. G. Carson, Chapman University, Orange, Calif.

The Science of Kissing
Organized by Albert H. Teich, AAAS Science and Policy Programs, Washington, D.C.

Social Emotion and the Brain
Organized by John T. Cacioppo, University of Chicago, Ill.

Evolution and Revolution

Animal Body Plan Evolution of Development
Organized by Christopher J. Lowe, University of Chicago, Ill.

Celebrating Darwin at 200: Explaining How Human Morality Evolved
Organized by Douglas Allchin, University of Minnesota, Minneapolis

Evolution Makes Sense of Biology
Organized by Eugenie C. Scott, National Center for Science Education, Oakland, Calif.

Evolution of Mammalian Retroelement Activity
Organized by Robert C. von Borstel, University of Alberta, Canada

Genetics Meets Anthropology: How DNA Unravels the Roots of Human Society
Organized by Eamonn Cahill, Office of the Chief Scientific Adviser to the Government of Ireland, Dublin

Host-Pathogen Interactions: Evolution of Immune Defenses
Organized by Nancy E. Beckage, University of California, Riverside

The Invisible Woman in Evolution: Natural Selection and Life-Cycle Events
Organized by Marquisa LaVelle, University of Rhode Island, Kingston

The Last Piece of Darwin's Puzzle: The Evolution of the Social Mind
Organized by Dario Maestripieri, University of Chicago, Ill.

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POSITIONS OPEN

DIRECTOR, IPCC WORKING GROUP II Technical Support Unit

Working Group II of the Intergovernmental Panel on Climate Change (IPCC) assesses the vulnerability of socio-economic and natural systems to climate change, potential negative and positive consequences, and options for adapting to it. The Technical Support Unit (TSU) provides technical and administrative services in support of assessment deliverables. A TSU Head is sought to provide senior-level scientific leadership for the assessment programs of IPCC Working Group II; to work with the co-chairs to structure, summarize, and disseminate scientific content of findings generated by and during the assessment process; and to provide scientific leadership and direction to TSU staff in support of IPCC-wide commitments. Primary emphasis will be on scoping, preparation, and review of the Working Group II contribution to the Fifth Assessment Report (AR5), to be published in 2014.

Requires Ph.D. in social or natural science discipline relevant to the assessment of global climate change and climate variability, as well as ten or more years of experience conducting research on climate impacts. Experience with management of multidisciplinary scientific groups and a distinguished impacts, adaptation, and vulnerability (IAV) publication record are required. Experience working within IPCC or comparable United Nations processes is desired.

This is a term position through 30 September 2009, with possibility of annual extensions through FY2014. It is located at the Carnegie Institution for Science in Stanford, California, U.S.A. Relocation expenses will be covered. To view the complete job description and apply, visit **website: http://www.fin.ucar.edu/hr/employment** (reference job #9047 under Current Job Openings/Scientific).

Initial consideration will be given to applications received prior to 23 January 2009. Thereafter, applications will be reviewed on an as-needed basis. Apply online at **website: http://www.fin.ucar.edu/hr/employment** (reference job #9047). *We value diversity. Affirmative Action/Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE/FULL PROFESSOR Cancer Pharmacology/Therapeutics

The Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center (TTUHSC), seeks applicants for an attractive new tenure-track faculty position at the Assistant/Associate/Full Professor level. The successful candidate will join a vibrant and expanding group of extramurally funded biomedical and pharmaceutical cancer researchers **(website: http://www.ttuhsc.edu/sop/pharmsci)** who are part of a newly developed University Cancer Biology Center. Applicants must have a doctoral degree with research experience in any aspect of cancer pharmacology, chemotherapeutics, drug discovery, or delivery. The candidates at Assistant Professor level are expected to develop an extramurally funded research program, whereas candidates at the Associate/Professor level should have current peer-reviewed funding and a track record of publishing. The successful candidate will be expected to teach Pharm.D. and Ph.D. students. Candidates who have training in pharmacology or drug therapy are particularly encouraged to apply. Competitive salary, startup package, and laboratory space are available. Applicants should submit documents online at **website: http://jobs.texastech.edu** (job requisition # 78130). Please include curriculum vitae, a summary of research accomplishments/interests, future directions, and names and addresses of three references. TTUHSC in Amarillo includes the School of Pharmacy, the School of Medicine, and the Harrington Cancer Research Center. The Department has 23 full-time faculty with interests in cancer biology, brain/vascular and pharmaceutical research, and state-of-the-art core facilities. For questions, contact the **Search Committee Chair, Dr. Sanjay K. Srivastava. E-mail: sanjay.srivastava@ttuhsc.edu. Telephone: 806-356-4015, extension 319.** *TTUHSC is an Equal Opportunity/Affirmative Action Institution. Minorities and women are encouraged to apply.*

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION Human Genetics

The Vanderbilt Center for Human Genetics Research (CHGR) is recruiting for a new tenure-track **ASSISTANT PROFESSOR** faculty position in genetic epidemiology, computational genetics/genomics, or statistical genetics. The successful candidates will be highly motivated and can demonstrate their potential to develop and maintain a strong independent research program. Applicants must hold a Doctorate in an appropriate field (e.g., genetics, human genetics, population genetics, computer science, biostatistics, epidemiology). The successful applicant will have a strong interest in one or more of the following: developing and applying statistical and/or computational methods of genetic analysis for complex traits, disease gene discovery, or population-based differences in disease etiology.

The CHGR is an interdisciplinary center with over 40 faculty representing numerous clinical and basic science departments. It has a highly active and interactive research program organized into three thematic programs: Disease Gene Discovery, Computational Genomics, and Translational Genetics. The CHGR has substantial core facilities for participant ascertainment; DNA banking, genotyping, and sequencing; and computational genomics, data management, and data analysis. We occupy over 14,000 square feet of newly appointed wet and dry laboratory space. The CHGR offers highly competitive startup packages and enjoys the substantial benefits of the collaborative Vanderbilt atmosphere. More information about the CHGR can be found at **website: http://chgr.mc.vanderbilt.edu.**

Applicants should submit curriculum vitae, three letters of reference, and a concise statement of research plans. Evaluation of applications will begin February 1, 2009. Please send all information or direct any inquiries to:

Faculty Search Committee
Center for Human Genetics Research
Vanderbilt University Medical Center
519 Light Hall
Nashville, TN 37232-0700
E-mail: recruiting@chgr.mc.vanderbilt.edu
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POSTDOCTORAL ASSOCIATE

The Institute for Terrestrial and Planetary Atmospheres at Stony Brook University, New York, operates a NASA-funded scientific center that serves an international research program of the World Climate Research Programme (WCRP) titled, Stratospheric Processes and Their Role in Climate (SPARC). We are seeking a Postdoctoral Research Associate who will be responsible for managing the Data Center, facilitating data exchanges among the various research groups and will also be conducting NASA- and NSF-funded research on stratosphere-troposphere interactions. For a full position description, application procedures, and to apply online, visit **website: http://www.stonybrook.edu/jobs** (reference #WC-R-5442-08-12-S). *Equal Opportunity/Affirmative Action Employer.*

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National Institute of Mental Health

Department of Health and Human Services
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Division of Intramural Research Programs

The National Institute of Mental Health (NIMH), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is seeking exceptional candidates for the position of Deputy Scientific Director, Division of Intramural Research Programs (DIRP). The Deputy Scientific Director will work with the Scientific Director to lead and manage a vibrant intramural research program with more than 50 independent principal investigators, an extensive network of core service facilities (http://intramural.nimh.nih.gov), and a budget above $160M.

The Deputy Scientific Director will assist in the recruitment of new faculty, oversee the expansion of the Division and its programs into several state-of-the-art research facilities on the NIH's Bethesda, Maryland campus and help manage the scientific resources at the NIMH DIRP.

Applicants must have a Ph.D., M.D., or equivalent degree in the biomedical sciences, with broad research experience and experience in direct administration of a multidisciplinary research program. Applicants may have the opportunity to perform independent and/or collaborative scientific studies. Applicants should be known and respected within their profession as distinguished individuals of outstanding scientific competence. Salary is commensurate with experience and accomplishments. Experience with NIH administrative policies, procedures, and operations is highly desirable but not essential.

Interested candidates should send a letter of interest, to include a complete description of research and administrative experience and rationale for applying for this particular position (2-3 pages maximum), contact information for at least six references, and a curriculum vitae and bibliography to: **Dr. Susan Swedo, Chair, Search Committee for Deputy Scientific Director, DIRP, NIMH, c/o steyerm@mail.nih.gov or to Bldg. 10, Rm. 4N-222, 9000 Rockville Pike, Bethesda, MD 20892-1381**. Review of applications will begin on **March 2, 2009**, but applications will continue to be accepted and considered until the position is filled.

National Heart Lung and Blood Institute

Proteomics Core Facility Director

Department of Health and Human Services
National Institutes of Health (NIH)
National Heart, Lung and Blood Institute

An expert is sought in the area of Proteomics with an emphasis on LC-MS/MS to direct the Proteomics Core Facility, Division of Intramural Research (DIR), National Heart, Lung and Blood Institute (NHLBI), NIH in Bethesda, Maryland USA. The successful applicant will interact with Principal Investigators in the DIR in the design and interpretation of experiments involving large-scale proteomic profiling, detection of post-translational modifications including protein phosphorylation, quantitative LC-MS/MS, as well as 2-D gel analysis. He/she will direct Core Facility personnel in the maintenance and operation of the Core mass spectrometers as well as instrumentation for protein and peptide separations. The Proteomics Core Facility is part of an NHLBI DIR Initiative in Systems Biology. Although the Core Facility is service-oriented, the Director has the opportunity to devote up to 20% of his/her time in original research in the area of technical development. The mission of the DIR is to improve the health of all Americans through basic and clinical research, research training, and translation of discoveries to new tools to be applied directly to the field of medicine.

We are seeking an experienced scientist (with Ph.D. or equivalent) with an outstanding track record in proteomics research and protein/peptide LC-MS/MS expertise. Prior experience in the operation of a service-oriented Proteomics Core Facility is highly desirable. Computational skills relevant to development of new algorithms for mass spectrometry and bioinformatics would also be advantageous. Salary will be commensurate with qualifications and experience. More detailed information about the NHLBI Division of Intramural Research may be found at: **http://dir.nhlbi.nih.gov/**.

Applicants should submit the following: cover letter highlighting key qualifications; current curriculum vitae with complete bibliography; names and addresses of four references; and a one-page summary of applicant's philosophy of core facility operation.

Applications must be received by **February 15, 2008**. PDF versions of documents sent by electronic mail are strongly preferred. Materials should be sent to **Dr. Robert S. Balaban c/o: Doug Price, Administrative Officer, NHLBI, by email: dwp@nih.gov; or by regular mail: Building 10, Room 7N220, 10 Center Drive MSC 1670, Bethesda, MD 20892-1670**.

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

The **Department of Physics, Mathematics and Computer Science of the Johannes Gutenberg-University Mainz** invites applications to fill, at the earliest possible date, the position of a

Professor of Experimental Quantum Optics / Atomic Physics

(Full Professor, Bes. Gr. W3 BBesG)

(Succession of Professor Immanuel Bloch)

at the Institute of Physics.

We are seeking to appoint an experienced scientist with an international reputation and an outstanding track record both in research on the fundamentals of quantum systems and in teaching. Relevant research interests might include: ultracold quantum gases, quantum computing and quantum information, ultrafast optics, micro- and nano-photonics.

The professorship is the chair of the scientific research group on quantum-, atomic- and neutron-physics (QUANTUM). Other groups at the Institute are devoted to both experimental and theoretical particle physics and condensed matter physics. Further collaboration opportunities include the DFG-funded Collaborative Research Centre SFB/TRR 49 "Condensed Matter Systems with Variable Many-Body Interactions", the Graduate School of Excellence "Material Science in Mainz (MAINZ)", the Research Centre "Elementary Forces and Mathematical Foundations", and the GSI Helmholtz-Centre for Heavy-Ion Research in Darmstadt (EMMI and FAIR).

Candidates are expected to teach courses covering all areas of experimental physics.

The Department of Physics, Mathematics and Computer Science aims at increasing the percentage of women in academic positions and strongly encourages women scientists to apply.

Applicants must have a PhD in physics and a proven first-rate research record. Moreover, the formal regulations set out in § 49 Hochschulgesetz Rheinland/Pfalz must be fulfilled.

The Johannes Gutenberg-University promotes a concept of intensive tutoring which requires a regular presence in Mainz.

The University is an equal opportunity employer: Applicants with physical disabilities will receive preferential consideration if their qualification and experience are equal to those of other applicants.

Informal enquiries may be directed to Dr. Holger Schinke, telephone: +49 6131 39 23654, e-mail: schinke@uni-mainz.de.

Qualified candidates are asked to submit their application to the:

universitätmainz

Johannes Gutenberg-University Mainz,
Dekan des Fachbereichs 08 – Physik, Mathematik und Informatik –,
D-55099 Mainz / Germany.

Closing date: 5 February 2009.

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the City College of New York

Department of Chemistry

Assistant, Associate or Full Professor

The Department of Chemistry at the City College of New York is seeking a faculty member at the Assistant, Associate or Full Professor rank to develop an active externally funded program that uses X-Ray crystallography to determine structure-function relationships in biomolecules. The faculty member, who will also join the CUNY Institute for Macromolecular Assemblies, is also expected to mentor graduate students and teach graduate and undergraduate courses. City College is part of the City University of New York. For more information about the City College of New York, see **www.ccny.cuny.edu**. For areas of departmental strengths, see **www.sci.ccny.cuny.edu/mma**.

Qualification Requirements:

Ph.D. and two years postdoctoral experience in structural biology or related field required. Additionally, a track record of independent research productivity and federal funding is required for consideration at senior ranks.

Send curriculum vitae, a description of current research and future research plans, and selected reprints and preprints to the address below. Also have three letters of reference sent by the closing date by conventional mail to: **Prof. Ruth E. Stark, Chair, Structural Biology Search Committee, The City College of New York, Department of Chemistry, 160 Convent Avenue, MR1208, New York, NY 10031.** Applications will be reviewed starting December 26, 2008 and review will continue until the positions are filled.

The City University of New York
An Equal Employment Opportunity/Affirmative Action/Immigration Reform and Control Act/ Americans with Disabilities Act Employer

CITY COLLEGE IS CUNY

Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. Of a number of recent indicators of the University's performance, one is its ranking at 26 among the top 200 universities in the world by the UK's Times Higher Education Supplement. The University has a comprehensive range of study programmes and research disciplines, with 20,000 undergraduate and postgraduate students from 50 countries, and a complement of 1,200 academic members of staff, many of whom are internationally renowned.

Assistant Professor in the Department of Anatomy (Ref.: RF-2008/2009-405)

Applications are invited for appointments as Assistant Professors (2 posts) in the Department of Anatomy, from as soon as possible, on a three-year fixed-term basis, with consideration for tenure after satisfactory completion of a second fixed-term contract.

The Department offers M.B.,B.S., B.NURS., B.Chin.Med.; M.Med.Sci., M.Res.Med.; M.Phil. and Ph.D. programmes. There is a full-time academic establishment of 13 faculty members, 1 research assistant professor, 1 scientific officer, 2 research officers and 6 post-doctoral fellows. Excellent research support and facilities, including state-of-the-art cellular and molecular biological equipment, are available. More information about the Department can be obtained at http://www.hku.hk/anatomy.

Applicants should possess a Ph.D. degree in biological or biomedical sciences, with post-doctoral experience and a strong record in teaching and research, preferably in cancer and cell biology, or neuroscience which are two of the four major areas of research conducted in the Department. The appointees are expected to undertake teaching duties at undergraduate and postgraduate levels as well as engage actively in high-quality research.

Annual salary for Assistant Professorship will be in the range of HK$504,480 – 779,640 (approximately US$1 = HK$ 7.8) (subject to review from time to time at the entire discretion of the University). At current rates, salaries tax does not exceed 15% of gross income. The appointment will attract a contract-end gratuity and University contribution to a retirement benefits scheme, totalling up to 15% of basic salary, as well as annual leave, and medical/dental benefits. Housing benefits will be provided as applicable.

Further particulars and application forms (152/708) can be obtained at https://www.hku.hk/apptunit/; or from the Appointments Unit (Senior), Human Resource Section, Registry, The University of Hong Kong, Hong Kong (**fax**: (852) 2540 6735 or 2559 2058; **e-mail**: senrappt@hkucc.hku.hk). **Closes February 9, 2009. Candidates who are not contacted within 3 months of the closing date may consider their applications unsuccessful.**

The University is an equal opportunity employer and is committed to a No-Smoking Policy

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Max Planck Institute for Plant Breeding Research

Max-Planck-Institut für Züchtungsforschung

International Max Planck Research School:

"The molecular basis of plant development and environmental interactions"

12 Ph. D. Studentships

The Max-Planck-Institute for Plant Breeding Research and University of Cologne invite applications for Ph.D. fellowships in the International Max Planck Research School (IMPRS) in Cologne, Germany.

The IMPRS is intended for highly motivated students with a strong training in molecular sciences. The constellation of participating institutions provides excellent conditions in the area of plant sciences with expertise in plant genetics, plant biochemistry, structural biology, bioinformatics, cell biology, and molecular microbiology.

The training includes regular seminars, yearly retreats, supervision of a thesis committee of the research school, and soft skill and practical courses on modern laboratory techniques.

The program is taught in English and open to students from all countries holding a Master's degree or a corresponding Diploma degree.

For detailed information about the application process and the Ph.D. program please visit the IMPRS homepage at www.mpiz-koeln.mpg.de/english/studentInformation/index.html.

Deadline for applications is March 31, 2009.

The Max Planck Society is an equal opportunity employer. We strongly encourage female scientists to apply for the program. After registration, the fellowship application should be mailed to:

Max Planck Institute for Plant Breeding Research
IMPRS – Molecular Basis of Plant Development
Scientific Coordinator
Carl-von-Linné-Weg 10
50829 Cologne / Germany

MAX-PLANCK-GESELLSCHAFT

KING ABDULLAH UNIVERSITY OF SCIENCE AND TECHNOLOGY (KAUST) AND THE AMERICAN UNIVERSITY IN CAIRO (AUC)

Within the framework of the joint collaboration between King Abdullah University of Science and Technology (KAUST), Kingdom of Saudi Arabia, and The American University in Cairo (AUC), Arab Republic of Egypt, applicants are invited to apply for post-doctoral positions.

KAUST (http://www.kaust.edu.sa) is an independent world-class, international graduate-level science and technology research university dedicated to inspiring a new age of scientific achievement in the Kingdom that will also benefit the region and the world. KAUST is governed by a self-perpetuating Board of Trustees and supported by a multi-billion dollar endowment. It reinvents the modern research university by establishing advanced research institutes that focus on interdisciplinary problems as the central organizing unit, and offering only graduate (M.S. and Ph.D.) degrees. As an independent and merit-based institution and one of the best endowed universities in the world, KAUST intends to become a major new contributor to the global network of collaborative research. The KAUST mission emphasizes research on applications of science and technology to problems of human need, social advancement, and economic development. It will enable researchers from around the globe to work together to solve challenging scientific and technological problems. The admission of students, the appointment, promotion and retention of faculty and staff, and all the educational, administrative and other activities of the University shall be conducted on the basis of equality, without regard to race, color, religion or gender.

KAUST's new 36 million square meter state-of-the-art campus in Thuwal (80 km north of Jeddah) will open its doors to students in Fall 2009. The campus includes a seaside town with housing, shops, schools, and numerous recreational amenities. KAUST welcomes exceptional researchers, faculty and students from around the world. To be competitive, KAUST will offer very attractive base salaries and a wide range of benefits.

AUC (http://www.aucegypt.edu) is a premier English-language institution of higher learning in its region. The university offers a strong liberal arts education in a cross-cultural environment and aspires to a tradition of life-long learning. AUC is committed to teaching and research of the highest caliber in diverse scholarly and professional fields, and to making significant contributions to Egypt and the international community. AUC is an independent, not-for-profit, equal-opportunity institution. Founded in 1919, AUC's campus has moved recently to a new, state-of-the-art campus in New Cairo beginning Fall Semester, 2008. AUC's degree programs are accredited by the Commission on Higher Education of the Middle States Association of Colleges and Schools. The Management program is accredited by AACSB. Construction, Electronics, and Mechanical Engineering Programs are accredited by the Engineering Accreditation Commission of ABET whereas the Computer Science Program is accredited by the Computing Accreditation Commission of ABET. All university academic programs are accredited by the Supreme Council of Egyptian Universities.

POST DOCTORAL POSITIONS

Within the framework of the joint research project on Environmental Genomics Approach for the Study of Marine Organisms in the Red Sea, applicants are invited to apply for four Post-Doctoral positions at KAUST. These positions are effective as soon as possible. The project will last until August 2012. Serious commitment for the whole period of the project is a priority in making the final selection. The selected candidate will become a member of the multidisciplinary team of marine, molecular, and computational biologists, and will have accessibility to a well-equipped molecular biology laboratory, and a cutting-edge genomics facility, including high-throughput DNA sequencer and high performance computational systems. Applicants must have a demonstrated potential for scholarly research in one of the following areas:

Molecular Biology

(EG# 1) Two positions are available to participate in establishing and analyzing metagenomic database from different environments in the Red Sea, including deep-sea, brine pools, and deep-sea sediments. Applicants should have a Ph.D. degree with a strong background in molecular biology and biochemical techniques.

Marine Biology:

(EG# 2) One position is available to participate in addressing fundamental questions in the field of marine biology including, but not limited to, microbial diversity and population genomics of the Red Sea, microbial dynamics in response to climate change, and microbial symbiosis in marine animals. Applicants should have a Ph.D. degree in marine biology.

Computational Genomics:

(EG# 3) One position is available to address the implementation of algorithms for clustering, alignment, annotation, and gene prediction from Red Sea metagenomic sequence data. Applicants should have a Ph.D. in bioinformatics, computational or system biology.

The successful candidate must have the ability to work independently and as part of a research team, demonstrated high work ethic, creativity, initiative, and experience in technical publication, and excellent English writing and communication and interpersonal skills. Appointed post-doctoral fellows are expected to share their time between AUC and KAUST. Their appointment will start at the American University in Cairo (Egypt) as soon as possible, except for the last position which is available beginning 2009-10, and then they will move to King Abdullah University of Science and Technology (KSA) for the rest of their contract according to the approved collaborative research plan. Deadline for applications is January 15, 2009 or until the position is filled.

All applicants must submit the following documents via the AUC online system a) an updated CV; b) a letter of interest; c) a completed AUC Personal Information Form (PIF); and d) names and contact information for at least three references familiar with the applicant's professional background.

http://tinyurl.com/auckaust

HARVARD UNIVERSITY
Assistant/Associate Professors
Department of Genetics and Complex Diseases

The Department of Genetics and Complex Diseases (GCD) at the Harvard School of Public Health (HSPH) invites applications for tenure-track positions at the level of assistant professor. Exceptional associate professor candidates will also be considered. GCD is a major new initiative in basic sciences in the HSPH Division of Biological Sciences. Through integrative programs combining biochemistry, cell biology, physiology and genetics, the GCD group seeks to generate basic research insights related to complex human diseases. Current areas of research include nutrient sensing, protein translation and degradation, nuclear hormone receptor signaling, vesicular transport, cellular stress responses, DNA repair, tumor metabolism, and regulation of carbohydrate, lipid, and energy homeostasis. Successful applicants will hold a PhD and/or MD degree and will have a record of outstanding productivity in an area that complements the existing research and training goals of the department. The candidate should possess the ability to work collaboratively with other scientists and the scholarly qualities required to mentor doctoral students in the graduate program in the Division of Biological Sciences. Generous start-up packages and state-of-the-art research facilities will be available. GCD has particular interest in recruiting individuals with research programs in the following areas:

- Organelle biology and dysfunction, particularly mitochondria or endoplasmic reticulum
- Mechanisms of signal transduction and integration, especially in the context of energy and nutrient sensing, metabolic homeostasis and stress response
- Tumor cell metabolism
- Inflammatory and stress responses with emphasis on chronic metabolic or degenerative diseases
- Systems approaches, applied at a mechanistic level, to problems of metabolic homeostasis, gene-environment interactions and/or adaptive responses

Please send a letter of application, including a statement of current and future research interests, curriculum vitae, sample publications, and the names of four references to the following address. Applicants should ask their four references to write independently to this address: **Chair, GCD Search, c/o Julie Gound, Department of Genetics & Complex Diseases, 655 Huntington Avenue, Building II, 107, Boston, MA 02115; gcddept@hsph.harvard.edu.**

The Harvard School of Public Health is committed to increasing the representation of women and minorities in its faculty, and encourages applications from such candidates.

UNT
UNIVERSITY OF NORTH TEXAS

Faculty Positions in BIOPHOTONICS

The Bio/Nano-photonics Research Cluster at the University of North Texas (UNT) seeks candidates for faculty positions in the broad area of biophotonics. The positions will be at the professor or associate professor rank but candidates at the assistant professor level will also be considered. Candidates will be expected to have an extramurally funded research program in biophotonics that can be integrated into the mission of the bio/nano-photonics cluster, and to help support the instructional needs of one or more cluster departments at the graduate and undergraduate levels. Preference will be given to candidates with expertise in imaging, bioconjugation, and spectroscopy of biological systems. The candidate can take advantage of new UNT facilities in nanoscale and ultrafast optical characterization, including near-field scanning and femtosecond time-resolved spectroscopy, and optical microscopy for live cell imaging using confocal fluorescence and Raman microscopy. The candidate will interact with current faculty in the biological sciences, chemistry, engineering, physics departments and/or the UNT Health Sciences Center. Competitive start-up funds and salary are available.

Send a cover letter highlighting qualifications and research interests, CV, 3 representative publications, and names of 3 references to: **Dr. Mohammad A. Omary, Search Committee Chair, University of North Texas, Department of Chemistry, 1155 Union Circle #305070, Denton, TX 76203**. In addition, please e-mail your CV to omary@unt.edu. Applications will be reviewed from Dec. 1, 2008 until the search is closed. UNT is a Doctoral/Research-Extensive Institution located in the Dallas/Fort Worth Metroplex. It is the fourth largest university in Texas, with nearly 35,000 students.

For further information, see http://www.unt.edu.
AA/ADA/EOE.

Structural and Functional Genomics at the University of Oklahoma

The Department of Chemistry and Biochemistry at the University of Oklahoma invites applications for a tenure-track or tenured faculty position in Biochemistry, at any rank. Applicants must have a Ph.D. degree and interests in problems at the forefront of structural/functional genomics research and/or technology, including but not restricted to DNA sequencing, proteomics, metabolomics, bioinformatics and/or systems biology. This recruitment relates to the University of Oklahoma's multi-departmental Integrative Life Sciences Initiative that complements and strengthens existing programs in chemistry, biochemistry, microbiology, botany, zoology, and bioengineering. The primary criterion for appointment is excellence in research and teaching. The successful candidate will contribute to biochemistry teaching programs at both the undergraduate and graduate levels.

Applicants at the rank of assistant professor must have postdoctoral experience; applicants for a senior faculty position must have a record of substantial and sustained externally funded research. Interested individuals should submit their curriculum vitae, a description of their research plans, and a statement of teaching interests and philosophy. Candidates for assistant professor should request three letters of recommendation and have them sent directly to the Chair of Genomics Search Committee; candidates at higher ranks should identify three individuals whom the Search Committee may contact for letters of recommendation. Send application materials or nominations to: **Professor Phillip E. Klebba, Chair of Genomics Faculty Search Committee, Department of Chemistry and Biochemistry, 620 Parrington Oval, Norman, OK 73019**. We will also accept completed applications in PDF format sent to: **sgfisher@ou.edu**. The review of applications will begin on **February 15, 2009** and will continue until the position is filled. Minorities and women are especially encouraged to apply.

The University of Oklahoma is responsive to the needs of dual-careers, and is an Affirmative Action Equal Opportunity Employer.

NUS
National University of Singapore

A Career in Neuroscience at the National University of Singapore (NUS)

The National University of Singapore, one of Asia's leading Universities but with a global outlook (http://www.nus.edu.sg), has strong basic and translational clinical programmes in Neuroscience. These are closely linked to research expertise in other biomedical areas, imaging, engineering and physical sciences. We are looking to further develop the Neurobiology group by recruiting senior scientists/clinician scientists with excellent track records in neuroscience research who can develop and lead new and existing programmes and secure competitive grant funding from the major sources available in Singapore. Areas we particularly wish to develop include dementia research, cognitive neuroscience, neuroengineering, and neuroeconomics, but applicants from other areas of neuroscience or whose research crosses the boundaries of these areas are welcome. Appropriate space, start-up funds and access to graduate students will be provided. The selected applicants will hold senior academic appointments in one or more relevant Departments/Faculties/Schools at NUS.

Applications (with full CV and names of at least 3 referees) and informal/confidential enquiries can be sent to:

Professor Barry Halliwell
Office of the Deputy President (Research and Technology)
National University of Singapore
University Hall
Lee Kong Chian Wing, UHL #05-02H
21 Lower Kent Ridge Road
Singapore 119077
Email: bchbh@nus.edu.sg

Closing date for applications: 15 February 2009

Wellcome Trust/DBT India Alliance

Biomedical Research Careers Programme for India

The Department of Biotechnology, Ministry of Science and Technology, India, and the Wellcome Trust, UK, have established an Alliance to fund biomedical research fellowships in India.

These fellowships will provide opportunities for support at key career stages and will fund science across the full spectrum of biomedical research, from fundamental molecular and cellular studies through to clinical and public health research.

Intermediate Fellowships for Researchers in India

For excellent scientists who wish to undertake high-quality research and to establish themselves as independent researchers in India.

The Fellowship provides five years' funding to support the Fellow's salary and research expenses. Fellows may spend up to 12 months outside their host institution either in other laboratories in India or elsewhere.

Candidates should have between three and six years' postdoctoral (or equivalent clinical) research experience at submission of a full application.

Deadlines

Preliminary applications must be received no later than **2 February 2009**.

Full applications will be invited by 2 March 2009.

Awards can be taken up from September 2009.

Early Career Fellowships for Researchers in India

For the most promising newly qualified postdoctoral researchers to make an early start in launching their independent research careers, working in the best laboratories in India.

The Fellowship provides four years' funding to support the Fellow's salary and research expenses. Fellows may spend up to 12 months outside their host institution either in other laboratories in India or elsewhere.

Candidates should:

- be Indian nationals, but do not have to be resident in India during the application process
- have no more than 12 months' postdoctoral (or equivalent clinical) experience at submission of a full application.

Deadlines

Preliminary applications must be received no later than **30 April 2009**.

Full applications will be invited by 1 June 2009.

Awards can be taken up from December 2009.

Further information and preliminary application forms are available at **www.wellcomedbt.org**

wellcome trust

Department of Biotechnology
Ministry of Science and Technology

UNIVERSITY OF MICHIGAN

Ara G. Paul Professor
Department of Pharmaceutical Sciences

The Department of Pharmaceutical Sciences is undergoing an exciting and new phase of growth and development, and invites applications for a senior Endowed Faculty Professorship. We are searching for a tenured scientist with outstanding leadership and research credentials, and a demonstrated track record of accomplishments in securing extramural funding. This position demands a commitment to graduate and professional education, and to professional service.

Applicants who are performing high-impact research in the following areas of interest, as applied to the pharmaceutical sciences, are preferred: (1) biomaterials and nanotechnology, (2) in vitro/in vivo drug targeting and biomedical imaging, (3) mechanisms of drug absorption, distribution and disposition, (4) structural biology (e.g., of drug transporters and metabolic enzymes), (5) cheminformatics, and (6) pharmacogenomics.

Interested individuals should send a letter of application, curriculum vitae, future research plans and contact information of 3 references (i.e., name, address, telephone, and email) to the Search Committee Chair via US Mail. A PDF file should also be sent to **jgetty@umich.edu** as an email attachment. The Search Committee will begin reviewing applications immediately and will continue to do so until the position is filled.

Submit the requested information to:
Professor Steven P. Schwendeman, Search Committee Chair
Department of Pharmaceutical Sciences
The University of Michigan
428 Church Street
Ann Arbor, MI 48109-1065

For more information, please contact the Search Committee Chair at:
Telephone: 734-615-6574; Facsimile: 734-615-6162;
Email: jgetty@umich.edu.

The University of Michigan is an Equal Opportunity/ Affirmative Action Employer.

SAINT LOUIS UNIVERSITY

CHAIRPERSON
Department of Biochemistry and Molecular Biology

Saint Louis University, a Catholic, Jesuit institution dedicated to student learning, research, healthcare, and service has initiated a national search for the Chairperson of the Edward A. Doisy Department of Biochemistry and Molecular Biology. The department has a strong tradition of excellence in research and education. It was founded in 1923 by Dr. Edward A. Doisy who received a Nobel Prize for his research on Vitamin K. The department occupies ample space in the recently built Doisy Research Center (**http://researchbuilding.slu.edu/**), with outstanding research equipment and core facilities. Departmental faculty are consistently funded in the areas of protein structure and function, macromolecular interactions, gene regulation, signal transduction, protein trafficking and turnover, cardiovascular research, and genetic disorders. Research strengths in other departments include neuroscience and aging, molecular virology, immunology and vaccine development, and liver disease. The School of Medicine is seeking a highly motivated academic leader who will cultivate an environment of excellence in research and teaching, and will foster an environment of collegiality and collaboration with other departments and schools. The successful applicant will provide strong leadership for all research and academic activities of the department and will have outstanding skills in communication and mentoring. She/he will have a Ph.D., a distinguished record of research productivity, teaching excellence, and leadership skills in academic program development. All applications must be made at: **http://jobs.slu.edu** and must include a curriculum vitae and cover letter describing their leadership style and their vision for developing a vibrant and collaborative research environment.

Letters of nomination(s) and curriculum vitae may be sent to:
William S. M. Wold, Ph.D.
Chairperson, Biochemistry Chairperson Search Committee
Saint Louis University School of Medicine
1402 South Grand Boulevard, M268
St. Louis, MO 63104
Email: woldws@slu.edu

Saint Louis University is an Affirmative Action/Equal Opportunity Employer and encourages nominations and applications from women and minorities.

www.abdn.ac.uk/jobs

1495 UNIVERSITY OF ABERDEEN

Research Fellow Reference: YMB156R

A bright postdoctoral research fellow, you will join a project studying stress responses in pathogenic Candida species. You should hold a PhD in molecular biology, microbiology, biochemistry or a related discipline.

Should you require a visa to undertake paid employment in the UK you will be required to fulfil the minimum points criteria to be granted a Certificate of Sponsorship and Tier 2 visa. As appropriate, at the time an offer of appointment is made you will be asked to demonstrate that you fulfil the criteria in respect of financial maintenance and competency in English. Please do not hesitate to contact Sacha MacLennan, HR Adviser, for further information on this.

As this post is funded by BBSRC it will be offered for a period of 54 months. Salary will be at the appropriate point on the Grade 6 (£29,704 – £31,513 per annum), with placement according to qualifications and experience. Informal enquiries to Professor Al Brown (al.brown@abdn.ac.uk).

Closing date: 23 January 2009.

The University of Aberdeen is a charity registered in Scotland, No SC013683.

For further information on these positions and to view all current vacancies at the University visit:

www.abdn.ac.uk/jobs

Promoting Diversity and Equal Opportunities throughout the University

EXPRESSION OF INTEREST FOR RESEARCH POSITIONS
At the NCSR "DEMOKRITOS"
Athens, Greece

The NCSR "D" announces its intention to hire a number of researchers at various levels, of excellent standing, in one or more of the following priority research areas of the center:

- Health, Biology and Biotechnology
- New Materials
- Nanoelectronics, Micro-Nano Systems
- Energy, Environment and Sustainable Development
- Information and Telecommunication Technologies
- Nuclear, Particle and Astroparticle Physics
- Nuclear Technology and Radiation Protection
- Cultural Heritage

The applicants should hold a PhD in a relevant subject and should be able to demonstrate sufficient prior experience in conducting research either in an academic and/or in collaboration with industrial environment.

The work in the above thematic areas is performed in the following Institutes of the NCSR "D"

- Institute of Nuclear Physics
- Institute of Nuclear Technology and Radiation Protection
- Institute of Materials Science
- Institute of Informatics and Telecommunication
- Institute of Microelectronics
- Institute of Physical Chemistry
- Institute of Biology
- Institute of Radioisotopes and Radiodiagnostic Products

Further information for NCSR "D" can be found at www.demokritos.gr

All expressions of interest should include:
a. Recent CV
b. Research Plan
and should be sent by the 28th of February 2009 to the following address:

Search Committee/Human Resources Department
National Center for Scientific Research "Demokritos"
Aghia Paraskevi, Attikis,153 10, Athens, Greece
e-mail: martaki@admin.demokritos.gr Fax: +30 210 6510594

HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

12 quai Saint-Jean, 67000 STRASBOURG, FRANCE

CALL FOR LETTERS OF INTENT FOR INTERDISCIPLINARY RESEARCH GRANTS: AWARD YEAR 2010

The Human Frontier Science Program supports **international** preferably **intercontinental** collaborations in basic life science research with emphasis placed on *novel*, **innovative** and **interdisciplinary** approaches to fundamental investigations. Applications are invited for grants to support new approaches to understanding **complex mechanisms of living organisms**. Preliminary results are not required. Applicants are expected to develop new lines of research distinct from their ongoing research.

There are two types of Grant: Young Investigators' Grants are for teams of scientists who are **all** within 5 years of establishing an independent laboratory and within 10 years of obtaining their PhDs. **Program Grants** are for independent scientists at all stages of their careers, although the participation of younger scientists is especially encouraged.

Grants provide 3 years support for 2 – 4 member teams, with not more than one member from any one country, unless critical for the interdisciplinary nature of the project, which is an essential selection criterion. Applicants may establish a local or national **interdisciplinary** collaboration as a component of an international team but will be considered as 1.5 team members for budgetary purposes. Awards are dependent upon team size and successful teams will receive up to $450,000 per year. The principal applicant must be located in one of the member countries (Australia, Canada, the European Union, France, Germany, India, Italy, Japan, New Zealand, Norway, the Republic of Korea, Switzerland, the United Kingdom and the United States) but co-investigators may be located in any country.

Guidelines and further instructions are available on the HFSP web site (www.hfsp.org). International teams of scientists must first submit a letter of intent online via the web site. Specific enquiries: grant@hfsp.org

Deadlines : Compulsory pre-registration, via the web site: March 20th 2009
Submission of Letters of Intent: March 31st 2009

UNIVERSITY OF ALBERTA
EDMONTON, ALBERTA, CANADA

www.careers.ualberta.ca

Assistant Professor, Anatomy

The Division of Anatomy in the Faculty of Medicine invites applications for a tenure-track position at the Assistant Professor level. We seek an individual capable of teaching human gross anatomy to medical and dental students with full dissection laboratories. The ability to teach embryology, histology and neuroanatomy will be an added advantage. The successful candidate will be expected to carry a substantial teaching load and engage in individual or collaborative research in any one of the anatomical disciplines or in the field of medical education.

Applicants must have an MD and/or PhD degree and significant experience in teaching all aspects of human anatomy, as well as a proven track record in independent or collaborative research.

The Division of Anatomy is primarily a teaching Division with responsibilities for teaching human anatomy to undergraduate and postgraduate students across campus. The Division of Anatomy also teaches postgraduate clinical anatomy courses to residents and fellows from a variety of clinical disciplines and specialties. We are dedicated to innovation and excellence in the teaching of anatomy and to the advancement of knowledge through research in basic and clinical anatomy, neurosciences, development, functional morphology and educational technology.

Interested candidates should submit a letter of application, an up to date curriculum vitae and teaching dossier as well as a two-page statement outlining your teaching philosophy and research interests. Three reference letters should also be forwarded. Please send your application package by February 28, 2009.

Interested applicants may apply to:

Dr. Anil H. Walji
Director, Division of Anatomy
Faculty of Medicine & Dentistry
5-05B Medical Sciences Building
University of Alberta
Edmonton, AB Canada T6G 2H7

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. If suitable Canadian citizens and permanent residents cannot be found, other individuals will be considered. The University of Alberta hires on the basis of merit. We are committed to the principle of equity in employment. We welcome diversity and encourage applications from all qualified women and men, including persons with disabilities, members of visible minorities, and Aboriginal persons.

Colorado State University

Senior Faculty and Leadership Positions in Infectious Diseases

Colorado State University and the Department of Microbiology, Immunology, and Pathology invite applications for three senior positions in infectious diseases. The three positions are:

- Bacteriologist Associate/Full Professor, Tenure Track
- Virologist Associate/Full Professor, Tenure Track
- Director Infectious Disease Supercluster and Infectious Disease Research Center

The successful candidates will join world class research and training programs in infectious diseases and will have opportunities to assume leadership roles in the innovative translational research activities at CSU, for example in the Infectious Disease Supercluster; MicroRx, the enterprise arm of the IDSC; the Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Diseases; the Mycobacterial Research Laboratory; the Arthropod-borne and Infectious Diseases Laboratory; the Prion and Molecular Diseases Program; and Program in Food Science/Safety.

The resources and facilities for infectious disease research and training at CSU are exceptional. Extramural funding for the Department of MIP was $25M in the past year. The facilities for ID and translational research at CSU include the Infectious Disease Research Center on the CSU Foothills campus, which encompasses the Rocky Mountain Regional Biocontainment Laboratory, the Bioenvironmental Research Building, virology, discovery, and large animal suites, and core support for animal models, cGMP manufacturing, and proteomics and genomics analyses. The impending Research Innovation Center will provide business incubator, discovery, and high throughput screening capacity for development of new knowledge and vaccines, therapeutics, and diagnostics for BSL-2 as well as BSL-3 pathogens.

The research and teaching environment at CSU is enriched by the highly interactive and collaborative environment with world-recognized ID programs and scientists on campus and nearby, for example, in the Centers for Disease Control-Division of Vector-Borne Infectious Diseases, the United States Department of Agriculture-National Wildlife Research Center and Centers for Epidemiology and Animal Health and the USDA Arthropod-borne Animal Diseases Research Laboratory in Laramie, WY.

Further information concerning the positions can be obtained by contacting the Chair of the respective committee and at **http://www.cvmbs.colostate.edu/mip/jobs/ids.htm**.

Applications and nominations will be considered until the position is filled; however, to ensure full consideration applications should be submitted by **February 16, 2009**. To apply, please send electronically a complete CV, a one or two page statement of research interests and goals, and the names and addresses of three professional references to the Chair of the respective committee.

Ralph E. Smith, Ph.D.
Chair, IDSC/IDRC Director Search
ralph.smith@colostate.edu
970-491-8382

Carol D. Blair, Ph.D.
Chair, Virologist Search
carol.blair@colostate.edu
970-491-6794

Patrick J. Brennan, Ph.D.Chair,
Chair, Bacteriologist Search
patrick.brennan@colostate.edu
970-491-6700

Colorado State University conducts background checks on all final candidates.
CSU is an Equal Opportunity/Affirmative Action Employer.

UAB COMPREHENSIVE CANCER CENTER

Basic and Translational Cancer-Relevant Research

The University of Alabama at Birmingham Comprehensive Cancer Center, in collaboration with departments in the School of Medicine and across the University, invites applications for multiple faculty positions at all levels in basic and translational cancer – related research. Specific areas of interest include, but are not limited to:

Cancer Immunology
Cancer Genetics
Cancer Virology
Cell Signaling and Growth Control
Drug Development
Animal Models of Cancer
Epigenetics
Molecular Epidemiology
Cancer Nutrition
Cancer Survivorship
Behavioral Intervention
Biomarkers & Imaging

This broad-based recruitment initiative is made possible by the UAB – CCC Cancer Research Fund, which provides approximately $60 million for cancer research activities. The major goal for this Fund is to recruit outstanding faculty at all levels to enhance and expand UAB's excellence in basic and translational science related to cancer.

Successful candidates will establish a rigorous research program, participate in graduate and medical education, and collaborate with basic and clinical cancer scientists. Collaborations at UAB are stimulated by the presence of numerous multidisciplinary Centers, including the Comprehensive Cancer Center, Autoimmunity Center of Excellence, Center for Clinical and Translational Science, Center for AIDS Research, and others. In addition UAB is ranked the 5th Best Place to work in academics according to the Scientist magazine.

Applicants for all positions should email their curriculum vitae, a description of research accomplishments and future plans, and the names and contact information of three references to:

Michael Bertram, Ph.D.
Associate Director for Administration
UAB Comprehensive Cancer Center
Michael.Bertram@ccc.uab.edu

Review of applications will begin immediately and will continue throughout 2009.

UAB is an Affirmative Action/Equal Opportunity Employer. Women and minorities under-represented in biomedical research are encouraged to apply.

KU THE UNIVERSITY OF KANSAS

Department of Pharmaceutical Chemistry Assistant Professor

The Department of Pharmaceutical Chemistry, University of Kansas (**http://www.pharmchem.ku.edu/**) invites applications for a full-time tenure-track faculty position at the Assistant Professor level for the 2009-2010 academic year. Candidates must have a Ph.D. or equivalent degree; demonstrated research activity and abilities in pharmaceutical chemistry (pharmaceutics), or related areas such as biomaterials and bioengineering areas related to drug delivery and formulation especially in cancer related areas. The candidate should demonstrate effective verbal and written communication and have demonstrated the ability to work with diverse people in a variety of situations. Postdoctoral experience is preferred. See **http://jobs.ku.edu** for the full announcement. The successful candidate will be expected to develop and sustain an independent, externally funded research program that includes multidisciplinary collaborations and to participate in teaching activities in the School of Pharmacy's professional program and in the Department's graduate program. Applications will be accepted until the position is filled. Screening of applications will begin **February 15, 2009**. Applications, including a letter of interest, a concise summary of past research and future research plans, a resume, and the names and addresses of three references should be sent to: **Dr. Teruna J. Siahaan, Department of Pharmaceutical Chemistry, The University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047-3729; (785) 864-7327**. *EO/AA Employer.*

UAB

University of Alabama at Birmingham
Faculty Positions in Virology and Immunology

The Department of Microbiology invites applications for up to six tenure-track junior or senior faculty positions in virology and immunology. Successful candidates will have demonstrated records of originality and productivity in research, existing or outstanding potential for consistent extramural research funding, and concerned interest in graduate and medical education. UAB ranks in the top 20 institutions in NIH funding for research and offers an unusually interactive research environment. Collaborations among the basic science disciplines and between basic and clinical faculty are stimulated by the presence of many multidisciplinary Centers, including a leading Center for AIDS Research, a Comprehensive Cancer Center, a Comprehensive Arthritis, Musculoskeletal and Autoimmuninty Center, a Comprehensive Diabetes Center, a Center for Clinical and Translational Science, a Center for Emerging Infections and Emergency Preparedness, a Liver Center and a campus-wide Program in Immunology. State-of-the-art BSL3/ABSL3 containment facilities are available.

Current areas of research strength are highlighted on the department web site (**http://www.microbio.uab.edu/interests.html**). Successful applicants can provide expertise in new, complementing areas or can extend existing strengths. Investigators in the areas of tumor virology, HIV, select agent research, cancer immunology, inflammation and cancer, immunology of diabetes, autoimmunity, and translplantation immunology are particularly encouraged to apply.

Review of applications will begin immediately and will continue until the positions are filled. Applicants are asked to submit (preferably electronically) their c.v., a 2-4 page summary of their research accomplishments and future plans, and the names and contact information of three references to: **David D. Chaplin, M.D., Ph.D., c/o Michael Settine, email: msettine@uab.edu; UAB Dept. of Microbiology, 845 19th Street S., Birmingham, AL 35294. Tel: (205) 934-3598**.

UAB is an Affirmative Action/Equal Opportunity Employer. Women and minorities under-represented in biomedical research are encouraged to apply.

Westlake International conference on Personalized Medicine
May 29-31, 2009
Zhejiang WTO-World Hotel, Hangzhou, China

www.westlakeconference.org

Themes include: Systems biology, Genomics and personalized genome, Proteomics and biomarkers, Nutrition, Drugs discovery, Bioinformatics and Regulatory issues in personalized medicine.

Organizers: Zhejiang-California International NanoSystems Institute (ZCNI), Zhejiang University, China

Conference co-chairs: Jianren Gu (顾健人院士), Lanjuan Li (李兰娟院士)

Conference executive chairs: Jun Yang (杨军), Biaoyang Lin (林标扬)

Keynote speaker: Leroy Hood

Yana Mi
Tel: +0086-571-86971506, 86971535
Fax: +0086-571-86971539
Email: amynmi@gmail.com

Medicines for Malaria Venture (MMV)
7th CALL FOR LETTERS OF INTEREST

Medicines for Malaria Venture is a not-for-profit Organization committed to the discovery, development and delivery of affordable anti-malarial drugs through public-private partnerships. We are looking towards the next generation of molecules which will power the agenda for the eradication of Malaria.

Three areas are highlighted. (a) The development of new non-Artemisinin Combination Therapies for uncomplicated malaria, to support the portfolio of medicines, should resistance to *P.falciparum* Malaria occur. (b) The development of new medicines to produce a radical cure by targeting the hypnozoite stages of *P.vivax* and (c) new medicines that in addition to working on the erythrocyte stages will also have activity against gametocytes and therefore play a role in transmission blocking.

Discovery projects are welcome at the stage where a target has been identified, and structures are available for medicinal chemistry evaluation. Projects based on molecules where the molecular basis of activity is not known are also welcome, but starting points should be sub-micromolar in whole parasite assays, selective against host, and with some evidence for bioavailability and in vivo activity in an infected rodent model. We are particularly interested in molecules that have long half lives.

Projects in clinical development are especially welcome. Medicines or new combinations that allow the development of new combination therapies, or target radical cure of *P.vivax* or transmission blocking are encouraged. Specifically, formulation development that increases the half-life and absorption of medicines are encouraged, especially for Primaquine or other agents with the potential for radical cure of *P.vivax* malaria.

Applications may be from single institutions or partnerships between an academic centre and a Pharmaceutical company.

The initial application should be by sending a letter of interest of no more than three pages to

Dr. Ian Bathurst
MMV, Rte de Pré-Bois, 20
P.O.Box 1826
CH-1215 Geneva 15, Switzerland
E-mail: proposals@mmv.org

Applications should reach us by March 15th 2009. Electronic submissions are preferred.

More details of the call can be found at

www.mmv.org

POSITIONS OPEN

DEAN of MISHER COLLEGE of ARTS and SCIENCES
University of the Sciences in Philadelphia

The University of the Sciences in Philadelphia (USP) is a unique and dynamic institution, focused on the critical areas of science and health. USP invites applications and nominations for its next Dean of Misher College of Arts and Sciences (MCAS). The Misher College of Arts and Sciences is largely responsible for offering the general educational requirements of all of the undergraduate students on campus. Many of the faculty in MCAS and its undergraduate and graduate students are heavily involved in the pursuit of research/scholarship.

The University seeks a leader who can help shape, communicate, and implement a bold vision and strategy for the College.

Opportunities and expectations for leadership: grow the College and its programs, both undergraduate and graduate; increase the College's visibility both on and off campus; be an effective advocate for the College, both internally and externally; foster interaction amongst all members of the College, including faculty, students, staff, and alumni; understand the challenge of assessment and lead the process in the College; unite the College departments with a shared purpose; and continue development of the College's interdisciplinary efforts.

Qualities and qualifications: terminal degree in a discipline associated with a college of arts and sciences; experience with creating and implementing plans grounded in data; experience with both undergraduate and graduate education; demonstrated commitment to excellence in teaching and support of faculty development; demonstrated research experience; demonstrated ability to work with those from other disciplines; demonstrated administrative experience commensurate with the demands of an academic Dean; collegial management style, coupled with the ability to be both fair and decisive; and excellent communication skills.

Procedures for candidacy: inquiries, nominations, and applications are invited. In order to ensure consideration, candidates should submit their materials by March 1, 2009. Candidate review will take place after that date, with selection expected during the spring semester. The successful candidate should be able to start on or after July 1, 2009.

Materials should include a cover letter describing interest in the position, curriculum vitae, and the names and contact information for five references; candidates will be notified before references are contacted. Materials should be submitted in confidence to **e-mail: r.wigent@usp.edu.** Material that cannot be e-mailed may be sent to the **University, Dr. Rodney Wigent, University of the Sciences in Philadelphia, 600 S. 43rd Street, Philadelphia, PA 19104.**

USP is an Equal Opportunity/Affirmative Action Employer.

The Department of Biological Sciences at the University of the Sciences in Philadelphia seeks applicants for a one-year (renewable) **POSTDOCTORAL TEACHING FELLOWSHIP** beginning in January 2009. The position requires 30 percent teaching and 70 percent research. The successful candidate will teach undergraduate introductory biology laboratories and participate in lectures. Significant mentoring will be provided by experienced educators. The successful applicant will join a dynamic research group studying the engineering of microbes for production of natural products. A strong background in molecular biology techniques, and experience with analytic analysis of compounds (i.e., high performance liquid chromatography, gas chromatography/mass spectrometry) is highly desired. Individuals with a background in yeast physiology are also encouraged to apply. Applicants should have received their Doctorate within the last two years in a relevant biological science. Please submit a cover letter, curriculum vitae, graduate transcripts (copies), and three letters of reference directly to the address below. All application materials must be sent to:

J.A. Teaching-Postdoctoral Fellow Search
Department of Biological Sciences
University of the Sciences in Philadelphia
600 S. 43rd Street
Philadelphia, PA 19104-4495

Cold Spring Harbor Laboratory 2009 Meetings & Courses

Meetings

Plant Genomes: Genes, Networks & Applications
March 4 - 7 abstracts due January 9

Systems Biology: Networks
March 18 - 22 abstracts due January 9

Computational Cell Biology
March 24 - 27 abstracts due January 16

Molecular and Cellular Biology of Plasminogen Activation
March 31 - April 4 abstracts due January 23

Synapses: From Molecules to Circuits & Behavior
April 14 - 18 abstracts due January 30

The Ubiquitin Family
April 21 - 25 abstracts due January 30

Telomeres & Telomerase
April 28 - May 2 abstracts due February 6

The Biology of Genomes
May 5 - 9 abstracts due February 13

Phosphorylation, Signaling & Disease
May 12 - 16 abstracts due February 20

Retroviruses
May 18 - 23 abstracts due February 27

74th Symposium: Evolution - The Molecular Landscape
May 27 - June 1 abstracts due March 6

Workshop on Single Cell Techniques
July 16 - 19 abstracts due April 17

Yeast Cell Biology
August 11 - 15 abstracts due May 22

Eukaryotic mRNA Processing
August 18 - 22 abstracts due May 29

Mechamisms of Eukaryotic Transcription
August 25 - 29 abstracts due June 5

Eukaryotic DNA Replication & Genome Maintenance
September 1 - 5 abstracts due June 12

Microbial Pathogenesis & Host Response
September 8 - 12 abstracts due June 19

Personal Genomes
September 14 - 17 abstracts due June 26

Stem Cell Biology
September 22 - 26 abstracts due July 3

Neurobiology of *Drosophila*
September 29 - October 3 abstracts due July 10

Cell Death
October 6 - 10 abstracts due July 17

Genome Informatics
October 27 - 30 abstracts due August 7

Harnessing Immunity to Prevent and Treat Disease
November 11 - 14 abstracts due August 21

In Vivo Barriers to Gene Delivery
November 18 - 21 abstracts due August 28

Rat Genomics & Models
December 2 - 5 abstracts due September 4

Courses

Protein Purification & Characterization
April 15 - 28

Cell & Developmental Biology of *Xenopus*
April 17 - 28

Molecular Neurology & Neuropathology
June 3 - 9

Advanced Bacterial Genetics
June 3 - 23

Ion Channel Physiology
June 3 - 23

Molecular Embryology of the Mouse
June 3 - 23

Integrative Statistical Analysis of Genome Scale Data
June 8 - 23

Workshop on Autism Spectrum Disorders
June 19 - 26

Computational Cell Biology
June 26 - July 16

Molecular Techniques in Plant Science
June 26 - July 16

Neurobiology of *Drosophila*
June 26 - July 16

Advanced Techniques in Molecular Neuroscience
June 30 - July 16

Structure Function & Development of the Visual System
July 1 - 14

Biology of Memory
July 16 - 29

Proteomics
July 17 - August 1

Imaging Structure & Function in the Nervous System
July 21 - August 10

Yeast Genetics & Genomics
July 21 - August 10

Eukaryotic Gene Expression
July 21 - August 10

Cellular Biology of Addiction
August 4 - 10

C. elegans
August 14 - 29

Programming for Biology
October 12 - 27

X-Ray Methods in Structural Biology
October 12 - 27

Workshop on Cereal Genomics
October 13 - 19

Advanced Sequencing Technologies & Applications
November 3 - 16

Immunocytochemistry, In Situ Hybridization & Live Cell Imaging
November 3 - 16

Phage Display of Proteins & Peptides
November 3 - 16

Computational & Comparative Genomics
November 4 - 10

The Genome Access Course
April 20 - 21, November 17 - 18

Cold Spring Harbor Laboratory, Meetings & Courses Program, 1 Bungtown Road, Cold Spring Harbor, NY 11724
Phone 516 367 8346, email meetings@cshl.edu, web www.cshl.edu/meetings

POSITIONS OPEN

BIOMOLECULAR NMR SPECTROSCOPIST
Indiana University

Indiana University has significantly expanded its nuclear magnetic resonance (NMR) capabilities with the installation of 600 and 800 megahertz (MHz) Varian Inova spectrometers in the new METACyt Biomolecular NMR Laboratory in Simon Hall. We now seek to appoint a Biomolecular NMR Spectroscopist at the **ASSISTANT SCIENTIST** (research-track) level, effective June 1, 2009. Candidates must have a Ph.D. in chemistry, biochemistry, structural biology, or a related field; two years of postdoctoral experience is desired. The successful candidate will have documented experience with the application and optimization of modern biological NMR spectroscopy to structural studies of proteins. Familiarity with aligned systems and nucleic acids is desirable. Experience with the operation and maintenance of high field NMR spectrometers (600 and 800 MHz) and cryogenic probe systems is required, and knowledge of hardware and Unix-based software maintenance is essential. Familiarity with Varian systems and methods of NMR data analysis through structure calculation and validation stages are also desirable but not necessary.

Applicants should submit a brief statement of interest, curriculum vitae, and arrange for three letters of recommendation to be sent to: **Prof. James P. Reilly, Chair of Search Committee, Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405.** Applications received by March 1, 2009, will be assured consideration. *Indiana University is an Equal Opportunity Affirmative Action Employer.*

RESEARCH ASSOCIATE 3, 4, or 5 (MICROSCOPY IMAGING SPECIALIST)
Department of Biological Sciences

The Socolofsky Microscopy Facility at Louisiana State University (LSU, **website: http://www.biology.lsu.edu/facilities/micro_fac/**) is a core facility serving the microscopy needs of the LSU research community. Required qualifications: (Research Associate 3) Bachelor's degree in biology or related field and three years of related experience or a Master's degree in a related field and one year of experience; (Research Associate 4) Master's degree in biology or related field and two years of related experience; (Research Associate 5) Master's degree in biology or related field and three years of related experience or a Ph.D. in a related field; (all levels) experience in electron microscopy. Responsibilities: prepares scanning electron microscopy and transmission electron microscope samples; performs image acquisition and analysis and user training; oversees scanning and transmission electron microscopes; supervises staff involved in light and electron microscopy. *An offer of employment is contingent on a satisfactory pre-employment background check.* Application deadline is January 30, 2009, or until candidate is selected. Send a resume (including e-mail address) and two letters of recommendation to: **Cheryl Thompson, Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Reference #006213, Baton Rouge, LA 70803. E-mail: cthomps@lsu.edu.** *LSU is an Equal Opportunity/Equal Access Employer.*

ASSISTANT PROFESSOR
Vertebrate Physiology/Environmental Science
Framingham State College

We seek a **VERTEBRATE PHYSIOLOGIST** whose work is applicable to environmental issues for a tenure-track faculty position. Teaching responsibilities will include comparative vertebrate physiology, introduction to environmental science, introductory biology courses, and a course in applicant's area of expertise. Minimum qualifications include a Ph.D. in biology and teaching experience. Application deadline is January 10, 2009. For complete application instructions and to apply online please visit **website: http://framingham.interviewexchange.com.**

ASSISTANT SPECIALIST in COOPERATIVE EXTENSION. An 11-month, career-track extension position which has 100 percent cooperative extension responsibilities. Candidate will be located at University of California, Davis Department of Plant Sciences. Candidate will develop and implement an extension and applied research program in vegetation management in perennial crops, particularly woody species, using ecologically sound approaches. Emphases will be on the ornamentals, floriculture, tree fruits, nuts, and vines grown in California. Candidate will provide statewide extension leadership, interact with numerous clientele groups, provide farm adviser training and advising, and develop an Affirmative Action program. Candidate will have the opportunity to participate in departmental teaching and in directing undergraduate and graduate research. Requirements include: a Ph.D. in weed science, pomology, horticulture, or related field with emphasis on vegetation management practices; leadership ability; management and communication skills; ability to conduct independent research in weed science must be demonstrated. Candidates should begin the application process by registering online at **website: http://recruitments.plantsciences.ucdavis.edu.**

Review of the applications for this position will begin February 20, 2009. The position will remain open until filled. **Dr. W. Thomas Lanini, Chair, Search Committee, Department of Plant Sciences, Mail Stop 4, One Shields Avenue, University of California, Davis, CA 95616; telephone: 530-752-4476; fax: 530-752-4604.** *UC Davis is an Affirmative Action/Equal Employment Opportunity Employer and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply; including women, minorities, veterans, and individuals with disabilities.*

FACULTY POSITION in NEUROBIOLOGY
University of Louisiana at Lafayette

The Department of Biology at the University of Louisiana at Lafayette invites applications for a tenure-track position in neurobiology at the **ASSISTANT PROFESSOR** level. Research interests may include but are not limited to neurophysiology, neural development, evolutionary neurobiology, and neuroethology. Applicants must have a Ph.D. in a biological science and postdoctoral experience. The successful candidate will establish a vigorous, externally funded research program that complements our doctoral program and will participate in teaching and advising. The Department consists of 30 faculty with broad research interests; resources include a state-of-the-art microscopy center. For additional information see **website: http://biology.louisiana.edu/.** The University has a diverse student body and embraces diversity among the faculty. Applicants should submit a cover letter, curriculum vitae, and statements of research and teaching interests to: **Biology Search Committee, Department of Biology, University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, LA 70504.** Three letters of recommendation should be sent to **e-mail: bef1918@louisiana.edu.**

Review of applications will begin on February 16, 2009.

UL Lafayette is an Equal Employment Opportunity/Affirmative Action Employer. Applications from minority candidates are especially encouraged.

POSTDOCTORAL POSITIONS are available to study the molecular mechanisms regulating immunoglobulin G and complement mediated neutrophil recruitment and effector functions using in vitro and in vivo approaches. Strong background in molecular biology/cell biology or small animal experimentation, especially intravital microscopy is desired. Publication(s) in international journals is a prerequisite. Please send curriculum vitae and names of three references to: **Tanya Mayadas, Ph.D., Associate Professor of Pathology, Brigham and Women's Hospital and Harvard Medical School, 77 Avenue Louis Pasteur, NRB 752O, Boston, MA 02115; e-mail: tmayadas2@rics.bwh.harvard.edu; fax: 617-525-4333.**

The Department of Biochemistry and Biophysics at the University of Pennsylvania School of Medicine seeks candidates for an **ASSISTANT PROFESSOR** position in the nontenure research track. The successful applicant will have experience in the field of structure/function studies of the energy transducing NADH-quinone oxidoreductases such as Complex 1 with a focus on mitochondria and bacteria. Responsibilities include participating in an intensive team effort of research on the catalytic mechanism of oxidoreductases and relevant issues in mitochondrial disease, for example. Applicants must have a Ph.D. or equivalent degree and have demonstrated excellent qualifications in research.

The candidate should have at least three years of postdoctoral experience in bioenergetics and a strong background in biochemistry and biophysics, exemplified by recent scholarly productivity. Technical expertise in current methods of molecular biology and electron paramagnetic resonance techniques is highly desirable.

Please submit curriculum vitae, a cover letter, three reference names, and a statement of research interests to: **Ruth Keris, Staff Assistant, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059; e-mail: keris@mail.med.upenn.edu**

The University of Pennsylvania is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.

MARKETPLACE