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Science

AAAS

Volume 321, Issue 5886

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Individuals with autism, a neurodevelopmental disorder, show impairment in social communication and language. Genetic analysis of closely related people reveals clues to the neural deficits that may underlie the disorder. See page 218.

Image: Kenneth Xavier/Xavier Studio

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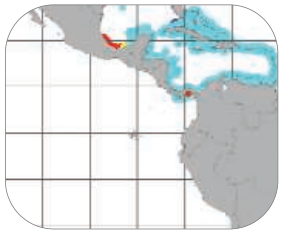

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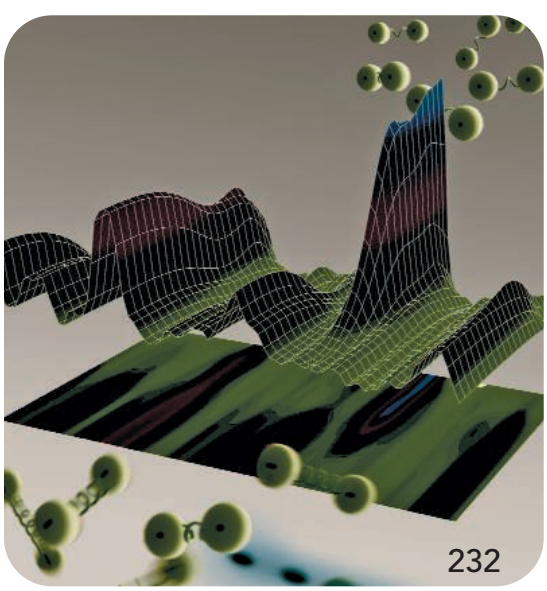

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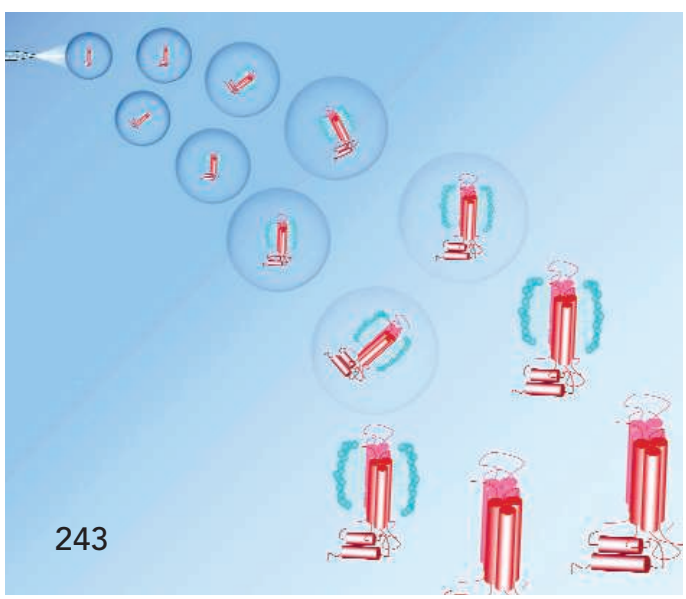

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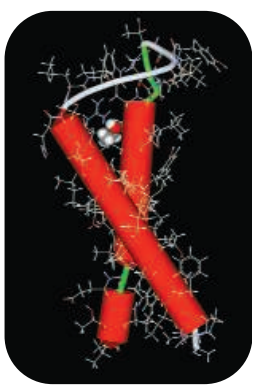

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FREE WEEKLY SHOW

Download the 11 July *Science* Podcast to hear about genetic links to autism, scientists' interactions with the mass media, bipolar disorder in children, and more.

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EDITED BY STELLA HURTLEY

Making Avalanches >>

Slab avalanches arise when weather changes cause a layer of low-density snow or ice to form between the base snow and a deep cover of snow. Such a layer can collapse under the weight of the snow cover, leading to a "wumph." However, under other conditions, the snow cover can slide off in a slab, producing a slab avalanche. It has been thought that the angle of the slope determined whether a wumph or an avalanche would occur. **Heierli *et al.*** (p. 240) analyze the snow fracture process using the concept of anticracks, where material displaces in an opposite manner to normal crack propagation, leading to a loss of cohesion and local density changes. The results of the analysis suggest that the angle of the slope is not the only factor in determining the avalanche type. Long-distance fracturing may be a cause for remote triggering of some avalanches.

Enzymes at Work

Recent technological advances in structural biology are now furnishing our first glimpses of the proteins that reside and work at the hydrophobic-hydrophilic boundaries provided by the lipid bilayers that constitute cellular membranes. **Forneris and Mattevi** (p. 213) review the sparse collection of membrane-associated enzymes whose crystal structures have been determined and classify them within a function-based framework that highlights how a diverse group of proteins have arrived at common strategies to cope with the challenges they face—of carrying out hydrolysis reactions within the interior of the membrane or of mediating the transfer of a hydrophobic substrate from the membrane into the enzyme's active site.

The Makings of Autism

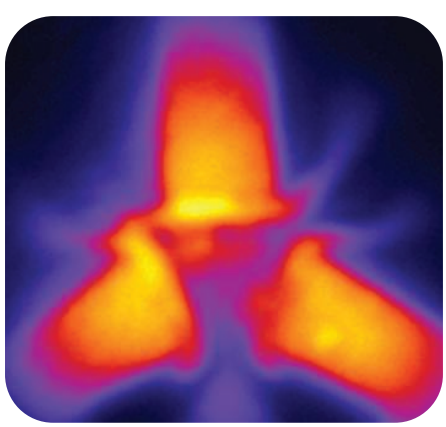
Autism is a common developmental disorder that impairs the acquisition of social skills and communication in children. The underlying causes of autism are unclear, but are likely to involve diverse and complex genetic factors. **Morrow *et al.*** (p. 218, cover; see the Perspective by **Sutcliffe**) have now used homozygosity mapping to identify genetic loci that correlate with susceptibility to autism. Results from a population enriched in consanguineous marriages highlighted the importance of autosomal recessive genes. The findings suggest that defects in activity-regulated gene expression may link many seemingly diverse autism mutations.

Going Supernova

Supernovae are massive stellar explosions that occur when a star runs out of nuclear fuel and collapses. Most are detected after the explosion has begun, which limits the amount of information available on how the explosion began and on the passage of the shock wave through the outer stellar material. **Schawinski *et al.*** (p. 223, published online 12 June) exploited an optical survey aimed at finding supernovae to search for ultraviolet light that preceded the optical light curve from the same star. For one recent supernova, a strong signal was observed 2 weeks before the optical detection. The data reveal the explosion just as it passes the shock wave at the star's surface and suggest that the supernova formed from a large red giant precursor star.

Excitonic Logic

In contrast to electrons, excitons (coupled electrons and holes held together by the Coulomb attraction) are optically active, which allows direct coupling between light and electronics. Compared to direct excitons that occur in bulk semiconductors, indirect excitons that are formed in coupled quantum well structures have longer lifetimes and can be spatially manipulated. **High *et al.*** (p. 229, published online 19 June) now show that "excitonic circuits" that exploit indirect excitons can be formed using patterned electrical contacts and that these circuits can perform simple logic processes using optical input and output signals.



Improving Solar Cells

Solar cells could generate more electricity if sunlight were to be collected over a wider area and concentrated on the cell. The use of mirrors can provide one solution, but can require tracking and lead to unwanted heating by also concentrating infrared radiation. An alternative approach is to use luminescent solar concentrators, waveguides that contain dye molecules that absorb light and then reemit at wavelengths that avoid reabsorption. In practice, self-absorption losses can be high. Now **Currie *et al.*** (p. 226) have created organic luminescent concentrators that avoid many of these losses by taking advantage of Förster energy transfer to lower dye concentrations and phosphorescent dyes that absorb more weakly in their emissive state.

The Geography of Rodinia

250 million years ago, all of the continents were assembled into a large supercontinent, Pangea. Its breakup had large effects on the geological evolution of the various fragments and affected evolution and Earth's climate. About 1 billion years ago, all the continents were also thought to be assembled into a supercontinent, Rodinia. The breakup of Rodinia controlled the geological and evolutionary history of the Paleozoic. The geography of Rodinia, and particularly connections across the western margin of North America, has been uncertain and widely debated. **Goodge *et al.*** (p. 235) present geochemical data on detritus in the Transantarctic Mountains and identify a rock clast there, which provides a link to a set of distinctive granitic rocks exposed in North America.

CREDITS (TOP TO BOTTOM): HEIERLI *ET AL.*; HIGH *ET AL.*

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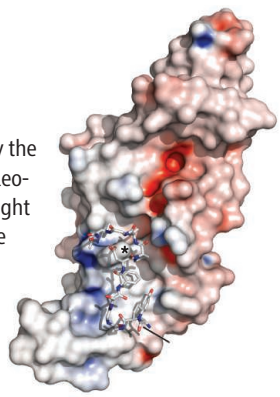
The data support a model in which Antarctica (to the south) and Australia were juxtaposed along western North America.

Enough's Enough

Achieving the efficient and specific uptake of extracellular molecules is only half the battle for any cell—there also needs to be a regulatory system that halts excessive accumulation. The bacterial membrane transporter ModBC hydrolyzes ATP to bring the oxyanions, molybdate and tungstate, into the cytoplasm. **Gerber *et al.*** (p. 246, published online 29 May) now show that a regulatory domain at the C-terminal end of the ModC subunit binds molybdate and in doing so blocks ATP hydrolysis by the rest of the ModC subunit. Within the crystal structure of the molybdate-inhibited ModBC complex, two molybdate ions are located at the interface between the two regulatory domains. Because the ATP-binding site is formed by the interface between these domains, the molybdate-bound ModBC cannot bind ATP.

Adapting from Co- to Posttranslational Membrane Targeting



Most membrane proteins are cotranslationally inserted into membranes by the Signal Recognition Particle (SRP) that has a universally conserved ribonucleoprotein core. However, the most abundant family of membrane proteins, light harvesting chlorophyll a/b–binding proteins (LHCPs), are imported into the chloroplast (cp) and then must be targeted to the thylakoid membrane by cpSRP. The latter contains no RNA, but is a heterodimer between cpSRP54, the chloroplast homolog of the SRP core protein, and cpSRP43, a protein dedicated to this targeting complex. Now **Stengel *et al.*** (p. 253) report the high-resolution structure of cpSRP43 alone and in complex with an internal signal peptide from LHCP. The overall shape and charge distribution of cpSRP43 resembles SRP RNA. In addition, SRP43 recognizes a specific motif in the LHCP peptide to adapt the conserved SRP system to achieve posttranslational targeting of LHCP proteins.

Know Thyself

Cells of the pathogenic bacterium *Proteus mirabilis* clump together to form colonies that, on meeting other colonies, can distinguish self from foreigners. *Proteus* detects then deters interlopers of the same species using polypeptide weapons called proticines. **Gibbs *et al.*** (p. 256) investigated the genetic basis of this self-nonself recognition system in *Proteus* and discovered a locus of six genes that seem to constitute general and specific recognition determinants, as well as accessory genes. Within infected hosts, *P. mirabilis* infections are usually clonal, and so this recognition system could be a way for an established clone to prevent superinfection by another clone and to avoid competition.

Resist or Persist

Many of the key immune pathways initiated in response to infection begin with the activation of the transcription factor NF-κb. Thus, it would not be unreasonable to expect that at least some pathogens would have evolved the means by which to impede this pathway. **Kravchenko *et al.*** (p. 259, published online 19 June) demonstrate that the opportunistic bacteria *Pseudomonas aeruginosa* produces a small signaling protein, C12, which impairs the turnover of proteins critical to the regulation of NF-κb activity. The resulting reduction in transcription of key immune response genes could have significant influence on persistent infection by this and other bacteria.

Exploiting Happy Coincidences

Treatment of human patients with therapeutic drugs and the annotation of side effects provide a large body of information about how these chemical agents affect human physiology. **Campillos *et al.*** (p. 263) analyzed data on more than 700 drugs currently on the market to determine whether shared side effects might be a useful predictor of drugs that, though chemically dissimilar, might share a similar mechanism of action on a target protein. Indeed, 13 of 20 pairs of drugs predicted to share a target were confirmed to show in vitro binding to a target protein. Although further analysis will be necessary, it seems existing drugs that have already been established to be safe in humans should possibly be able to be used for additional therapeutic purposes.

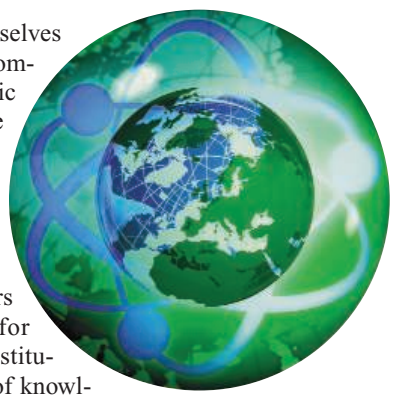
CREDIT: STENGEL *ET AL.*

Published by AAAS

Enric Banda is president of Euroscience and co-chair of the Euroscience Open Forum 2008.

European Research, 10 Years On

NEXT WEEK, BARCELONA WILL HOST THE EUROSCIENCE OPEN FORUM, EUROPE'S MAJOR interdisciplinary meeting, to discuss the latest trends in science, technology, the humanities, and policy. Euroscience, the founding organization of this meeting, was born more than 10 years ago, at a time when there was much debate on the need for new research structures and the desire to establish a common research "space" across Europe. In 2000, the European Union (EU) launched the European Research Area (ERA), a concept that aims to increase the impact of research efforts by strengthening the coherence of research activities and policies in Europe. In 2007, the EU launched the European Research Council (ERC), the first pan-European funding structure for frontier research in all fields of knowledge. So, is European science better off now than 10 years ago? The answer contains both good news and bad news.



Creating the ERA was an excellent idea, but we must remind ourselves that it is a work in progress. Many of its objectives have not been accomplished. For instance, investment in terms of percent of gross domestic product has remained flat for the last decade at around 1.8 to 1.9% (the goal is 3% for 2010). Although the ERA is theoretically the sum of 27 national programs, most member states have not delivered as far as restructuring their own systems to coordinate with a broader European network, dealing with inflexible academic structures, and addressing insufficient research funding.

The ERA Green Paper of 2007 only confirms this view. It appears to tackle some of the main issues: "good framework conditions for research, well trained and mobile researchers, excellent research institutions and research infrastructures, as well as an efficient exchange of knowledge between public research and industry." However, the Green Paper would have had more impact had it addressed the fundamental issues underlying the actual development of the ERA. For instance, its broad conclusion that EU funds aimed at structure and cohesion should increasingly aim at innovation and research simply restates what was said at ERA's inception. Statements like this will get us nowhere. We need action—a specific road map on how to achieve this lofty goal.

With or without a Green Paper, EU research ministers have shown on several occasions that they know where the problems are. The European Commission has the Framework Programme as its main mechanism to achieve Europe's research objectives. But when compared with the total public resources for research in Europe, it represents marginal (though very influential) funding. Therefore, the member states must act. The problem lies largely with them and not necessarily with the Commission. A European vision of a common research space continues to be an intimidating concept for some member states, who consider such notions a threat to their national sovereignty. Unfortunately, acting means providing resources and giving up some national hobbyhorses, and this is where we get stuck.

There is cause for optimism, however. The ERC can be qualified as a great success. It is the first model of its kind to be tried at a European level, and it has already accomplished one of its key goals of investing in, and raising the profile of, young researchers. It also has established good procedures for tying funding to research excellence. Interestingly, its formation was forced upon both the EC and member states by a bottom-up movement. The engagement of several institutions, such as the European Science Foundation and Euroscience, and the quality of the project, left the EU with few arguments to turn it down. Science will profit from the ERC, whose budget is projected to grow to several billion euros within the next few years.

The future of European research lies precisely in a European vision. Bottom-up success stories like the ERC are an encouraging sign that we are slowly moving in the right direction. But more than ever, it is the member states who will decide the course we run. It is time they showed courage and act as Europeans, united.

– Enric Banda

10.1126/science.1161888

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EDITED BY GILBERT CHIN AND JAKE YESTON

ECOLOGY

The Ups and Downs of Island Life

The equilibrium theory of island biogeography, developed in the 1960s by MacArthur and Wilson, has been the principal reference point for ecologists investigating how the dynamic processes of colonization, speciation, and extinction affect biodiversity in insular habitats. However, the theory has been less successful when applied to longer-term evolution on oceanic islands, where geological dynamics such as erosion come into play. Whittaker *et al.* have developed a general dynamic model (GDM) that takes account of the humped trend in an oceanic island's carrying capacity over its entire life span. The model generates predictions about the biotic properties (species diversity, rates of speciation and extinction) of oceanic islands that fit snugly with data from oceanic archipelagoes, including the Hawaiian islands and the Galápagos (shown above) among others. By unifying evolutionary and ecological time scales and processes in a single theoretical framework, the GDM adds to the understanding of island biology. — AMS

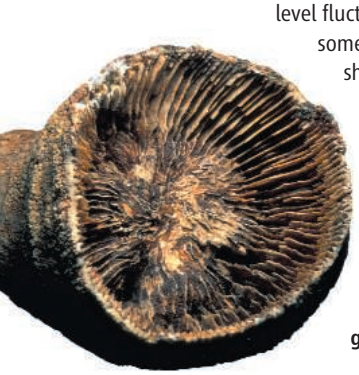
J. Biogeogr. **35**, 977 (2008).

GEOLOGY

Weighing Sverdrup Sediment

The huge extinction at the end of the Permian period (~252 million years ago) is marked globally by a large drop in the carbon isotope ratio ($^{13}C/^{12}C$) of organic matter preserved in sedimentary rocks. Low values persist for millions of years after the extinction, in part marking the delayed recovery of Earth's biota. In addition to providing a marker of the event, the magnitude and abruptness of the drop are important constraints on the likely cause(s) and pace of the extinction. Many sections show a relatively abrupt decrease, though some of these may be partly compressed by erosion; the Permian was a time when most of the land masses were assembled together in one supercontinent (Pangea), and sea level fluctuated markedly. Yet some sections seem to show a more complicated or gradual decrease. Grasby and Beauchamp describe several isotope records preserved across the Sverdrup Basin, Arctic Canada, which is now known to contain thick sections of Permian and Triassic rocks. Sections at the margin of the basin, where some erosion is documented to have occurred, show an abrupt carbon isotope drop at the boundary. Sections in the center, which seem to record continuous deposition and a thicker boundary layer than most other sections globally, show a more gradual decline over about 3 m, after a period of relative stability that is not well resolved elsewhere. Although the sections thus provide important detail on the extinction record and perhaps a period just before it, detailed dates are not yet available to calibrate absolute rates of change. — BH

Chem. Geol. 10.1016/j.chemgeo.2008.05.005 (2008).



Going, going, gone: extinct coral.

DEVELOPMENT

The Roots of Hair Growth

Adult hair follicles sustain repeated cycles of hair loss and regrowth. Stem cells reside within a small niche, called the bulge, located in the upper part of the hair follicle. These stem cells are responsible for driving this cycle of growth and can repopulate follicles and surrounding epidermis damaged by wounding. However, the first hair follicles in mice arise from the developing epidermis, not from preexisting bulge stem cells, and these first-time hair follicles do not exhibit the conspicuous bulge that only becomes apparent some weeks after birth.

Nowak *et al.* have analyzed the origins of the hair follicle stem cell niche in developing mice. The stem cells of the bulge are in fact established much earlier than previously suspected and begin to form in the embryo. These cells, which are characterized by expression of the transcription factor Sox9, not only contribute to the formation of the initial hair follicle, but also give rise to the adult bulge stem cells that are responsible for the maintenance of the hair follicle itself. Ablation of Sox9 left the embryos without bulge cells, and the mice never grew any hair and did not have any sebaceous glands. Moreover, the skin did not repair epidermal wounds well when Sox9 was missing. These results implicate Sox9 in establishment of the hair follicle stem cell population and show that early stem cells can contribute to skin morphogenesis before assuming their role as adult stem cells. — PJH

Cell Stem Cell **3**, 33 (2008).

PHYSICS

Every Little Second Counts

Part of the appeal of nuclear magnetic resonance phenomena is that the two-level system being manipulated (nuclear spin up, or nuclear spin

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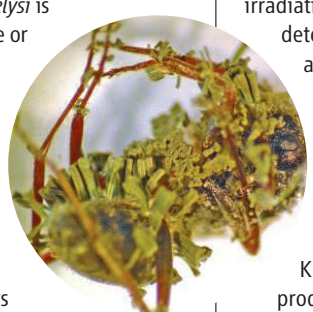
down) is comparatively easy to model in a quantum mechanical framework. In this context, application of a very short and strong ("hard") electromagnetic pulse to a spin ensemble can be approximated as having an instantaneous effect—a hard pi pulse, for instance, immediately rotating the aggregate spin vector 180°. Of course, such pulses are not precisely instantaneous, and Dong *et al.* show that it is possible to exploit their small but still finite durations to manipulate coherence in experiments that apply many of them, one after the other, in a train. The technique substantially reduces linewidth in inhomogeneously broadened samples, most strikingly by nearly five orders of magnitude for ^{29}Si resonances in antimony-doped silicon powder. The authors also apply the technique to ^{13}C probing in C_{60} samples. — JSY

Phys. Rev. Lett. **100**, 247601 (2008).

ECOLOGY

Resistance to Infection

In 1987, W. D. Hamilton wrote that the pressure of parasites is one of the factors favoring genetic diversity. The ant *Formica selysi* is found in colonies with single or multiple queens. Colonies with one queen have low genetic diversity, but workers tend to live longer than in polygynous colonies with high genetic diversity. In the wild, the multiqueen colonies are large, and individual workers are small and have short lives, mainly it seems because they do not provision so efficiently. So why does polygyny persist? The advantages of diversity became apparent when Reber *et al.* brought ants into experimentally controlled conditions to reduce the impact of compensating factors such as environmental variables. Artificial ant colonies revealed a strong advantage conferred by diversity when challenged by a fungal parasite; colonies of lower genetic diversity were decimated by infection. — CA



Ecol. Lett. **11**, 682 (2008).

CHEMISTRY

Radical Stabilization

The properties and reactions of single hydrogen atoms are of interest because of their inherent quantum mechanical behavior; experimentally, they can be generated and stabilized at very low temperatures (4 K) by high-energy irradiation of solid molecular hydrogen. Yeon *et al.* show that icy organic hydrates, which contain small cages that can trap guest molecules, can be used to create and trap H atoms at higher temperatures. They trapped H_2 in deuterated tetrahydrofuran hydrates (D_2O and THF-*d*8) at 123 K, using γ-ray irradiation to form stabilized H atoms that were detected by electron spin resonance (ESR) and magic-angle spinning proton nuclear magnetic resonance (MAS NMR) spectroscopy. Irradiating THF afforded ESR assignments for free D atoms and THF radicals that were also created. The temperature evolution of the MAS NMR signals from 173 to 183 K indicated that the formation of radical products H_2^+ and H_2^- likely occurred directly as opposed to being mediated by reaction with the ice framework. — PDS

J. Am. Chem. Soc. **130**, 10.1021/ja802952p (2008).

Science Signaling
AAAS

<< A Ligand, Not a Carrier

Volatile odorants are generally thought to bind to G protein–coupled receptors, and this event then activates downstream signaling pathways. In *Drosophila*, various odorant-binding proteins are secreted into the lymph around subsets of olfactory neurons; the function of these pheromone- and odorant-binding proteins, however, has been unclear. Building on earlier work that showed that the odorant-binding protein LUSH is required for sensitivity to the pheromone 11-*cis* vaccenyl acetate (cVA), Laughlin *et al*. have compared the x-ray crystal structure of LUSH bound to cVA with the previously determined structure of uncomplexed LUSH and found that binding of cVA (which was almost completely enclosed by LUSH) induced a conformational change in LUSH. A LUSH mutant bearing an amino acid substitution predicted to minimize this conformational change was less effective than the wild-type protein at conferring cVA sensitivity to T1 neurons, which mediate the response to this pheromone; in contrast, a mutation predicted to enhance the conformational change produced a more potent ligand. Moreover, a mutation yielding an uncomplexed LUSH conformation that resembled that of the cVA-bound form stimulated T1 neurons even in the absence of cVA. Thus, the authors conclude that LUSH is an inactive ligand that is converted into an active form through cVA binding. — EMA

Cell **133**, 1255 (2008).

CREDIT: ANABELLE REBER AND GRÉGOIRE CASTELLA

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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

Chopin's Heart

Frédéric Chopin died in France in 1849 at the age of 39 of what his death certificate recorded as "tuberculosis of the lungs and larynx." After his death, friends had the composer's heart removed, submerged in a jar of cognac, and placed in a Warsaw church in his native Poland in accordance with his wishes.

Now Polish scientists want to reopen the jar to see whether Chopin actually died of cystic fibrosis. Michal Witt of Warsaw's International Institute of Molecular and Cell Biology has argued that Chopin had childhood symptoms matching a mild form of the genetic illness, including respiratory infections, weakness, and delayed puberty. As an adult, Chopin was slight of stature, had a hard time climbing stairs, and occasionally had to be carried offstage after concerts. "If it turned out that Chopin had cystic fibrosis, this would be very special news for all those affected with CF," Witt says.

Witt hopes to persuade Polish authorities to open the niche where Chopin's heart is stored by 2010, the 200th anniversary of his birth. "It's a good moment to check, and once we have it in our hands it's a small matter to do a CT [computed tomography] scan and DNA test," says Tadeusz Dobosz, a geneticist at Wrocław Medical University. Poland's Culture Ministry is considering the request.

Was China an Early Emitter?

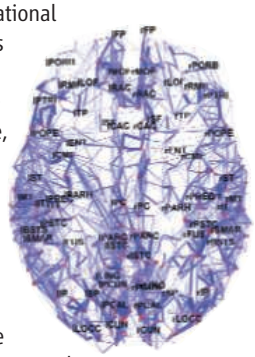
Last year, China overtook the United States as the world's leading emitter of greenhouse gases. But archaeological evidence suggests that the Chinese are old hands at global warming: Rice farmers may have begun making significant contributions thousands of years ago.

A Chinese-U.S. team led by William Ruddiman, a paleoclimatologist emeritus at the University of Virginia, Charlottesville, surveyed 311 archaeological sites in rice-growing regions of China. It found that between 6000 and 4000 years ago, the number of sites increased almost 10-fold. The timing coincides with evidence from other studies that atmospheric levels of methane, a byproduct of many farming activities, including wetland rice cultivation, began to increase about 5000 years ago. These findings, published in the July issue of *Quaternary Science Reviews*, are in line with Ruddiman's controversial earlier claims that human contributions to global warming began long before the industrialization of the 19th century (*Science*, 16 January 2004, p. 306).

Dorian Fuller of University College London, an expert in prehistoric Chinese agriculture, says the study adds "important and compelling" information in support of Ruddiman's hypothesis. He says climate modelers should also start looking at other early sources of atmospheric methane, such as cattle herding, which likewise increased dramatically about 5000 years ago.

Brain Traffic

The "connectome" of the human cortex has been produced by an international team of brain scientists and imagers led by Patric Hagmann of the University of Lausanne, Switzerland. Specialized regions of the neocortex are linked by a dense network of neural pathways, with several distinct nodes, like airline hubs, the researchers reported last week in *PLoS Biology*. The data were based on imaging brains of five male volunteers.



The Invisible Hand

Researchers in the United Kingdom are trying to help amputees speed up the process of getting used to prostheses by harnessing a well-known illusion.

In the "rubber hand" illusion, a person's hand and an adjacent rubber hand are both brushed gently. The real hand is kept out of sight. Before long, the subject's brain creates a new spatial link, imagining that the sensation in the real hand is arising where the rubber hand is.

Graduate student Matthew Mulvey of Leeds Metropolitan University has now shown that the effect will work if the researchers deliver transcutaneous electrical nerve stimulation (TENS) not to the hidden hand but to the wrist. After being primed with the illusion, subjects perceive the impulses—which hijack the nerve pathways between hand and brain—as a tingling located in the rubber hand. The researchers predict that with an amputee, a TENS signal from above the site of amputation would seem to come from the fake limb.

The team, which showed its results at the Royal Society's Summer Science Exhibition last week, hopes TENS can help amputees adapt faster to prostheses and possibly counter phantom limb pain, a major problem. Kate MacIver, a research nurse at the Pain Research Institute at the University of Liverpool in the U.K., says the idea is "harmless, … so it's worth a try."

CREDITS (TOP TO BOTTOM): THE GALLERY COLLECTION/CORBIS; INDIANA UNIVERSITY; COURTESY MARK JOHNSON

MOVERS

SPEAK UP. At 22, Tom Holder is already a veteran of a U.K. student-led campaign to counter demonstrations and vandalism by animal-rights activists. Now he's hoping to apply lessons learned from those battles to help scientists speak out about the benefits of animal research in the United States, where attacks by animal-rights extremists have been on the rise (*Science*, 21 December 2007, p. 1856).

"When a lot of people stand up together, the attacks go right down," says Holder, who founded the nonprofit group Speaking of Research (speakingofresearch.org) in March.

It's a message U.S. audiences need to hear, says the recent University of Oxford philosophy graduate, citing the presence of an armed police officer outside the hall at one recent university speech he gave. "It really sent the wrong message," Holder says. "I'm out there saying you're not going to get attacked for speaking up about this." The organization is also working with public schools to counter information from groups such as People for the Ethical Treatment of Animals.

Mary Hanley, executive vice president of the National Association for Biomedical Research in Washington, D.C., applauds Holder's efforts. "I wish there was an army of him," she says.

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

CREDITS (TOP TO BOTTOM): SOURCE: TOM HOLDER; SOURCE: NOLAN GASSER; LING CHEN; SOCIETY FOR WOMEN'S HEALTH RESEARCH

IN THE NEWS

CELESTIAL SYMPHONY. Composer Nolan Gasser admits he was "a little bit intimidated" when he was asked to write a musical accompaniment for last month's launch of the Gamma-ray Large Area Space Telescope (GLAST) (*Science*, 23 May, p. 1008). But his 9-minute *GLAST Prelude* for brass quintet delighted project scientists at NASA's Goddard Space Flight Center in Greenbelt, Maryland. The piece features notes timed to depict the electromagnetic spectrum and a crescendo of trombones and trumpets symbolizing the mission's energetic target of gamma ray bursts.

What he learned while researching the origins of supernovae and black holes has prepared him for his next commission: a 40-minute composition, with spoken and video segments, that will detail the history of the universe.

"The more we can bring an appreciation of science to the public, the better," says physics philanthropist Pierre Schwob, founder of classicalarchives.com and a cowriter of the work's libretto. Gasser's *Cosmic Reflection* will be performed by the Boston Symphony Orchestra in Washington, D.C., in September 2009.

IN THE FIELD

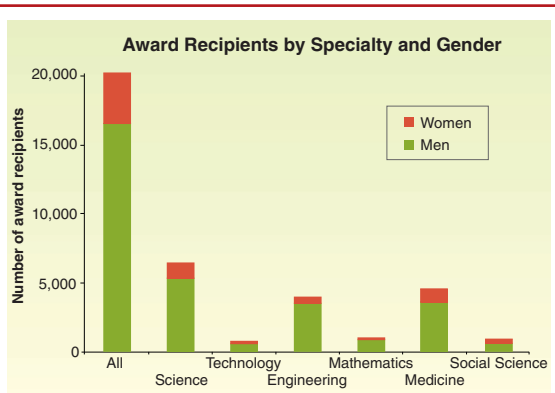
BATTING BUGS. Katharina Dittmar de la Cruz spent half of her evenings last month atop farmhouses and barns trying to figure out what's killing hundreds of thousands of bats in the northeastern United States. The evolutionary biologist at the University of Buffalo in New York state doesn't have an answer yet, but she's convinced that the flying mammals get a bum rap.

De la Cruz is trying to track down the cause of white-nose syndrome, a mysterious disease discovered last year that weakens a bat's immune system. She suspects that bat flies and other blood-sucking parasites may be carriers of the disease, and she plans to study their DNA to better understand how that transmission might occur. But the only way that she and undergraduate Doug Brummell could obtain enough samples was to perch on rooftops and pluck the insects off their hosts.

An avid caver with a fascination for organisms that lurk in dark corners, de la Cruz says bats' reputation as spooky beasts is misplaced. "I have been swarmed a few times, but they are more scared of me than I am of them."

Data Point >>

RAISING AWARENESS. Women now make up 33% of the U.S. science and engineering workforce, but the community has been slow to recognize their accomplishments. The RAISE project (www.raiseproject.org), an initiative to increase awareness of women's achievements in science, has analyzed 1011 award programs that have honored 20,373 people since 1981. Only 17% of the winners are women. And one-third of those were awarded by programs reserved for women. Even worse, women make up fewer than 1% of the honorees for 32% of the awards. The project is sponsored by the Society for Women's Health Research.



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FOOD SAFETY

Arsenic and Paddy Rice: A Neglected Cancer Risk?

BEIJING—Rice is the staff of life for 3 billion people, predominantly in Asia. But does the food that sustains half of humanity also increase the risk of cancer for some? That question arises from three sets of findings—including data now in press—that report elevated arsenic levels in rice and products such as rice bran and rice crackers.

Much of the arsenic found in these studies is in an inorganic form—the oxides arsenate and arsenite—known to sicken people exposed via drinking water. Cancer runs high in this population. "The problem is big," says Steve McGrath, a biogeochemist at Rothamsted Research in Harpenden, U.K., who studies contaminants in crops and is familiar with the new findings. Because rice accumulates arsenic, he says, even the background level "is a problem for people who eat much rice in their diet."

Experts caution that there are no data linking rice and cancer. Although there's "a definite need to reduce arsenic levels in rice," says Richard Loeppert, a crop scientist at Texas A&M University in College Station, "it's not an immediate hazard." A lead researcher, environmental biologist Zhu Yong-Guan of the Research Center for Eco-environmental Sciences in Beijing, acknowledges that "we still don't have all the answers." "But arsenic *is* arsenic," he says.

China agrees: It's one of a handful of countries that regulate arsenic levels in food. In 2005, the government lowered the acceptable limit in rice from 700 to 150 micrograms (μg) of inorganic arsenic per kilogram. Fish and other seafood contain an organic compound, arsenobetaine, that's largely benign at dietary levels. In a guidance document issued for shellfish consumption in 1993, the U.S. Food and Drug Administration recommended a "tolerable daily intake" of inorganic arsenic of 130 μg. But most governments, including the United States and the European Union, have not set legal limits on inorganic arsenic in food. The recent findings could provide an impetus for regulators to move faster.

Pick your poison? Zhu Yong-Guan's group is attempting to reduce arsenic levels in rice at this plot in China. In five varieties (inset) sampled in Zhu's lab, levels of inorganic arsenic ranged from 63 micrograms per kilogram (μg/kg) in Chinese white rice (far right) to 421 μg/kg in French red rice (center).

CREDITS: ZHU YONG-GUAN

Zhu and others are not waiting; they're already exploring ways to defang rice, which contains at least 10-fold higher arsenic concentrations than wheat and other cereals. Possibilities include altering farm practices—growing paddy rice in raised beds, for instance—and engineering rice plants to shed arsenic. The task is urgent, some say, because the global food crisis is increasing rice cultivation near mines or smelters, or on land formerly used to grow cotton or other crops that are often heavily treated with arsenic-based pesticides. Paul Williams, a postdoctoral researcher working with Zhu and Andrew Meharg, an environmental chemist at the University of Aberdeen, U.K., says, "We fear that more and more marginal land contaminated with arsenic will be used for growing rice."

Inorganic arsenic in a single dose of about 100 milligrams can kill by shutting down energy metabolism. Its chronic, low-dose effects are more insidious and first came to light in the early 1980s in India and Bangladesh, where many people who relied on arsenic-tainted wells developed arsenicosis, an ailment marked by rough skin that is often a prelude to serious diseases such as skin or bladder cancer. Tainted wells typically contain hundreds of micrograms of arsenic per liter, well above the maximum contaminant level of 10 μg per liter set by the World Health Organization (WHO) and adopted by most countries. Regions with high natural arsenic levels have been trying to develop alternative water supplies (*Science*, 23 March 2007, p. 1659). "Billions of dollars are spent to decrease arsenic levels in water," says Zhu. "Even if we solve that problem," he says, "it still gets into rice."

Paddy rice takes up arsenite readily from waterlogged soil, from which the element is liberated by anaerobic microbes, McGrath and colleagues reported online last month in *Environmental Science & Technology*. (Other crops grown in watery environments such as lotus, water chestnut, and water spinach also tend to have high arsenic levels.) WHO's limit for arsenic in water equates to a daily intake of 10 μg in food, Zhu, Williams, and Meharg note in an article this month in *Environmental Pollution*. Assuming an average daily rice consumption of 200 grams—a lowball estimate in Asia—the researchers calculate that arsenic levels would have to be as low as 50 μg per kilogram to remain below the WHO limit for water. However, Zhu and his colleagues report, surveys around the world have found that arsenic levels in rice "commonly exceed" the 50-μg threshold and can reach ▶

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400 μg per kilogram.

Last April, Meharg's group caused a sensation when it reported disturbing levels of arsenic in rice porridge sold in U.K. supermarkets for weaning infants. According to their findings in *Environmental Pollution*, 35% of baby rice samples they tested had arsenic levels exceeding China's permissible level (sciencenow.sciencemag.org/cgi/content/full/2008/430/1).

Two upcoming reports from the team could cause another stir. They found what Zhu calls "extremely high" levels of inorganic arsenic in rice bran, a common item in health food stores and a popular supplement for malnourished children in international aid programs. The samples of rice bran products they tested came from Japan and the United States. "Their research has shown the size of the problem and its international dimension," says McGrath, who calls their analyses "state of the art."

The food industry has sought to allay concerns. For example, after news reports last autumn about arsenic in U.S. rice, a top official at NutraCea, a company based in Phoenix, Arizona, that sells rice bran and bran extracts, in a letter to customers wrote that "the levels found in U.S. rice are well within food tolerances established by the Food and Drug Administration. U.S. rice has been consumed for over a hundred years with no reported human health problems." Williams argues that "there are no standards" in the United States for permissible maximum amounts of inorganic arsenic in food.

Experts have floated several mitigation strategies. Arsenic levels are lower in rice from certain regions, including California and parts of India; rice from these sources could be blended with higher arsenic rice before sale. But blending "may be difficult in poor areas with little infrastructure and subsistence diets," McGrath says. Another tack would be to tilt production toward upland rice, which is grown on dry land and absorbs far less arsenic than paddy rice. A third approach—growing paddy rice aerobically in raised beds—reduces the mobilization of soil arsenite and "can dramatically decrease arsenic transfer from soil to grain," McGrath says. But that would require a fundamental change in farming practices in Asia.

One attractive possibility is to tweak rice's metabolism. "Arsenic that accumulates in grain is effectively under genetic control," say Zhu. A decade ago, a team led by Barry Rosen, a molecular biologist at Wayne State University in Detroit, Michigan, showed that a family of proteins called aquaglyceroporins transports arsenic and other metalloids across cell membranes. Building on that work, Thomas Jahn's group at the University of Copenhagen in Denmark last month unveiled in *BMC Biology* an aquaglyceroporin subfamily, nodulin26-like intrinsic proteins (NIPs), in plants, including rice. It may be possible, says team member Gerd Bienert, to engineer plants to express NIPs that resist taking up arsenic—although that will be tricky, as NIPs facilitate the uptake of vital nutrients such as boron or silicon. Rosen's lab hopes to target arsenic by engineering aquaglyceroporins to discriminate between metalloids.

Another approach pursued by Rosen and Zhu is to create transgenic rice equipped with a bacterial enzyme—arsenite S-adenosylmethyltransferase—that converts inorganic arsenic to methylated species, including a volatile compound. "We propose that rice expressing the enzyme will volatilize arsenic, producing rice grains with reduced arsenic content," Rosen says. Field testing will begin in China soon. Rosen, Zhu, and colleagues are also using conventional breeding techniques to select for cultivars that accumulate little arsenic. To be a hit on the farm, any new varieties will have to have decent yields: A hypothetical cancer risk pales in comparison with an empty stomach. **–RICHARD STONE**

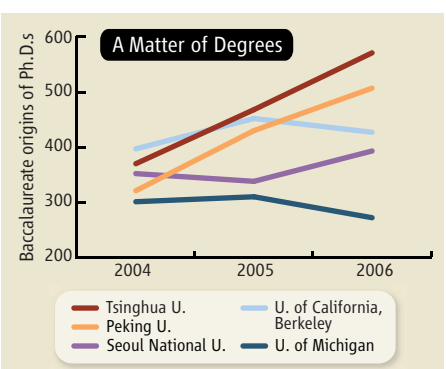
SOURCE: CPST ANALYSIS OF SED, 2006

U.S. GRADUATE TRAINING

Top Ph.D. Feeder Schools Are Now Chinese

The summer Olympics don't start until next month. But Chinese universities can already claim gold and silver medals in one important global competition involving institutions of higher learning.

A new study has found that the most likely undergraduate alma mater for those who earned a Ph.D. in 2006 from a U.S. university was … Tsinghua University. Peking University, its neighbor in the Chinese capital, ranks second. Between 2004 and 2006, those two schools overtook the University of California, Berkeley, as the most fertile training ground for U.S. Ph.D.s (see graph). South Korea's Seoul National University occupies fourth place behind Berkeley, followed by Cornell University and the University of Michigan, Ann Arbor.



Leading the pack. Tsinghua and Peking universities top Berkeley in having their graduates complete U.S. doctoral programs.

"The United States is very attractive to Chinese students, and they are certainly taking advantage of the opportunity to study here," says Judy Sui, director of data for Berkeley's graduate division. "At the same time, Chinese officials are trying hard to improve their system of higher education so that their students don't have to go abroad for graduate training."

The rankings were compiled by the Commission on Professionals in Science and Technology from a survey conducted by the U.S. National Science Foundation. In part, they reflect the fact that 37% of doctoral recipients from U.S. universities are not U.S. citizens. Sui says they also point to the wider choice of good careers available to U.S. students who hold a bachelor's or master's degree; foreign-born students are more likely to need a Ph.D. to find a good job, she says.

Berkeley still retains its top ranking for the number of undergraduates who went on to earn Ph.D.s over the past 10 years (1997 to 2006). But its total of 4298 isn't that far ahead of Seoul's 3420. And Tsinghua and Peking could well surpass their Korean rival in upcoming decadal tallies. **–JEFFREY MERVIS**

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U.S. BUDGET

2008 Supplemental Helps Fermilab By Putting Jobs Before Research

The only remaining U.S. lab dedicated to particle physics won't have to impose involuntary layoffs this month after getting a last-minute infusion of government money. The reprieve, for up to 100 scientists and technicians at the Department of Energy's (DOE's) Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois, is the result of a $186 billion supplemental spending bill to fund the wars in Iraq and Afghanistan.

The bill, signed 30 June by President George W. Bush after a compromise with the Democratic majority in Congress, contains $24 billion in domestic spending for 2008, including $62.5 million each for DOE science, NASA, and the National Science Foundation and $150 million for the National Institutes of Health (*Science*, 27 June, p. 1706). For the other three agencies, the extra money will mean a bit more for research. But for DOE, the focus is on preserving jobs. The agency, for example, still won't be able to make its promised contribution this year to ITER, a fusion reactor experiment being built in France. "Obviously, you can't run anything without the workforce, so the emphasis on jobs is probably correct," says Michael Lubell, a lobbyist with the American Physical Society in Washington, D.C.

In December, an unexpectedly small budget increase for DOE science forced officials at Fermilab, home of the Tevatron collider, to announce plans for layoffs affecting about 200 of its 1950 employees (*Science*, 11 January, p. 142). In February, lab officials instituted a rolling furlough that forced every employee to take 1 week every 2 months as unpaid leave. About 100 people have left since the beginning of the year, including about 50 who took voluntary layoffs last month.

In late May, the picture brightened when an anonymous donor gave $5 million to the University of Chicago to be spent at Fermilab. That ended the furloughs. And last week, Director Pier Oddone called off the involuntary layoffs.

The supplemental "was clearly targeted toward layoffs, and Fermilab was the poster child for layoffs," says a congressional staffer. Specifically, legislators told DOE to use the money to eliminate all layoffs "which are a direct result of budgetary constraints" and to resolve that problem before spending any money on research.

Some observers say the lab also benefited from a desire by Democratic leaders to help Representative Bill Foster (D–IL), a former Fermilab physicist, retain in the November elections the seat he won in a special election this spring. "Foster certainly can go back to his district and say, 'Look what I did [for you],' " Lubell says.

High-energy physics gets $32 million, all but a few million to be spent at Fermilab. Another $13.5 million goes to basic energy sciences, which supports x-ray sources and other "user facilities" for materials science, structural biology, and other fields. More than half goes to the Advanced Photon Source at Argonne National Laboratory in Illinois, preventing layoffs amounting to about 10% of the 500-person APS staff. Nuclear physics receives $1.5 million.

The ITER program gets a $15.5 million slice of the supplemental. That's far less than the $160 million that DOE had told its six partners it would spend this year, the majority of which would have bought materials for the reactor parts the United States will build. The December spending bill contained only $11 million for ITER. DOE ▶

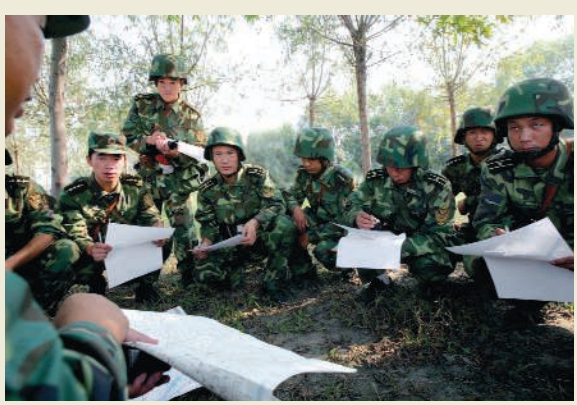
SOCIAL SCIENCES

Defense, NSF Team Up on National Security Research

The U.S. Department of Defense (DOD) hopes to spend $20 million this year on social science research aimed at understanding real and potential threats to national security. Part of the money will be funneled through the National Science Foundation's (NSF's) acclaimed merit-review process in an attempt to attract the best scientists—and to defuse criticism of DOD funding for such research.

"The relationship between [the Pentagon] and the social sciences—humanities in particular—for decades has covered the spectrum from cooperative to hostile," Defense Secretary Robert Gates said in an April speech to the Association of American Universities (AAU). His policy aide, Thomas Mahnken, says that NSF's role will help "make this program as attractive to the largest number of people as possible."

Inked last week, the 3-year agreement between the two agencies broadens a Pentagon initiative launched last month, called Minerva, that aims to bring academic social scientists into the defense research fold. Gates hopes that the 5-year, $100 million program, which he unveiled in his AAU speech, will build a firmer "intellectual foundation" in five areas: the ideological roots of terrorism, the changing face of Islam, the history of the Iraqi military, the vast unclassified literature on China's army, and miscellaneous other approaches to strengthening national security.



Better directions? U.S. defense officials hope social scientists can help them learn more about the Chinese military, shown in this 2007 exercise in Henan Province.

CREDIT: XINHUA/LANDOV

The program will have two arms of equal size. One will be managed by Defense officials and the other by NSF, with some Pentagon input on the selection of reviewers. "There are several topics of mutual interest" within the Minerva areas, says David Lightfoot, who heads NSF's social sciences directorate. "Securing the national defense was part of our charter in 1950," he adds.

Pentagon research official William Rees emphasizes that the money, which he hopes to award by December, is for "basic research

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Hug the messengers. Representative Bill Foster (at podium) and other dignitaries delivered good news last week to Fermilab scientists.

CREDIT: FERMILAB

reprogrammed $9 million of that amount this spring to cover salaries for the U.S. ITER offices, most of which are at Oak Ridge National Laboratory (ORNL) in Tennessee. Foster calls the $15.5 million a "symbolic" gesture that keeps the United States in the game. But the money won't go for equipment, thanks to a likely delay in adoption of the 2009 budget beyond the 1 October start of the next fiscal year. "We have to hold it in reserve to avoid laying off the whole project team on October 1," says ORNL Director Thom Mason.

Persis Drell, director of the Stanford Linear Accelerator Center in Menlo Park, California, says she would have preferred Congress to have given DOE more leeway in how to spend the money. Its decision to cut funding for ITER and Fermilab, she says, "was the cause of the disaster in the 2008 budget, and I'm not any happier with [language] in the supplemental" that focuses on jobs. And Lubell and others warn that Fermi and other labs may have to reinstitute layoffs if DOE's science budget does not increase significantly next year.

–ADRIAN CHO

With reporting by Eli Kintisch.

to form the basis of knowledge" on various topics. But political scientist Howard Silver, executive director of the Consortium of Social Science Associations in Washington, D.C., thinks the infusion of NSF-caliber researchers could reap more immediate benefits. "If [the U.S. government] had done a better job of listening to the cultural and language needs in Iraq, things might have worked very differently," says Silver.

Will NSF's involvement provide sufficient cover for the Pentagon? Silver thinks "the proper protections" are in place, including promises that the Pentagon-supported research will be unclassified and that scientists will be able to publish without interference. Cognitive psychologist Baruch Fischhoff of Carnegie Mellon University in Pittsburgh, Pennsylvania, says academic reviewers should ensure top-notch applicants. But Brown University anthropologist Catherine Lutz fears that the Pentagon dollars will militarize her field by potentially "pulling people off" other projects that are unrelated to defense. She and many colleagues are upset by another DOD program, called Human Terrain Teams, that has partnered social scientists with U.S. troops in Iraq and Afghanistan in an effort to better understand those cultures.

The Pentagon's involvement in the social sciences could reach beyond areas of interest to the military. The NSF-DOD memorandum allows defense officials to consider funding some proposals submitted to NSF's $38-million-per-year Human and Social Dynamics (HSD) program in risks and human behavior and decision-making. That would make NSF's dollars go farther.

University of California, Irvine, psychologist Roxane Cohen Silver, whose current HSD grant expires next year, says she'd have no problem taking no-strings-attached Pentagon dollars, especially "if that then opened up additional funding for social sciences." Fischhoff, who chairs the Department of Homeland Security's scientific advisory board, says that social scientists can sharpen their thinking by working with officials in defense and other "real life" fields. "They will press you on the quality of your data," he says.

–ELI KINTISCH

Dutch Limit Iranian Access

Iranian-born scientists and students are upset by new Dutch regulations, announced last week, that ban them from nine fields of study and five research facilities where they might have access to nuclear technology. The Dutch government says the rules are an implementation of U.N. Security Council resolution 1737, which seeks to limit Iran's access to nuclear technology (*Science*, 1 February, p. 556).

But the new rules are the strictest of any country and are unfairly singling out one group, critics say. "This stigmatizes the next generation of Iranian scientists," says Nasser Kalantar of the Nuclear Physics Accelerator Institute in Groningen, the Netherlands, who says he plans to investigate whether the measure is constitutional. Peyman Jafari of the International Institute of Social History in Amsterdam hopes the Dutch parliament will intervene. The Netherlands is particularly sensitive to the issue because Abdul Qadeer Khan, the so-called father of Pakistan's nuclear program, passed on highly classified material to Pakistan while working at a Dutch uranium-enrichment plant in the 1970s.

–MARTIN ENSERINK

Yellow Light for British Science

After months of hearing from anxious astronomers and physicists, the United Kingdom's Science and Technology Facilities Council (STFC) has detailed its plans to spend $3.9 billion over the next 3 years. Despite new investments in projects that include the proposed Extremely Large Telescope and the FAIR nuclear center in Germany, many researchers are angry that STFC will still reduce university research grants and ax support to fields such as gamma-ray astronomy and ground-based solar-terrestrial physics.

Because of commitments it inherited when formed last year from two U.K. funding bodies, STFC was short of cash and announced a swath of cuts (*Science*, 21 December 2007, p. 1851). Researchers were up in arms and parliamentarians joined the chorus of criticism, prompting the council to embark on a 3-month consultation.

In STFC's updated plans, some cuts have evaporated, such as those to the Gemini telescopes in Hawaii and Chile, and a financial lifeline has been thrown to the iconic Jodrell Bank telescope near Manchester, although partners will be needed to achieve full funding. But cuts remain. "The U.K. should be playing a leading role instead of hanging on to the coattails of others," says nuclear physicist William Gelletly of the University of Surrey.

–DANIEL CLERY

Published by AAAS

NUCLEAR CONTROL

Iraq Embarks on Demolition of Saddam-Era Nuclear Labs

When Ronald Chesser arrived by military helicopter at the Al-Tuwaitha Nuclear Center south of Baghdad on 2 July, the radioecologist from Texas Tech University in Lubbock was thrilled by what he saw. Few would consider it a pretty sight: dozens of buildings riddled with radioactivity, some reduced to rubble by Coalition bombs. What filled Chesser with hope was a clutch of trailers, including one for decontamination showers, freshly installed for an urgent task: the dismantlement of a sprawling facility where physicists in the 1970s and 1980s tried in vain to build an atomic bomb for Saddam Hussein. "I thought, 'Wow, this is really going to work,'" Chesser says.

At a ceremony in Baghdad on 7 July, Iraqi officials launched a cleanup effort that's expected to take at least 15 years and cost millions of dollars. "This marks the beginning of closure on Saddam's nuclear weapons program," says Carleton Phillips, a Texas Tech biologist who advised the Coalition Provisional Authority on nonproliferation issues until 2004. Saddam's nuclear program was much less advanced than the Bush Administration made it out to be in the runup to war in 2003. But no one disputes that Tuwaitha is a radioactive disaster zone that poses daunting challenges. "It's quite a high-tech project in a war-torn country," says Mark Hannan, Tuwaitha project manager at the International Atomic Energy Agency in Vienna, which has been training Iraqi specialists in nuclear decommissioning.

The cleanup is also a major milestone in efforts "to redirect former Iraqi weapons scientists to big projects important for rebuilding their country," says Phillips. Others agree. "This project will also build skills that the Iraqis can then apply to other environmental problems facing Iraq," says John Cochran, an expert on radioactive waste at Sandia National Laboratories in Albuquerque, New Mexico, who is also assisting the Iraqis.

The dismantlement of Saddam's main nuclear weapons complex comes after years of painstaking preparation. A big challenge lies in its chaotic state. Tuwaitha's two research reactors and other key buildings, including facilities for fuel fabrication and plutonium separation, were bombed in 1981 and 1991. During the Iraq war in April 2003, Tuwaitha staff fled and the center was looted. Unaware of the health risk, people hauled off scores of drums of uranium oxide extract—dumping some of the "yellowcake" on the grounds of Tuwaitha—to use the barrels for catching rain or washing clothes. By June, nuclear inspectors had accounted for virtually all the missing drums and uranium. Two years later, Chesser, Phillips, and Brenda Rodgers of Texas Tech, with Iraq's science ministry, surveyed schools in villages near Tuwaitha. "To our relief, we found they were not contaminated," says Phillips. In a secret operation described earlier this week by the Associated Press, U.S. forces last April removed 550 tons of yellowcake from Tuwaitha. Iraq sold the uranium to a Canadian company for processing into fuel for civilian power reactors.

Down and dirty. Ron Chesser (*left*) and Iraqi colleagues checking contamination at the "Russian silos"—rad-waste storage wells—last week at Tuwaitha.

To pinpoint where looters had dumped yellowcake and where bombs had dispersed radioactive materials, Phillips and Chesser, with colleagues from Iraq and Ukraine, in 2005 analyzed more than 400 soil samples, compiling a rough map of radioactivity at the 9300-hectare Tuwaitha compound. The contamination map laid the groundwork for a U.K.-funded "Train and Engage" program that the Texas Tech duo ran last month for 27 Iraqi scientists, including several former weaponeers. They met at the International Radioecology Laboratory in Slavutych, Ukraine, and in nearby Pripyat, a city drenched in fallout and abandoned when a reactor at the Chernobyl nuclear power plant exploded in 1986. "It's a reasonable analog for Tuwaitha," says Hannan.

At Tuwaitha, the first job will be to take apart the Active Metallurgy Testing Laboratory (LAMA). The facility was designed to extract enriched uranium from fuel rods and handle radioisotopes in hot cells: rooms with meter-thick concrete walls and robotic manipulating arms. "Supposedly, it was used only for a single experiment before Coalition forces bombed it" during the Gulf War in 1991, says Phillips, meaning it is lightly contaminated. But LAMA is huge—at 62,000 square meters, it covers an area equal to six football fields—and is strewn with rubble, necessitating a slow pace. A 50-strong team is expected to take at least a year and a half to finish the job.

Last week, the Texas Tech–Iraq team took additional soil samples and probed so-called Russian silos: covered storage wells for radioactive waste that are 4 meters deep with largely unknown contents. They also scouted for a place to store low-level rad waste from LAMA and other buildings. Later, Iraqi authorities must establish a repository for hotter waste when more hazardous buildings are dismantled.

The demolition job at Tuwaitha is by far the largest single project for redirecting former Iraqi nuclear personnel into civilian work. Part of the challenge has been to overcome what Phillips calls "the legacy of living in a dictatorship." For example, he says, many of his Iraqi colleagues had been accustomed to shaping data and analyses to meet the expectations of superiors. "We hope that this project will be a huge step in restoring their credibility with the international scientific community," Phillips says.

Not all of Tuwaitha is destined for the scrapheap. The science ministry hopes to convert several intact, clean buildings into a science park. For a nuclear reserve with a checkered past and a radioactive present, that would be a remarkable future indeed. **–RICHARD STONE**

CREDIT: CARLETON PHILLIPS

Published by AAAS

ACOUSTICAL SCIENCE

Major European Cities Are Quietly Missing Antinoise Deadline

PARIS—The Europeans have many words for noise—bruit, Lärm, fracasso—but few plans for reducing it. At a conference* here in France's noisy capital last week, European acoustical scientists admitted that they and most policymakers are not close to meeting an 18 July deadline to develop action plans to shush the European Union's (E.U.'s) largest cities.

Chronic noise has increasingly been linked to sleep problems, poor education, and even serious heart disease. Yet urban noise reduction is a daunting—and expensive—task; most scientists are still struggling just to locate noise hot spots.

The action plan deadline stems from a 2002 E.U. antinoise directive. "Europe has a bigger noise problem than the United States," says Gaetano Licitra, an environmental acoustics consultant helping the Italian region of Tuscany muffle its noise. "Instead of spreading out in suburbs, we tend to both live and work in the same area, and our cities more often have railroads going right through the center and nearby airports."

The first stage of the E.U. directive required mapping noise levels in all cities with at least 250,000 people. This is largely done with virtual models of cities that estimate people's average exposure to loud sound sources such as automobile, railroad, and airplane traffic and industry. One problem is that an urban noise map is a moving target, with infrastructure and traffic patterns constantly changing. Another is that "noise is not the same thing as loudness," says Brigitte Schulte-Fortkamp, an environmental acoustician at the Technical University in Berlin. "Loudness is physical and can be measured in decibels with a sound meter, but noise is a psychological phenomenon."

People are far more tolerant of sound levels depending on the context and source, researchers noted at the meeting. Relatively loud natural sounds from birds and water, for example, can put people at ease, whereas quieter sources, such as an electrical buzz, cause stress. Surveys have also found large variation in noise tolerance among people and even between whole communities.

So far, despite a June 2007 deadline for the noise maps, only a handful of major European cities have charted their soundscapes. Even fewer are close to proposing an antinoise action plan. "Most realize they will miss the deadline," says Schulte-Fortkamp. "Now there is a scramble to finish" because failure to comply will result in stiff fines in a few years. An exception is Berlin. Not only has the city mapped its noise, but an action plan is already in public consultation.

Most of the noise reduction in cities will come from changing transportation infrastructure, strictly regulating where trucks can travel, and relocating speed bumps and traffic lights, for example. One high-tech solution discussed at the meeting is to make the noise sources quieter. Nils-Åke Nilsson, an acoustic engineer based in Täby, Sweden, reported that asphalt containing grains of rubber hushed traffic significantly in sections of the Swedish city of Göteborg. Another strategy noted is insulating buildings better from outside noise. Pierre Leroy, a materials scientist at the French National Center for Scientific Research in Marseille, introduced a "smart foam" that efficiently dampens not only high-frequency sounds, such as the screech of brakes, but also the more difficult low-frequency sounds made by truck engines and underground trains. The foam could be incorporated into walls and road barriers.

The complexity of dealing with noise is daunting, but E.U. cities are also dragging their feet, says Licitra, because "once you have an action plan, then you have to start spending real money to address the problem, and that will cost billions." **–JOHN BOHANNON**

Taking action. Berlin (*above*) has a plan to reduce urban noise from sources such as traffic.

CREDIT: GALLUP/GETTY IMAGES

*Acoustics'08, Paris, 29 June to 4 July.

Academic Hackers in Court

A Dutch court is set to decide whether academic researchers can reveal how they cracked one of the most widely used security cards in the world. Chip producer NXP of the Netherlands has sued to prevent computer scientists from Radboud University in Nijmegen from discussing the topic at an October symposium in Spain. As part of a program to identify security weaknesses, the researchers announced in March that they had figured out how to "clone" MIFARE Classic, a chip used in hundreds of millions of building security and transit cards. Bart Jacobs, who led the work, says that academic freedom is at stake; NXP is "trying to kill the messenger," he says. A company spokesperson declined to comment. A verdict is expected before 14 July, the deadline to submit final papers for the Málaga meeting.

–MARTIN ENSERINK

Postdocs Unionize

The 5000-odd postdocs at the University of California (UC) may be on the verge of forming the biggest postdoc union in the United States. More than 3000 UC postdocs have signed cards to be represented by the United Automobile, Aerospace and Agricultural Implement Workers of America (UAW), according to Matthew O'Connor, a bioengineering postdoc at UC Berkeley who helped collect the signatures. "Many of us realized that [popular] postdoctoral associations are great for professional networking and career development but are not as well equipped to deal with issues like wages and benefits," says O'Connor. Previous attempts to unionize have failed to gain state certification (*Science*, 10 November 2006, p. 909). If the postdocs clear that hurdle, the next step is collective bargaining with UC officials.

–YUDHIJIT BHATTACHARJEE

CNRS Reforms Adopted

PARIS—A controversial plan to create a series of new institutes within France's National Center for Scientific Research (CNRS) was approved by the center's board on 1 July. Scientist labor unions gave up their resistance after last-minute concessions from the French government, including dropping the idea to give some of the new institutes a privileged "national" status. The plan is a general outline, however; Jean-Luc Mazet of union SNCS-FSU predicts that "the battle will resume" when details are hammered out in a contract between CNRS and the government this fall.

–MARTIN ENSERINK

Published by AAAS

Steering Harvard Toward Collaborative Science

Provost Steven Hyman is at the forefront of the university's dramatic push to transform the way it carries out research

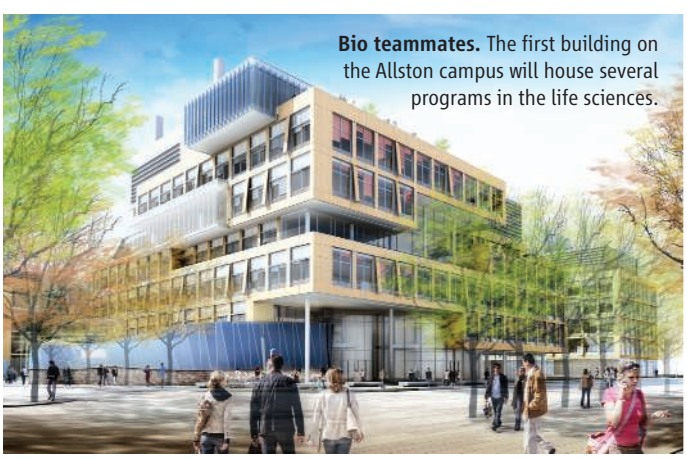
Lawrence Summers conceived of a new science complex during his stormy tenure as Harvard University's president, and the idea has been endorsed by his successor, historian Drew Faust. But it's Steven Hyman who will make sure that the ribbon is cut on the massive building in Allston in 2011. Fueled by espresso and a passion for collaborative research, the Harvard provost is leading the university's belated rush to catch up and surpass other U.S. universities in reorienting basic science. He's also trying to bridge the gap between research and applications and strengthen faculty ties with industry.

The 55-year-old researcher, hired by Summers in 2001 and retained by Faust, is himself an interdisciplinary mix of psychiatry and molecular biology. He wants to turn the oldest and most prestigious university in the United States into the locus of work to eliminate diseases, understand how the brain functions, and expand the possibilities of medical genomics. To succeed, he must attract new talent and persuade Harvard's famously independent fiefdoms to work in concert. The vehicle for that transformation is the multibillion-dollar campus in Allston, a working-class suburb across the Charles River from Harvard Yard, that for the first time will concentrate a host of science and engineering disciplines under a single roof. "We're trying a very different way in answer to our critics, who have seen us as irretrievably Balkanized," says Hyman.

Some critics have labeled the Allston campus a real-estate boondoggle, and others complain that Hyman's approach threatens to abandon Harvard's tradition of curiosity-driven research. But scientists both inside and outside the university say Hyman is making bold, long-needed changes that will allow Harvard to compete more ably with institutions such as Stanford University and the neighboring Massachusetts Institute of Technology (MIT), which have been at the forefront of connecting basic and applied sciences. They add that Hyman's political savvy, willingness to listen, and amiability—traits not associated with his former boss—give him a better shot at nudging Harvard in a new direction.

"He has a force of will, intelligence, and a lot of chutzpah," says Alan Kraut, executive director of the Association for Psychological Science in Washington, D.C., who has followed Hyman's career for more than a decade. "And even when he's gruff he has a lot of humor."

Significantly altering an institution as complex and crusty as Harvard ranks among the toughest tasks in American academe. The university's faculty of arts and sciences, numbering nearly 500, is centered on the 372-year-old Cambridge campus. The medical school, across the river in the Longwood section of Boston, is home to 6400 faculty members



Bio teammates. The first building on the Allston campus will house several programs in the life sciences.

CREDITS (TOP TO BOTTOM): JUSTIN IDE/HARVARD UNIVERSITY NEWS OFFICE; BEHNISCH ARCHITEKTEN

Published by AAAS

Building excellence. Provost Steven Hyman hopes the Allston campus will transform research at Harvard.

and even more postdocs. Additional faculty are scattered among hospitals and institutes in the area. That geographical separation, combined with a tradition of independence, has allowed Harvard researchers to resist collaboration to a greater extent than researchers at most universities.

"It's clear that Harvard has lagged behind other universities in making connections within its faculty," says Yale University molecular biologist Joan Steitz, who is on Harvard's Board of Overseers. She notes that Yale, by contrast, has for decades given joint appointments between its medical school and its arts and sciences faculty. Harvard schools even now balk at that approach, adds Harvard chemist Gregory Verdine, who failed to win approval for such an appointment just 3 years ago.

Although basic science has flourished within this system of fiefdoms, the number of discoveries moving from the lab bench to the clinic is too small, says Hyman. Statistics bear that out. Harvard averaged $21 million a year in licensing revenues between 2001 and 2007, and faculty formed 32 new companies. MIT, in contrasts, boasts of twice the yearly revenue and three times the rate of entrepreneurship.

Hyman admits that changing that mindset will require him to reconcile differences in salary structure, sources of funding, and territorial claims among the various Harvard cultures. "I am not naïve; there will be air pockets and lumps and bumps," he admits. But many researchers agree that if anyone can succeed, it is Hyman.

Multiton tanker

Throughout his career, Hyman has developed a reputation for encouraging collaboration and reforming staid institutions. Harold Varmus, while director of the National Institutes of Health, plucked Hyman from his position as a Harvard professor in 1996 to direct NIH's National Institute of Mental Health (NIMH) in Bethesda, Maryland. "Like Harvard, NIMH is a multiton tanker, but he was incredibly successful there," says Kraut.

At NIMH, Hyman shifted funds from long-running projects to a new generation of mental health researchers excited about breakthroughs in areas such as neurobiology. Those moves infuriated the old guard. "He was a little like a bull in a china shop," Kraut recalls. Darrel Regier, then an NIMH scientist and now head of research at the American Psychiatric Association in Arlington, Virginia, felt Hyman's ax come down on his own epidemiological project. "But the changes were long overdue, and by and large, he did a good job," he says.

Hyman also knew how to navigate the treacherous political currents in Washington. "I was struck by how well Steve translated the science and made it compelling," says one former staffer for Representative Patrick Kennedy (D–RI). "Unlike some scientists who bury their nose in the bench, he understood people."

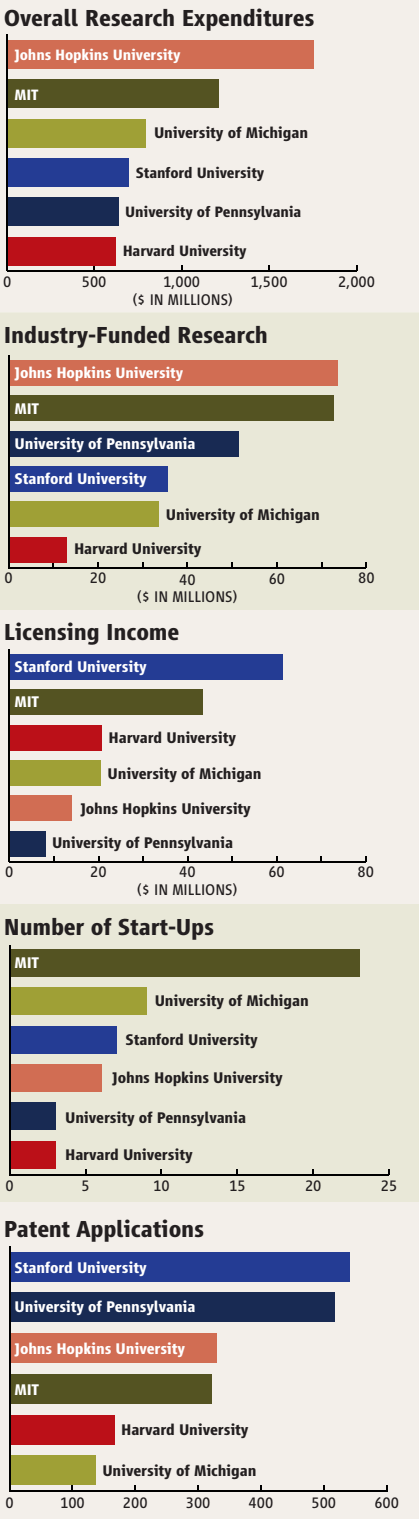
The staffer recalls how Hyman helped his boss to blunt an attempt by one lawmaker to limit the way funds could be used for basic science; Kennedy patched in Hyman to a conference call with the lawmaker. "He was so good—he knew all the nuances—and backed her off a draconian position," the former staffer recalls. "She dropped her plans for the amendment." His successful track record in Washington has spawned rumors that he could succeed Elias Zerhouni as NIH director in either an Obama or a McCain Administration.

Kraut and others say Hyman pushed NIMH into supporting more interdisciplinary as well as translational research, the latter a current buzzword for connecting basic science with applications. Hyman has tried to bridge that gap throughout his professional career. After graduating from Yale and studying the philosophy of science at the University of Cambridge, he received his M.D. from Harvard Medical School and also worked at Harvard College. His interest in molecular biology—a fascination unusual and potentially career damaging for a psychiatrist at the time—made him the ideal candidate to pull together a mind, brain, and behavior initiative at Harvard that would link researchers from various disciplines. The initiative, from then-president Neil Rudenstine, still thrives today. But it failed to establish deep roots. "It was very much a top-down initiative from people like me—central administrators—and its activities didn't become part of anybody's day job," Hyman says.

After Summers brought him back to Harvard, the two men agreed that the university needed to play a bigger role in scientific areas from neuroscience to stem cells. To do that, they proposed a new campus within walking distance of both the medical school and the Harvard campus. The massive construction project spooked residents, who were wary of overdevelopment, and researchers, who were unhappy with the prospect of relocating to a new site. Under the current blueprint, the first building will be a 93,000-square-meter facility for a new

WHERE HARVARD RANKS

Hyman wants to make Harvard a powerhouse in collaborative research and a hotbed of entrepreneurship. Here's how it compared in 2006 with some of its academic peers on several leading indicators.



SOURCE: ASSOCIATION OF UNIVERSITY TECHNOLOGY MANAGERS

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stem cell and regenerative biology department, a stem cell institute, the systems biology department, and bioengineering.

Given the promise of stem cells—and the growth of opportunities in California, Singapore, and Israel—Hyman says it is critical that Harvard researchers pool their talents: "The way to hold the community together is to create the best intellectual critical mass that we possibly can." Harvard's schools of public health and education will also likely be housed in Allston, but there are no firm plans to move other science and engineering sections there.

Hyman argues that building a neutral place—"like Switzerland," he jokes—provides Harvard with its best shot at persuading reluctant partners to collaborate. Summers envisioned Allston as the center of an East Coast Silicon Valley, and Verdine hopes someday to see a line of entrepreneurial companies eventually clustered along the area's Soldiers Field Road. "If you are organized as a community entirely of small curiosity-driven labs, you are not organized to move advances through the pipeline to application," says Hyman. And if solving real-world problems is the goal, he adds, "you don't want to be organized as a colony of curiosity-driven researchers."

Such talk worries some physical and life scientists at Harvard, who requested anonymity for fear of retribution. "He's Mr. Translational Research," says one senior faculty member about Hyman. "And he's attempting to put everyone in one boat, which does not serve the university or basic research." Another professor emphasizes that "there has to be a balance" between research conducted for curiosity and that which has a specific goal. Both researchers say they fear that Harvard's real motive in the Allston project is largely economic: more grants and closer ties with industry. And there is big money to be had. In May, NIH awarded Harvard Medical School $117.5 million as part of a larger initiative to conduct bench-to-bedside research with help from university faculty and affiliated medical centers.

"It is not about the money," insists Hyman. "We have a cultural history that devalues technology transfer and connections with industry to some degree. I've set about reforming that, and to have a closer connection to industry." The purpose, he adds, is to tie research efforts to combating diseases and mental health disorders. But closer ties to industry can backfire, too. Last month, a Senate investigation revealed that a well-known Harvard child psychiatrist and a medical school colleague did not report large sums in consulting fees from drug companies as university rules require (*Science*, 27 June, p. 1708).

Some scientists remain unconvinced that Allston provides the best way for Harvard to assure preeminence in the life and physical sciences. Last fall, biologist and Nobelist James Watson dismissed the new campus as an "almost Soviet-style fantasy," a misguided attempt to create a centrally planned mass of buildings to fix a problem that he said has more to do with long-term lack of funding for basic research. And Harvard molecular biologist John Dowling says that although Allston is "a terrific idea, at the moment we don't need it," noting that a 46,452-square-meter building opening this fall on the main campus will unite neuroscientists and biologists. (The new facility, although welcomed by neuroscientists, comes 3 years after MIT dedicated its brain and cognitive science building.) "Beyond stem cells, it is not clear what role Allston will play," notes Dowling.

Yet most criticism of the new campus has waned since Summers's departure 2 years ago. It helps that Faust has promised a more collaborative and slower approach. "You have to start somewhere, and Allston is on a very, very good trajectory to serve real programmatic needs," says Hyman.

That positive trajectory allows Hyman time to feed a voracious intellectual appetite. Along with his administrative duties, he teaches an undergraduate course on neuroscience ethics, edits an annual review of neuroscience, and reviews for several journals on the side. In one corner of his wood-paneled office in Massachusetts Hall sits a well-used coffee machine. "I'm down to 20 espressos a day," he says. "There's just not enough time to brew it."

–ANDREW LAWLER

STEVEN HYMAN ON ...

... FUNDING: It was wonderful for NIH [National Institutes of Health] to have had a budget doubling, but this feast followed by protracted famine is damaging. A young person right now watching the fate of their midcareer mentors in any of the sciences—whether life science, physics, or astronomy—sees a rather discouraging picture. It really turns off a generation.

... THE 2008 ELECTION: Neither candidate has that much of an empirical track record. What's most striking is how little science has figured in the campaign, with the exception of stem cells. The buzzwords may be a knowledge-based economy, but there hasn't been enough recognition of the role of science and technology in creating this kind of modern economy.

... FOREIGN STUDENTS: We should be passport blind when it comes to bringing people to the United States. The visa situation has gotten better, but we all have anecdotes of a Chinese graduate student who has to return home for a wedding or a funeral and is very frightened about whether they can come back. [Among universities] there hasn't been a concerted effort to change this.

... HIS PASSIONS: Social neuroscience has burst on the scene. It can be criticized in terms of rigor, but it's wonderful to see people asking questions about human brain function and economic behavior or moral reasoning.

... THE WORKFORCE: We're more likely to be able to train and retain in the United States a generation of scientists and engineers if they were born here and call this home. As China and Singapore and other countries become alert to the extraordinary possibilities of science and engineering in an increasingly knowledge-based global economy, they will have pulls on people born in those countries.

... HARVARD'S $34 BILLION ENDOWMENT: People don't understand endowments. I wish we had a giant bag of money underneath this building. In fact, we have something like 11,400 individual accounts, and when people donate money, they do indeed restrict it to a variety of uses—some of which look brilliant with time and some of which age. We are working very hard to liberate what we can [while retaining] full respect for gift terms.

CREDIT: JUSTIN IDE/HARVARD UNIVERSITY NEWS OFFICE

CREDIT: IMAGEZOO/IMAGES.COM; SOURCE: CDC

BIPOLAR DISORDER

Poles Apart

The number of children and adolescents diagnosed with bipolar disorder has been rising sharply, prompting debate and research on how the illness should be characterized in young people

Bipolar disorder used to be considered a disease of adulthood. Most mental health professionals assumed that the first episode of mania—the defining event for the illness—rarely occurred before people reached their 20s and might not hit until middle age. It came as a surprise to some, then, when two studies published last year documented a dramatic increase over a decade in the number of children identified with the disease.

A U.S. survey* revealed that in 2003, 1% of the population under 20 received the diagnosis—a 40-fold leap since 1994. Another study† indicated that up to five times as many U.S. children and adolescents were hospitalized for bipolar illness in 2004 as in 1996. No one knows for sure "whether there is an increase in these very disturbed kids or whether they are being relabeled," says psychiatrist Gabrielle Carlson of Stony Brook University School of Medicine on Long Island.

Online
sciencemag.org
Podcast interview with the author of this article.

Critics of psychiatry such as psychiatrist David Healy of Cardiff University in the U.K. attribute at least part of the increase to the influence of the pharmaceutical industry, which they believe leads to overdiagnosis by encouraging doctors to prescribe the latest drugs for problem children.

The debate has been fueled by recent allegations that Harvard University psychiatrist Joseph Biederman, a leading proponent of the idea that many childhood disorders are actually bipolar illness, has received substantial support from drug companies. Biederman, who says "there were no conflict-of-interest violations" in his ties to industry, insists that the increase in diagnoses more closely reflects the real incidence of the disease. "I spend my days dealing with these populations" of troubled children at Massachusetts General Hospital (MGH) in Boston. "I can tell you it's a very desperate state of affairs."

Other researchers agree that the numbers don't exaggerate the problem. "We don't go out in the street and offer meds to children," says psychiatrist Christoph Correll of the Albert Einstein College of Medicine in New York city. "They are sent to us."

The stakes are high. A wrong diagnosis could consign a person to decades of inappropriate drug treatment; failure to spot the disease can lead to many years of misery—as was the case with child actress Patty Duke, who recounted at this year's annual meeting of the American Psychiatric Association (APA) in Washington, D.C., that she suffered for more than 15 years before being diagnosed with bipolar disorder at age 35. But getting the diagnosis right is difficult because there are no objective tests for bipolar disorder, and the disease in children can look very different from that in adults.

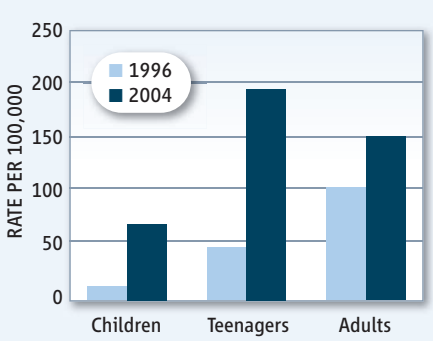
An upward trend

The disorder used to be known as manic depression. The change in name was sparked in part to avoid the somewhat derogatory word "manic" but also for accuracy. A person's primary tilt may be toward one or the other mood extreme—more often, depression—although mania is the tip-off. The trouble is, it's often hard to distinguish mania from a welter of other symptoms and problems, especially in children.

Even in adults, bipolar disorder, estimated to afflict about 1% of the population, has been called "the great impersonator" because it comes in many guises and often coexists with other problems, says psychiatrist Husseini Manji of the U.S. National Institute of Mental Health (NIMH) in Bethesda, Maryland. And it's especially complicated because it's marked by two drastically different mood states: profoundly debilitating and often-suicidal depressions and intense phases of manic hyperactivity.

With children, the disease is even more complex. They're "a moving target" when it comes to mental disorders, says Correll.

Rates of bipolar diagnoses among U.S. inpatients



* C. Moreno *et al.*, *Archives of General Psychiatry*, September 2007.

† J. C. Blader and G. A. Carlson, *Biological Psychiatry*, 15 July 2007.

Published by AAAS

Their fast-developing brains are unpredictable. They have difficulty expressing themselves, they have little or no history to guide the clinician, and it's often impossible to tell whether some behavior is pathological or just a twist in the path of normal development. What's more, children's symptoms do not simply mimic those of adults. Children are more likely to have "mixed" states that combine euphoric and destructive behavior.

Partly for these reasons, it was not until 1995 that members of the Pediatric Psychopharmacology Unit at MGH published a paper suggesting that many children who were then being identified as hyperactive or having conduct disorders were in fact bipolar. Judging by the leap in diagnoses over the following decade, that finding resonated with pediatricians, says co-author Janet Wozniak, head of MGH's pediatric bipolar research program. It also jibes with recent studies of adult bipolar patients indicating that about 60% of them experienced the first symptoms before age 18.

These troubled children are not just fidgety, noisy, whiny, sulky, or contrarian. They may be angry and self-destructive, prone to violent rages or dangerous impulses such as jumping out of a moving car during an argument, exhibit sexually inappropriate behavior, and risk losing touch with reality, says psychiatrist Barbara Geller of Washington University in St. Louis, Missouri. Geller adds that mania in a child may initially be hard to differentiate from childish giddiness. These children are "never serious, always acting up—they've been described by many parents as behaving as if they're little Jim Carreys," she says. But there's an added element of grandiosity: "They'll get up and start teaching the class or tell coaches how to coach."

Such symptoms may seem distinctive, but mania is hard to recognize when it's mixed with a half-dozen other problems that youth is heir to. *The Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*), psychiatry's bible, contains many labels that could fit a troubled, acting-out child. Most common is attention deficit hyperactivity disorder (ADHD). Others include conduct disorder; anxiety disorders, including obsessive-compulsive disorder; and newer diagnoses such as "oppositional defiant disorder" and "intermittent explosive disorder."

Delayed diagnosis. Former child star Patty Duke, now 61, was diagnosed with bipolar disorder at the age of 35. In her late teens, she told psychiatrists at their May meeting, she experienced "eruptions of temper alternating with taking to bed for months at a time, getting up only to go to the bathroom and attempt suicide."

CREDIT: HULTON ARCHIVE/GETTY IMAGES

Narrow versus broad constructionists

Clinicians roughly divide into two schools of thought over how broad a range of behaviors should be classified as bipolar, says Carlson. In an online seminar this year sponsored by the American Academy of Child and Adolescent Psychiatry ("What those in the know, know," www.aacap.org), Carlson described two cases that illustrate the challenge.

One child, 13-year-old "Nicola," is brought in by her parents because of a sudden behavior change over the previous 2 or 3 weeks. Formerly quiet, she is suddenly loud and grandiose, donning sexy clothing, talking constantly, and sleeping little. Her moods have become volatile, and she is easily driven to hysterical laughter or frustrated rages.

The other, "Lynda," 11, has been hyperactive since preschool, a condition that has been helped somewhat by stimulants (Ritalin). But lately it's gotten worse, and she has become unruly, explosive, aggressive, and provocative in her dress and behavior. She's developed some new habits including downloading porn on the computer and smoking marijuana. At the same time she's anxious and depressed, and she's falling behind in school.

Are one or both of these girls bipolar? Some researchers, including a group at NIMH, take a conservative approach to the diagnosis. In the seminar, psychiatrist Ellen Leibenluft says she would diagnose Nicola as bipolar, tipped off by the sudden change in her personality. But Lynda's symptoms are not episodic, which the NIMH group believes is central to a bipolar diagnosis. To them, the problem looks like severe mood dysregulation (SMD), a category that Leibenluft's group has created to describe mood and behavior problems that persist over a number of years.

The group at MGH, however, would diagnose both girls as bipolar. Wozniak puts more stock in Lynda's chronic irritability and explosive rages. Biederman, her boss, says such children have often been labeled as having ADHD. Yet, he says, ADHD is not fundamentally an emotional problem, and children like Lynda "are all the time in [emotional] turmoil." It's "very clear," says Biederman, that "these children had every symptom in *DSM* of mania." It's just that they may have a lot of other things wrong as well.

Psychiatrist Boris Birmaher of the University of Pittsburgh School of Medicine in Pennsylvania says, "I see the same [type of] kids as Biederman," but he doesn't believe they're all bipolar. One way to assess the probability in a given case, he says, is to look at what's going on with a child's first-degree relatives. The disease is highly heritable, and studies have shown that the risk goes up 10-fold if a person has a bipolar parent.

Carlson is also skeptical of Biederman's assessment. Bipolar children tend to grow into bipolar adults, she notes. Yet, "so far, what we know from long-term studies of ADHD and aggressive children" suggests that many of the type who might now be diagnosed as bipolar children grow up to be substance abusers, hotheads, and antisocial personalities—but not bipolar. Carlson is co-author of a forthcoming paper on a 23-year follow-up of 101 "high-risk" children into adulthood. Although all had big problems, she says, only "one-third of those with what some call a 'bipolar phenotype' developed adult-style bipolar disorder."

"Getting this [bipolar] diagnosis means putting people on meds for life," says Manji, "so you kind of want to be sure we're treating the right thing." Missing a diagnosis of bipolar disorder can also have serious repercussions: If a bipolar child is given medication for just ADHD, that can trigger mania, as can antidepressants. If someone is suspected of being bipolar, says Wozniak, they should be started on a mood stabilizer (lithium or an antiseizure drug) before getting medications for other conditions. And other drugs—for depression, anxiety, or

ADHD—are almost always necessary. The average pediatric bipolar patient, clinicians say, is usually on three or four drugs.

The bipolar brain

Right now, physicians must make a diagnosis solely on behavioral symptoms: There is no test available for bipolar illness. Gene hunts have failed to come up with more than a list of possible suspects. And brain-imaging studies often produce conflicting information.

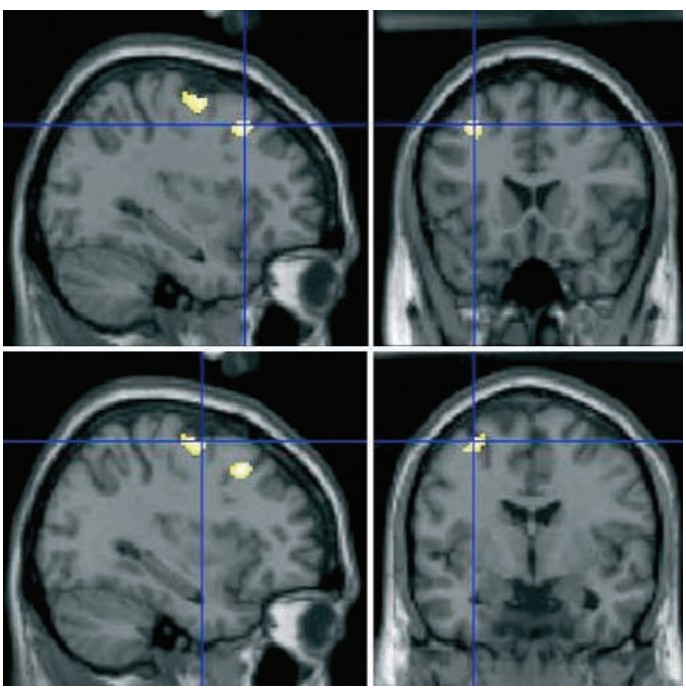
Nonetheless, comparisons of brain functions between bipolar children and those with other diagnoses are yielding some intriguing clues that could eventually help in diagnosis. Psychiatrist Daniel Dickstein, formerly with Leibenluft's NIMH group and now at Brown University, thinks bipolar children have a deficit in the brain's "reward machinery." Mania, he says, is a "hyperhedonic" state in which the brain is "excessively reward-sensitive." Depression is the converse. He believes that teasing out different brain responses to a test involving rewards may help differentiate the brain mechanisms of bipolar illness from those of other psychiatric illnesses in children.

The task Dickstein has used to probe these brain mechanisms assesses "reversal learning." In this test, two groups of subjects are compared on a task in which they are rewarded for matching certain cards according to color, number, or shape; they have to learn by trial and error when rules are covertly changed by the experimenter. Fifty children aged 7 to 18 who exhibited classic cyclical bipolar illness—the kind readily recognizable in the *DSM-IV*—were compared with 44 others whom the NIMH group labeled as SMD: chronically irritable, agitated, and with ADHD-like symptoms. Both groups did worse than the controls on the tests, but the bipolar group—even though they were tested when in a normal mood—made more errors and took longer to learn the new rule (*Journal of the American Academy of Child and Adolescent Psychiatry*, March 2007). This indicates they have a harder time inhibiting a learned response that is no longer rewarded and replacing it with a new one that is. That, says Dickstein, suggests impaired "cognitive flexibility."

In as-yet-unpublished work, the NIMH group claims that brain imaging further bolsters this theory. Leibenluft reported at this year's APA meeting that the two groups could be distinguished on the basis of parietal lobe activity in response to a "change task" that requires subjects to switch gears cognitively, inhibiting one response and substituting a new one.

Another type of task is designed to reveal poor regulation in both cognitive and emotional circuitry—as evidenced by lack of cognitive flexibility and emotional overreactivity. This is a frustrating computer game in which subjects are initially rewarded for speedily hitting the right button in a test; later they discover that no matter how fast they respond, they are told they lost because they weren't fast enough. During the test, Leibenluft and Brendan Rich compared a particular brain wave—the P3 wave, which reflects focused attention in the parietal lobe—in bipolar and SMD children compared with a group of normal controls. Both the bipolar and SMD groups reacted with more frustration than did the controls, the group reported in February 2007 in *The American Journal of Psychiatry*. But the brain waves of the bipolar children looked different. The amplitude of a much-studied brain wave, P3, was lower in this group, suggesting, says Leibenluft, that they weren't able to "mount the extra attentional effort" needed to overcome their emotional frustration.

Other probes reinforce the picture of poor emotional regulation with bipolar illness. In a study also headed by Rich and published 6 June 2006 in the *Proceedings of the National Academy of Sciences*, Leibenluft's group compared brain images of 22 adolescents with 21 controls in reaction to images of facial expressions. There were no differences between the two groups in looking at a nonemotional facial feature: nose width. But those with the bipolar diagnosis saw more anger in the neutral faces and reported more fear when viewing them—showing correspondingly more activation in emotional brain areas, particularly the amygdala, the seat of fear.



Bipolar brain. Images show significantly greater activation in both the prefrontal cortex (top two panels) and primary motor cortex (bottom two) in 25 young people diagnosed with bipolar disorder than in controls matched by age, sex, and IQ. Activation in the prefrontal area also differentiated bipolar youth from those with severe mood disorder (SMD), according to research in press.

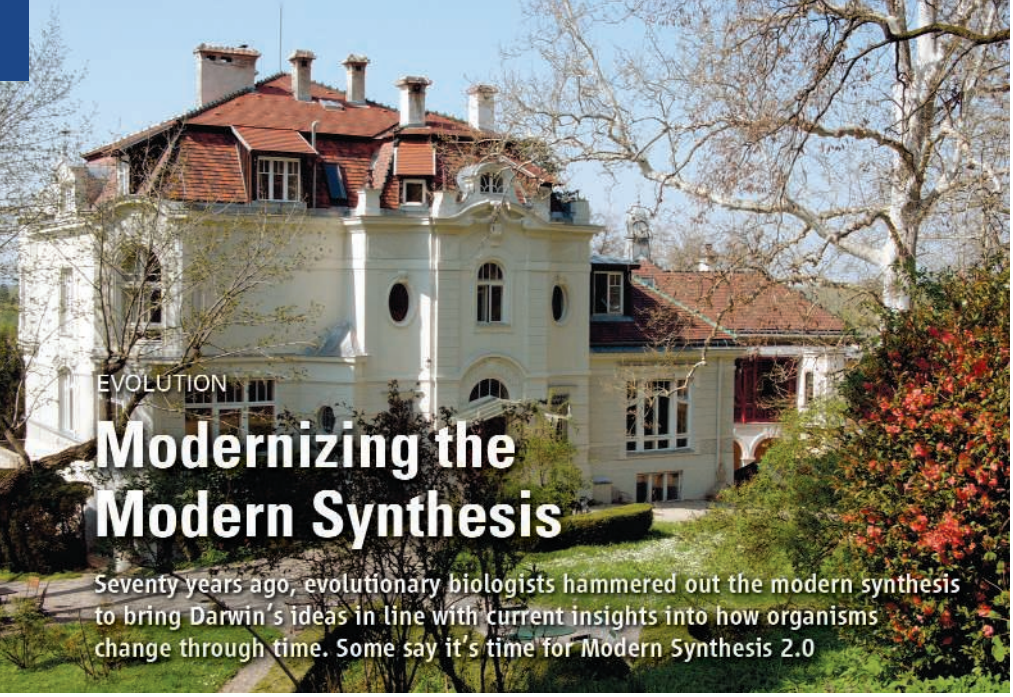
CREDIT: NELSON *ET AL.*, *BIPOLAR DISORDERS* 9, 810–819 (2007)

Some researchers suspect that bipolar disorder is linked to more far-flung perturbations in the nervous system. Dickstein, for example, thinks it may affect fine motor movements. He led a study comparing bipolar children, bipolar children who also had ADHD, and children diagnosed with just ADHD. The results, published in *Biological Psychiatry* in October 2005, indicated that the bipolar groups—both with and without ADHD—were slower than the ADHD group on a test that involved touching one's thumb to each of the four other digits in sequence and repeating the process four times, a chore that requires both motor and cognitive flexibility.

Some scientists also believe that bipolar illness is linked to problems that extend beyond the nervous system to the endocrine and immune systems—as hinted by the fact that bipolar people have more than their share of other health problems, such as obesity, diabetes, hypothyroidism, migraine, and multiple sclerosis.

Researchers believe that studying the disease in its earliest stages will be necessary to figure out what's really going on. Says Wozniak: "If it comes on in childhood, it stands to reason that starting our studies with children will yield better results. Symptoms in adults are complicated by meds and by years and years of illness. Studying them is like studying cancer only in the end stage."

–CONSTANCE HOLDEN



Woodstock? Austria's Konrad Lorenz Institute for Evolution and Cognition Research is hosting a much-discussed evolutionary biology meeting.

EVOLUTION

Modernizing the Modern Synthesis

Seventy years ago, evolutionary biologists hammered out the modern synthesis to bring Darwin's ideas in line with current insights into how organisms change through time. Some say it's time for Modern Synthesis 2.0

Massimo Pigliucci is no Jimi Hendrix. This soft-spoken evolutionary biologist from Stony Brook University in New York state looks nothing like that radical hard-rock musician whose dramatic guitar solos helped revolutionize rock 'n' roll. But to Suzan Mazur, a veteran journalist who occasionally covers science, Pigliucci is the headliner this week at a small meeting she believes will be the equivalent of Woodstock for evolutionary biology. The invitation-only conference, being held in Altenberg, Austria, "promises to be far more transforming for the world" than the 1969 music festival, Mazur wrote online in March for Scoop.co.nz, an independent news publication in New Zealand.

That hyperbole has reverberated throughout the evolutionary biology community, putting Pigliucci and the 15 other participants at the forefront of a debate over whether ideas about evolution need updating. The mere mention of the "Altenberg 16," as Mazur dubbed the group, causes some evolutionary biologists to roll their eyes. It's a joke, says Jerry Coyne of the University of Chicago in Illinois. "I don't think there's anything that needs fixing." Mazur's attention, Pigliucci admits, "frankly caused me embarrassment."

Yet Pigliucci and others argue that the so-called modern synthesis, which has guided evolutionary thought and research for about 70 years, needs freshening up. A lot has happened in the past half-century. DNA's structure was revealed, genomes were sequenced, and developmental biologists turned their sights on evolutionary questions. Researchers have come to realize that heredity is not simply a matter of passing genes from parent to offspring, as the environment, chemical modification of DNA, and other factors come into play as well. Organisms vary not only in how they adapt to changing conditions but also in how they evolve.

Evolution is much more nuanced than the founders of the modern synthesis fully appreciated, says Pigliucci. That doesn't mean that the overall theory of evolution is wrong, as some intelligent design proponents have tried to assert using Mazur's story as support, but rather that the modern synthesis needs to better incorporate modern science and the data revealed by it. More than genes pass on information from one generation to the next, for example, and development seems to help shape evolution's course. "Many things need fixing," emphasizes one invited speaker, Eva Jablonka of Tel Aviv University in Israel. "I think that a new evolutionary synthesis is long overdue."

Daring duo. Massimo Pigliucci (*right*) and Gerd Müller want to update the modern synthesis.

Modern tradition

The modern synthesis essentially represents a marriage of the 19th century concept of evolution with Mendelian genetics, which was rediscovered at the beginning of the 20th century; the birth of population genetics in the 1920s added to the intellectual mix. By the 1940s, biologists had worked out a set of ideas that put natural selection and adaptation at evolution's core. Julian Huxley's 1942 book, *Evolution: The modern synthesis*, brought together this work for a broad audience.

Simply put, the modern synthesis holds that organisms have a repertoire of traits that are passed down through the generations. Mutations in genes alter those traits bit by bit, and if conditions are such that those alterations make an individual more fit, then the altered trait becomes more common over time. This process is called natural selection. In some cases, the new feature can replace an old one; in other instances, natural selection also leads to speciation.

However, several concepts have arisen since then that make the modern synthesis seem too simplistic to some, Pigliucci among them. In a 2007 *Evolution* paper, he called for the development of an "extended evolutionary synthesis." His plea coincided with a similar one made that year by Gerd Müller, a theoretical biologist at the University of Vienna. Together, with support from the Konrad Lorenz Institute for Evolution and Cognition Research in Altenberg, they organized this week's conference, inviting many who share the view that the modern synthesis is incomplete. "What's happening now in evolutionary theory is as exciting and foundational as during the early days," says David Wilson of Binghamton University in New York, another attendee.

Beyond genes

Insights from ecology, developmental biology, and genomics in particular are nudging evolutionary biology away from a focus on population genetics—how the distribution of genes changes across groups of individuals—and toward an understanding of the molecular underpinnings of these changes. Better family trees that give researchers greater confidence about the relatedness among organisms have helped promote a credible, comparative approach to these mechanisms, says invitee Günter Wagner, an evolutionary developmental biologist at Yale University.

Some studies, for example, indicate that development constrains evolution. From the modern synthesis perspective, Wagner explains, "the body plan is a historical residue of evolutionary time, the afterglow of the evo-

CREDITS: (TOP AND BOTTOM RIGHT) COURTESY OF GERD B. MÜLLER; (BOTTOM LEFT) COURTESY OF MASSIMO PIGLIUCCI

lutionary process" such that more closely related organisms share more features. The alternative view, he says, is that "body plans have internal inertia," and evolution works around this stability.

This perspective fits in well with that of Stuart Newman, another invitee to the conference. A developmental biologist at New York Medical College in Valhalla, Newman and Müller have focused on physical processes that guide how cells organize limbs, livers, hearts, and other tissues. The stickiness, elasticity, and chemical reactions within and between cells, for example, all influence where cells wind up in an organism. The duo thinks these processes helped define early multicellular life, a time when genetic systems were still quite primitive and body shapes were presumably more plastic than now.

Their work suggests that body plans with interior spaces, segments, appendages, and multiple layers of tissue are inevitable. That's "heresy for the modern synthesis but inescapable if you incorporate physics into the picture," says Newman. Studies of development that suggest how evolution proceeded—the so-called evo-devo approach—have yielded other insights, among them that genes and proteins are arranged in networks that have their own set of properties. "There are lots of interdependencies that allow only certain patterns of evolution to happen," says Wagner.

Much like networks, "regulation" is a new buzzword in biology circles; yet it's another concept virtually ignored in the modern synthesis. Scientists now grasp that gene activity, RNAs, and proteins are all under regulatory controls and that shifts in those controls likely drive evolution as much as traditional gene mutations that alter a protein's form. Harvard University's Marc Kirschner, for example, contends that organisms have long possessed "core" components—the machinery for energy metabolism, pattern formation during development, making cytoskeletons, or cell signaling—that have persisted relatively intact through time. But he proposes that genetic changes that alter when and where in the developing body these components are used have helped create modern diversity.

Wagner thinks that by virtue of the breadth of genes they influence, transcription factors may be central to the type of evolutionary shifts Kirschner proposes. Changing the regulation of a few factors, even one, could help coordinate the systemic changes needed to make a new trait, helping to ensure that larger muscles coevolve with bigger jawbones for a more powerful bite, for example. Bottom line: "New traits contain very little that is new in the way of functional components, whereas regulatory change is crucial," Kirschner and John Gerhart of the University of California, Berkeley, wrote in a supplement to the 15 May 2007 issue of the *Proceedings of the National Academy of Sciences*.

The modern synthesis also doesn't take into account epigenetics. A small chemical modification of a DNA base—the addition of a methyl group, for example—can turn a gene off or on as easily as a mutation. Molecular biologists have long known about such epigenetic effects, but only recently have they demonstrated that

methylation tags and other epigenetic marks that silence or activate genes can travel from one generation to the next. That potentially creates a "bewildering increase in the complexity of the entire inheritance system," Pigliucci asserted in his 2007 call to arms.

Certain environmental conditions, such as diet during gestation, can alter the epigenetic patterns of the resulting offspring, and new traits that result can last for generations, says Jablonka, who has been striving to get recognition for this mode of inheritance for years. For example, in a study conducted several years ago, pregnant mice injected with an endocrine disrupter gave birth to males with reduced fertility, whose subsequent sons, grandsons, and even great-grandsons were likewise affected. Each generation had inherited the same altered methylation pattern of DNA (*Science*, 3 June 2005, p. 1466). "It's beginning to be accepted that [epigenetics] may actually have something to contribute to evolution," says Jablonka.

She argues that because these chemical modifications change how tightly wound DNA is, they also influence other properties of a genome that are relevant to evolution. The coiling of a DNA strand, she points out, can alter the rate of mutation, the ease by which mobile elements can move around, the duplication of genes, and even how much gene exchange occurs between matching chromosomes.

Beyond reason?

As the Altenberg 16 seek to modernize the modern synthesis, other unconventional ideas will be on the table. One is evolvability, the inherent capacity of an organism or a population, even a species, to respond to a changing environment. Introduced about 20 years ago, the concept can help explain why certain groups of organisms readily and rapidly diversified. Consider vertebrate toes: Amphibians have a wider range in digit number than, say, reptiles, which may indicate that the former are more evolvable for that trait, Pigliucci points out. But the question remains whether natural selection favors more evolvable organisms. If the idea of evolvability wasn't radical enough, a few researchers have proposed that organisms can stock up mutations whose effects manifest themselves only when the right circumstances arise.

Both ideas have their skeptics. "I don't believe organisms have a closet where they maintain all this genetic variation," says Douglas Schemske, an evolutionary biologist at Michigan State University in East Lansing.

Even among those coming to Altenberg, there's far from universal agreement. Wagner finds epigenetic inheritance hard to swallow. "I haven't been convinced," he says. And some outside the Altenberg 16 don't see what all the fuss is about. "I'm happy" with the modern synthesis, says George Weiblen, an evolutionary biologist at the University of Minnesota, Minneapolis. Others note that some of the items on the meeting's agenda, such as the role of plasticity in looks and behavior in evolution, have fallen in and out of favor for decades. "It's like selling old wine in new bottles," says Thomas Flatt of Brown University.

But these criticisms don't faze Altenberg's organizers. The modern synthesis emerged from at least a decade's worth of discussions. "The crucial point of the workshop is bringing these concepts together," says Müller. And no one truly expects a scientific Woodstock. "Woodstock was an immensely popular event celebrating a new musical mainstream," says Newman. "I imagine this will be more like a jam session circa 1962."

–ELIZABETH PENNISI

LETTERS

edited by Jennifer Sills

Biofuels: Effects on Land and Fire

IN THEIR REPORTS IN THE 29 FEBRUARY ISSUE ("LAND CLEARING AND THE BIOFUEL CARBON debt," J. Fargione *et al*., p. 1235, and "Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change," T. Searchinger *et al.*, p. 1238), the authors do not provide adequate support for their claim that biofuels cause high emissions due to land-use change. The conclusions of both papers depend on the misleading premise that biofuel production causes forests and grasslands to be converted to agriculture. However, field research, including a meta-analysis of 152 case studies, consistently finds that land-use change and associated carbon emissions are driven by interactions among cultural, technological, biophysical, political, economic, and demographic forces within a spatial and temporal context rather than by a single crop market (*1*–*3*).

Fired up. Biofuel production may have indirect effects on the use of fire as a land-management tool in the Amazon.

Searchinger *et al*. assert that soybean prices accelerate clearing of rainforest based on a single citation (*4*) for a study not designed to identify the causal factors of land clearing. The study (*4*) analyzed satellite imagery from a single state in Brazil over a 4-year period and focused on land classification after deforestation. Satellite imagery can measure what changed but does little to tell us why. Similarly, Fargione *et al.* do not rely on primary empirical studies of causes of land-use change.

Furthermore, neither fire nor soil carbon sequestration was properly considered in the Reports. Fire's escalating contribution to global climate change is largely a result of burning in tropical savannas and forests (*5*, *6*). Searchinger *et al*. postulate that 10.8 million hectares could be needed for future biofuel, a fraction of the 250 to 400 million hectares burned each year between 2000 and 2005 (*5*, *6*). By offering enhanced employment and incomes, biofuels can help establish economic stability and thus reduce the recurring use of fire on previously cleared land as well as pressures to clear more land (*7*–*9*). Neither Searchinger *et al.* nor Fargione *et al.* consider fire as an ongoing land-management tool. In addition, deep-rooted perennial biofuel feedstocks in the tropics could enhance soil carbon storage by 0.5 to 1 metric ton per hectare per year (*10*). An improved understanding of the forces behind land-use change leads to more favorable conclusions regarding the potential for biofuels to reduce greenhouse gas emissions.

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CREDIT: VIRGINIA H. DALE

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Response

ALTHOUGH WE SHARE KLINE AND DALE'S interest in the underlying social, political, and cultural causes of land clearing, the focus of our study was primarily on land clearing's effects. We considered the greenhouse gas impacts of producing various biofuels on newly cleared land or on degraded land that has already been cleared. We found that any newly cleared natural ecosystem used to produce current food-based biofuels releases large amounts of carbon dioxide to the atmosphere. This carbon debt can be minimized or avoided by producing biofuels from some types of waste biomass or from some perennial crops grown on agriculturally degraded land. We showed that a full and accurate accounting of the greenhouse gas impacts of land-use change is needed to determine the extent to which a given biofuel may or may not provide greenhouse gas benefits.

Our analyses explicitly included both carbon release and carbon storage. We reported the potential for perennial crops to store carbon on degraded land with an example of perennial crops grown on U.S. degraded cropland. African grasses grown in South America [(*1*), as cited by Kline and Dale] are an additional example that supports our point, although the magnitude of the effect cited by Kline and Dale is debated (*2*).

The points raised by Kline and Dale do not in any way lead us to draw "more favorable conclusions regarding the potential for biofuels to reduce greenhouse gas emissions." If existing cropland is insufficient to meet imminent food demands, then any dedicated biofuel crop production will necessarily create demand for additional land (*3*–*5*). Some of this land could come from

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previously degraded land no longer used for food production (*6*). Policies guiding biofuel production toward this land and away from natural ecosystems would offer substantial greenhouse gas and other benefits. As Kline and Dale point out, many factors contribute to land clearing. This observation does not diminish the fact that biofuels also contribute to land clearing if they are produced on existing cropland or on newly cleared lands (*7*–*10*).

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Response

KLINE AND DALE CONFUSE THE MUCH-STUDIED question of why some tropical forests are converted in some locations and not others, which depends on multiple factors, with the role of agriculture as an underlying economic driver worldwide. For example, the infrastructure to support agricultural expansion exists in some parts of the Brazilian Amazon, but less in the Peruvian Amazon, which is why our study predicts that biofuels will increase deforestation more in Brazil than in Peru. Some deforestation remains untied to agricultural demand, but as the meta-analysis cited by Kline and Dale actually found, "agricultural expansion is, by far, the leading land use change associated with nearly all deforestation cases" (*1*). Its related report found "economic factors" to be "the most important and robust underlying forces of tropical deforestation" (*2*). Other studies have found that "demand for agricultural commodities appears to be driving substantial increases in deforestation rates" (*3*, *4*).

We cited the study of Mato Grosso, Brazil (*5*), only to highlight proof of the principle that as economic returns for a land use increase, that land use expands. That basic principle of land economics is confirmed by studies showing that agricultural conversion of forests rises with the price of beef (*2*) as well as with access to roads, which reduces shipping costs and increases the effective crop price (*6*). The estimate of land-use change in our study was actually based on a well-established model showing the relationship between price and agricultural production in different countries around the world, and data showing the broad mix of forest and grasslands converted to agriculture in the 1990s. These patterns of conversion by definition reflect the complexity of factors referenced by Kline and Dale that influence where conversion occurs. These factors implicitly explain our average emission of 351 tons of CO_2 per hectare (t/ha), which is closer to grass and savannah conversion of 75 to 300 t/ha than forest conversion of 600 to 1100 t/ha.

Indeed, if farmers did not convert land to replace food diverted to biofuels, corn ethanol would cause fewer greenhouse gas emissions but at the expense of more world hunger.

Kline and Dale also suggest that because large-scale burning already occurs in the tropics, conversion to biofuel production could be good for global warming. But the great bulk of this fire comes from the annual burning of tropical grassland, which only releases aboveground carbon taken up by the grassland that year and therefore does not increase carbon in the atmosphere annually. By contrast, conversion of forest and grassland to cropland adds carbon to the atmosphere.

The assertion that biofuels produced in the developing world can contribute to economic stability and reduce deforestation, which might be true in some circumstances, is irrelevant to our study. It focused on biofuel production in the United States, not the tropics, and the resulting ripple effects in the tropics through food production. More broadly, none of the studies cited by Kline and Dale support the judgment that making deforestation more profitable, through higher crop prices or otherwise, would reduce deforestation—the precise economic effect of many biofuel policies.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "Building the tree of life, genome by genome" by E. Pennisi (27 June, p. 1716). The photo identified as a nudibranch is actually a shelled snail, Cyphoma, which is in a different subclass from the nudibranch.

News of the Week: "Heinz Center wants Feds to build ecosystem indicator partnership" by E. Stokstad (20 June, p. 1575). The amount of carbon stored in agricultural soils did not increase by 11 million tons from 1995 to 2005; it increased by 16.5 million tons during the 1990s.

Special Issue on Plant Genomes: Perspectives: "The epigenetic landscape of plants" by X. Zhang (25 April, p. 489). The last sentence in the first paragraph of the Conclusions section should read, "The function(s) of DNA methylation that are enriched in different fractions of the gene space in *Arabidopsis* (3′ half of transcribed regions) and rice (promoter regions), as well as DNA demethylation by the DEMETER (DME) family of DNA glycosylases (*53*), are not yet understood and warrant further functional studies."

Reports: "Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors" by E. S. Witze *et al*. (18 April, p. 365). In the paragraph beginning "Fz3, a noncanonical Wnt receptor," on p. 368, the mention of Fig. 4, E and F, should be Fig. 3, E and F.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Genetically Determined Differences in Learning from Errors"

Michael Lucht and Dieter Rosskopf

Klein *et al*. (Reports, 7 December 2007, p. 1642) used individuals with a polymorphism adjacent to the dopamine receptor 2 gene as naturally occurring models for reduced brain dopamine receptor density in a probabilistic learning task. We raise the concern that this polymorphism resides in the gene for the kinase ANKK1, where it causes a nonconservative amino acid exchange.

Full text at www.sciencemag.org/cgi/content/full/321/5886/200a

RESPONSE TO COMMENT ON "Genetically Determined Differences in Learning from Errors"

Tilmann A. Klein, Martin Reuter, D. Yves von Cramon, Markus Ullsperger

Since the publication of our findings, further genetic and pharmacological studies have bolstered our conclusion that dopamine D2 receptors are essential for performance monitoring and learning. Although the functionally complex dopamine D2 receptor gene polymorphism DRD2-TAQ-IA may also affect cellular signaling components, the accumulated evidence supports the notion that our findings were mediated by differential D2 receptor density.

Full text at www.sciencemag.org/cgi/content/full/321/5886/200b

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Biofuels: One of Many Claims to Resources

IN THEIR REPORT, "LAND CLEARING AND THE biofuel carbon debt," (29 February, p. 1235), J. Fargione *et al*. raise the important issue of competing land use needs in the planning of climate change mitigation strategies incorporating biofuel production. Research pointing out probable negative impacts of poorly planned policies is needed, but many recent works simplistically present biofuel as a disturbance in an otherwise optimally functioning system. Fargione *et al*. seem to assume that all land for bioenergy feedstock production either would be taken from the natural resource pool or would drive other land uses directly into it. Contrary to Fargione *et al*.'s thesis, we contend that not all current forms of land use are critical to society. In fact, uses can change without necessarily negatively affecting livelihoods and food security.

Even if current agricultural land use were indeed inelastic, it would be incorrect to attribute all effects of "displacement" to biofuel. Livestock feed mill companies, livestock producers, and consumers themselves have a range of options and should bear some responsibility for the consequences of their choices. Today, the bioenergy sector is the subject of substantial scrutiny, whereas this year the largely unscrutinized feed industry will divert an amount of cereals from humans to animals that is well over 7 times globally that diverted by biofuel use (*1*). Consequently, we question initiatives of specific treatment for biofuel feedstock, as called for by Fargione *et al*. Upon what grounds is it appropriate to enforce sustainability of soy oil imports for biodiesel, while not applying this to soy meal imports for feed?

We suggest that researchers stop presenting bioenergy as an aggressive intruder on an agrarian utopia and instead admit that bioenergy is just one of many agricultural products that use natural resources. Alarmist articles may do more harm than good to current decision-making in the EU and the UN.

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Letters to the Editor

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

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Comment on "Genetically Determined Differences in Learning from Errors"

Michael Lucht[1]* and Dieter Rosskopf[2]

Klein *et al*. (Reports, 7 December 2007, p. 1642) used individuals with a polymorphism adjacent to the dopamine receptor 2 gene as naturally occurring models for reduced brain dopamine receptor density in a probabilistic learning task. We raise the concern that this polymorphism resides in the gene for the kinase ANKK1, where it causes a nonconservative amino acid exchange.

Klein *et al*. (*1*) reported an association between the dopamine receptor 2 gene (DRD2) TAQ-IA (rs1800497) polymorphism and a probabilistic learning task. To explain these results, they noted that reduced D2 receptor expression is associated with the TAQ-I A1 allele. Although these findings are intriguing, the causal reasoning deserves some cautionary notes. (i) The association of the TAQ-I A1 allele with receptor availability—a measure with considerable variability—was not always replicated (*2*). Others reported that TAQ-I-A1 is associated with higher 3,4-dihydroxyphenylalanine uptake and increased decarboxylase activity, potentially resulting in higher dopamine levels antagonizing the lower receptor availability (*3*). (ii) The DRD2 gene is confined to a cluster of genes including neural cell adhesion molecule 1 (NCAM1); tetratricopeptide repeat protein 12 (TTC12), a potential scaffolding protein for multiprotein complexes; ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1), a kinase presumably involved in cell signaling; and, finally, DRD2. Importantly, the TAQ-IA polymorphism is located 9489 base pairs downstream from the 3′ end of DRD2, within the last exon of ANKK1, and causes a nonconservative amino acid exchange ($Glu^{713}Lys$) in a conserved ankyrin repeat (*4*, *5*) (Fig. 1). The genetic block of the TAQ-IA polymorphism comprises portions of DRD2 and ANKK1. Dubertret *et al*. cloned ANKK1 from whole-brain RNA and reported transcript expression in adult and fetal brain, cerebellum, and spinal cord (*5*). (iii) In large part, the identification and characterization of DRD2-TAQ-IA results from numerous association studies on alcohol dependence with rather controversial results [reviewed in (*6*)]. Recent replication studies considering many variants in the NCAM1-TTC12-ANKK1-DRD2 region have provided evidence for stronger associations of polymorphisms located in TCC12 and ANKK1 with dependence than for those in DRD2 (*6*, *7*). Indeed, there is good evidence for the contribution of the dopaminergic system to learning processes, as shown in a recent study of Parkinson's patients on and off dopaminergic treatment (*8*). However, the idea that signaling components—as suggested for ANKK1 and TCC12—also contribute to neural function and, ultimately, to learning, is also plausible. A refined genetic analysis that includes polymorphisms in TCC12 and ANKK1, as well as other functional DRD2 variants, could help to resolve this issue. The DRD2 C957T variant (rs6277) is one such polymorphism located within the DRD2 gene that has been associated with D2 receptor density and negative feedback learning (*9*). Pharmacological approaches and single-photon computerized tomography studies could further corroborate the findings by Klein *et al*. (*1*). In the absence of such data and in light of the complexities of this genetic locus, we suggest caution when considering the straightforward reasoning tightly linking TAQ-IA variants, dopamine receptor 2 expression, and the observed neuropsychological phenotype.

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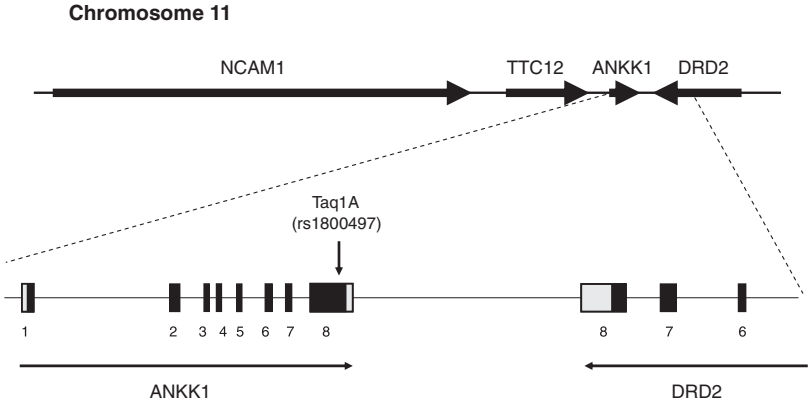

Fig. 1. (**Top**) Genetic locus comprising the genes for NCAM1, TTC12, ANKK1, and DRD2 on human chromosome 11. (**Bottom**) Enlarged illustration for ANKK1 and part of DRD2. Arrows refer to the transcriptional orientation of these genes. Numbers indicate exons, black boxes coding exons, gray boxes nontranslated parts of these exons. TAQ-IA refers to the localization of this polymorphism in the last exon of ANKK1 within the coding sequence.

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10.1126/science.1155372

Response to Comment on "Genetically Determined Differences in Learning from Errors"

Tilmann A. Klein,[1]* Martin Reuter,[2] D. Yves von Cramon,[1,3] Markus Ullsperger[1,3]*

Since the publication of our findings, further genetic and pharmacological studies have bolstered our conclusion that dopamine D2 receptors are essential for performance monitoring and learning. Although the functionally complex dopamine D2 receptor gene polymorphism DRD2-TAQ-IA may also affect cellular signaling components, the accumulated evidence supports the notion that our findings were mediated by differential D2 receptor density.

We thank Lucht and Rosskopf (*1*) for pointing out the inherent complexity of the genetic locus under investigation in our recent report (*2*). In fact, the way by which the DRD2-TAQ-IA polymorphism exerts its functional impact is not completely understood. Nevertheless, the main conclusion of our study—that dopamine plays a major role in learning from errors—remains robust.

The vast majority of studies have shown a reduction of dopamine D2 receptors for subjects carrying the A1 allele (*3*–*8*). Contradictory findings (*9*) may be explained by the particular populations used (e.g., schizophrenia patients, in which pathological changes in receptor densities may mask genetic effects) and different, potentially less sensitive, imaging techniques (such as single-photon computerized tomography instead of positron emission tomography or autoradiography).

The question remains, however, how a mutation located less than 10 kilobases downstream of DRD2 within a protein-coding region of the adjacent ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1) gene (*10*) can affect receptor expression. A recent study by Zhang *et al*. (*11*) investigated 23 polymorphisms within the D2 gene. The authors report that expression of the short splice variant of the D2 receptor was less than that of the long splice variant; the difference was related to two intronic single-nucleotide polymorphisms (SNPs), rs2283265 and rs1076560. Both SNPs were associated with an increased functional magnetic resonance imaging signal in the striatum and the prefrontal cortex during a working memory task. At the same time, these SNPs were associated with reduced performance in the working memory task. The minor allele of the two SNPs shows strong linkage disequilibrium with the A1 allele of the DRD2-TAQ-IA polymorphism [D' = 0.855 (*11*)]. It may be this linkage that causes DRD2-TAQ-IA to be a marker for dopamine receptor density, as indicated by numerous studies (*3*–*8*). As we discussed in (*2*), the finding of a higher 3,4-dihydroxyphenylalanine uptake of A1 allele carriers (*12*) adds evidence supporting a functional role of this polymorphism for dopaminergic transmission.

At the behavioral level, converging evidence supporting our findings showing genetic influences on preference and avoidance learning in humans comes from a recent study (*13*). It shows an influence of the C957T polymorphism (rs6277 located in exon 7) of the DRD2 gene on preference/avoidance learning in human volunteers. Subjects carrying at least one C allele (C/C homozygous or C/T heterozygous, associated with lower D2 receptor availability) showed impairments on avoidance learning, consistent with our findings in the A1+ subjects. These correlative findings are bolstered by a study in which pharmacological challenge with a D2 receptor agonist impaired reinforcement learning (*14*).

Taking these converging findings into account, our study yields strong evidence for the role of dopamine in feedback-based reinforcement learning. Hence, although the genetic regulation of D2 receptor density is highly complex and requires further investigation, the DRD2-TAQ-IA polymorphism in our opinion provides a useful marker for dopamine activity, enabling hypotheses testing of dopaminergic transmission in cognitive functions.

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DEVELOPMENT

X Inactivation Is a Good Thing

Jennifer A. Marshall Graves

Females Are Mosaics
X Inactivation and Sex Differences in Disease

by Barbara R. Migeon

Oxford University Press, Oxford, 2007. 291 pp. $59.95, £35.99. ISBN 9780195188127.

I've often wondered why there haven't been a dozen books about X-chromosome inactivation. The topic has got everything: sex and discrimination, gruesome diseases, not to mention epigenetic silencing. Since its discovery in 1961, X inactivation has posed fundamental questions. How does silencing work at the molecular level? How did such a complicated system evolve? And how is phenotype determined in females, who are mosaics of cells expressing genes on one or other randomly inactivated X chromosome?

Perhaps prospective authors (or their publishers) have shied away from the topic because the answers are complicated and becoming more so. Barbara Migeon is well qualified to take on the challenge, having been a consistent contributor to the field for 40 years. Migeon (a human geneticist at the Johns Hopkins School of Medicine) has produced an impressive range of data, particularly on the human X chromosome and its contribution to genetic disease. She has been a forceful advocate for studying X inactivation in animals other than the mouse, and her work on human (and a dalliance with marsupial) X-chromosome inactivation complements data from that model system. In *Females Are Mosaics*, she demonstrates again that the mouse does not tell us the whole X-inactivation story. Human X-chromosome inactivation differs from that of the mouse at both the phenotypic and molecular levels, and imprinted inactivation in marsupials is very different from what is known from either of these eutherians.

Thus, the book covers a lot of ground. Nicely structured, it begins with a description of the mammal X chromosome, an overview of dosage compensation, and a history of the discovery of chromosome inactivation and the principal experimental models. The second section zeros in on the mechanics of inactivation, then the book reaches its peak in its discussions of the phenotypic and health consequences to women of being mosaics of two cell populations.

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Migeon's eyewitness account of the discovery of X inactivation and the investigations of its mechanism is engaging and interesting. But curiously, she doesn't discuss one thing that has always fascinated me: Why are so many important contributors, starting with Mary Lyon (and Liane Russell), women? Is it something about the femaleness of X inactivation that attracted Marilyn Monk, Sohaila Rastan, Gail Martin, Christine Disteche, Carolyn Brown, Laura Carrel, Edith Heard, and Jeannie Lee, not to mention the author herself?

To understand X-chromosome inactivation, we need to know the genetic makeup of sex chromosomes and understand how X and Y differentiated. Migeon works hard to summarize a rather Y-centric literature, but this is the least convincing part of the book (in places, her account is embarrassingly wrong). Disappointingly, she barely mentions the evidence for a biased gene content of the human X, although its overrepresentation of genes involved in reproduction and intelligence must influence the phenotypic effects of X inactivation. Nor does she really tackle how such a complex system as X inactivation evolved or why it has evolved to be so different in humans, mice, and kangaroos.

CREDIT: BARBARA MIGEON

The complexity of X-chromosome inactivation continues to confound molecular biologists. Our simplistic early idea that differences in DNA methylation could explain heritable silencing was trashed by the discovery of variant histones, then histone modifications, and especially of the Xist gene that supposedly controls inactivation (but which is, curiously, absent from marsupials). Migeon's chapters on the mechanisms of X inactivation are thorough and clearly written; many geneticists will be grateful for her explanations of the molecular complexities and conflicting data in fairly simple terms. Perhaps her account is a little dogmatic in the interests of clarity (many controversies are relegated to footnotes and some current debates given short shrift). The author is not scared to put forward her own hypotheses, which are always informed and interesting—although sometimes they are simply her opinions about what "seems more likely" or is "difficult to imagine."

As promised by the full title of the book, Migeon's focus is how the cell mosaicism enforced by random X-chromosome inactivation influences the female phenotype and the expression of disease. She asserts that the extra variation in mosaics is "a good thing," because females "had to give up" heterozygosity to be on a par with hemizygous males. (I would have said to avoid the consequences of up-regulation of X-borne genes.) She discusses the sources of variation, in the degree of skew (the sources of which she incisively analyses), cell selection, and the possible interactions between the two populations of cells. This is a novel and spirited section, peppered by the author's own observations and ideas. Particularly useful are the clear explanations of phenotypes that can be puzzling, such as the immunodeficiency, centromere instability, and facial anomalies syndrome (ICF, which is not on the X but affects X inactivation) and Rett syndrome (female-specific, because affected males die). The appendix is clear and useful to flip to, although it overlaps somewhat with the main text. The strength of this section of the book may help at last to overturn the attitude held by many doctors and health-funding agencies that nobody dies from X-chromosome inactivation and to encourage recognition that X-chromosome inactivation is crucial to women's health and survival.

There are some odd features about the book's production. Some of the black-and-white figures are crudely drawn or puzzling because of inadequate legends. Several poor-quality black-and-white illustrations (particularly of chromosomes and tissues) duplicate figures also presented in gorgeous color. Readers should turn straight to the color plates.

Females Are Mosaics is aimed at geneticists (or at least biologists), medical scientists, and health professionals. Biologists will appreciate the breadth of the book and the thorough referencing. Health professionals may find the first part of the book

heavy going but will enjoy the section on the medical consequences of X inactivation. Generally, readers will agree that the book is a good thing.

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ECONOMICS

Tilt the Table Toward Good Choices

Eric J. Johnson

As described by behavioral economics, our choices and the preferences they represent are a curious mixture. In part they reflect what we want to do and have, but they also reflect the influence of subtle differences in the way the choice is being presented. The problem is not in knowing what good things we want: We all know we want more money when we retire, but we also want to spend now. The challenge is knowing how to trade off these two desirable things: How many expensive dinners would you give up this month for an extra day at a luxurious hotel in 20 years? Because we are uncertain about our tradeoffs, our choices, and the preferences they imply, are influenced by many subtle details of how the question is asked. The most well-documented is the effect of choice defaults: Designating one option as the one that is selected if no action is taken has a material effect on the rate of organ donations across countries and the amount set aside in retirement plans, to give just two prominent examples.

In *Nudge: Improving Decisions About Health, Wealth, and Happiness*, Richard Thaler, a founder of behavioral economics, and Cass Sunstein, a leading legal scholar and advocate of adding "behavioral" as a prefix to the growing area of economics and the law, come face to face with a dilemma generated by this modified view of preferences: How does government help people when they may be unsure precisely what they want?

Thaler and Sunstein present two separable but related ideas. The first is that the many factors that influence choice represent a choice architecture. The analogy is to the fact that the architect of a building determines quite a bit of the behavior of the building's users through the placement of doors, hallways, offices, and perhaps even bathrooms. Architects of choice can influence what is chosen by adjusting the number of options presented, the salience of different types of information, and the selection of defaults. While it is tempting to suggest that choices ought to be presented in a "neutral" architecture, Thaler and Sunstein point out this is not an option: Every way of presenting a choice will influence the decision-maker in some way. For example, all ways of presenting a choice have a (usually implicit) default, and these options will be chosen more often than if other defaults had been selected by the architect.

Nudge
Improving Decisions About Health, Wealth, and Happiness

by Richard H. Thaler and Cass R. Sunstein

Yale University Press, New Haven, CT, 2008. 303 pp. $26, £18. ISBN 9780300122237.

What makes *Nudge* important is not the book's foundational research ideas. Thaler and Sunstein are integrating 30 years of work in the psychology and behavioral economics of decision-making. Rather, it is the realization that anyone who poses a choice is a choice architect—the role is performed by supermarket, stockbroker, doctor, and government agency alike. The concept of choice architecture is a big idea, one clearly worthy of a book on its own, and more than the sketch provided by this review.

Nudge's other big idea covers how government should address the responsibility of influencing choice, an approach the authors call libertarian paternalism. They suggest that government should, often, offer people a choice in matters of public policy, but that this choice be provided with an architecture that favors people's best interest. It is difficult to disagree with some of Thaler and Sunstein's examples. Given that Americans do not save enough toward retirement, it seems responsible to change the default (as has been done in some retirement plans) to a reasonable savings rate rather than the original default of no savings, but give everyone making this decision the option of changing that level. The libertarian part of the term is providing a choice, the paternalistic part is the choice of the default. In the case of retirement savings, this architectural change has been made with broad bipartisan support. While the book contains many good ideas, not all the authors' suggestions are as persuasive, nor do they always follow from choice architecture or libertarian paternalism.

Some might find the idea of government designing an architecture disquieting. Critics on the right might find this version of government "too hard" because it allows government to influence people's choices. Those on the left may claim it to be "too soft" because it allows people the freedom to make mistakes. Yet because it implies a pragmatic and minimalist course, many readers will find that the ideas suggested in *Nudge* are just right. In an era of limited governmental monetary resources, these ideas seem efficient: they greatly affect behavior with little cost. In an era of partisanship, they reflect a scientifically grounded concept of how a responsible government should pose choices.

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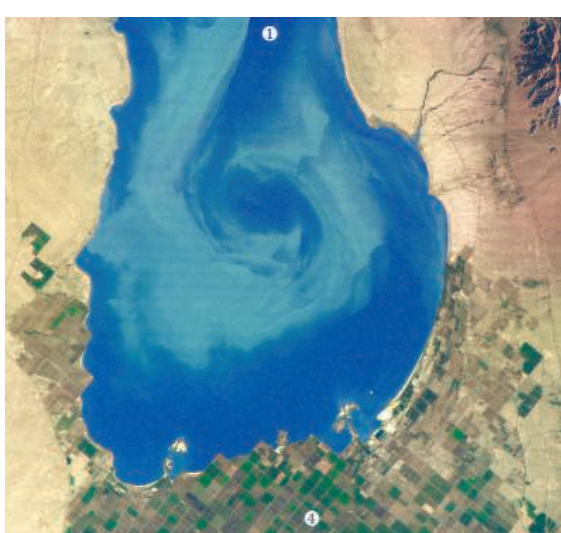
BROWSINGS

America from the Air. A Guide to the Landscape Along Your Route. Daniel Mathews and James S. Jackson. Houghton Mifflin, Boston, 2007. 400 pp + CD-ROM. Paper, $19.95, C$26.95. ISBN 9780618706037.
Those willing to forgo an aisle seat will enjoy this guide to sights along 14 flight corridors over the continental United States. The authors pair aerial photos annotated to identify landscape features with essays that discuss the geology, natural history, and traces of human activities passing beneath the plane (right, the Salton Sea with an algal bloom and irrigated croplands of the Imperial Valley, California). Localities are marked on a map of preferred flight paths, and the CD offers passengers easy access to the entire book through their laptops.



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SCIENCE COMMUNICATION

Interactions with the Mass Media

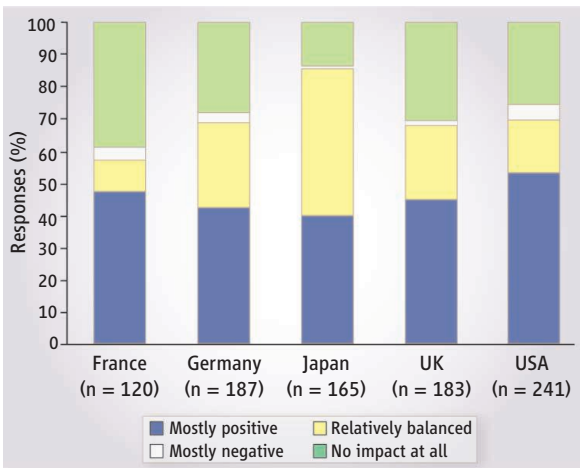
A survey reveals that media contacts of scientists in top R&D countries are more frequent and smooth than was previously thought.

Hans Peter Peters,[1]* Dominique Brossard,[2] Suzanne de Cheveigné,[3] Sharon Dunwoody,[2] Monika Kallfass,[1] Steve Miller,[4] Shoji Tsuchida[5]

Previous research, as well as anecdotal evidence among researchers and journalists, often leads to perceptions of "barriers" to a more active involvement of scientists in public communication [e.g. (*1*–*3*)] or of a "gap" between science and journalism (*4*) or to areas of potential conflict between the two professions (*5*, *6*). Recently, researchers have begun to recognize the symbiotic character of many scientist-journalist interactions (*7*). Nonetheless, negative experiences with the media still dominate peer communication about science-media relations. On the basis of extensive survey data, we now challenge several of the negative impressions of science-media interactions that are still all too common.

Although surveys of scientists' interactions with the media have been conducted in several countries [e.g. (*1*, *4*, *6*)], little empirical research has compared scientists' public communication attitudes and activities across countries in a rigorous way. One might expect cross-cultural differences in the science-media interface for several reasons, among them possible differential benefits of public visibility because of variance in competitive research funding environments, differences in the nature of professionalism in science journalism or science public relations, or cultural differences in public acceptance of science and technology across countries.

Our analysis was based on a mail survey in 2005–06 of 1354 researchers in the United States (n = 358), Japan (n = 239), Germany (n = 283), United Kingdom (n = 281), and France (n = 193), the top countries for research and development (R&D) at the time of the study. Averaged across countries, the response rate was 43%; the sampling bias is unlikely to invalidate our main findings [for details, see (*8*)]. We used two research fields—epidemiology and stem cell research—as case studies. The sample comprises 648 epidemiologists and 706 stem cell researchers who had published during 2002–04 in peer-reviewed journals. With few exceptions, the results for the two research fields studied were quite similar. We, therefore, present aggregated research field results here and focus on cross-country comparisons. The supporting material (*8*) will provide the reader with breakdowns by field.



Perceived impact of media contacts on career by country. Distribution of answers to the question: "Consider the totality of your media contacts over your career. How great has their positive or negative impact been on you professionally?" Only respondents reporting media contact(s) in the past 3 years are included in the graph. See table S4 for a breakdown of responses by country and research field.

Across the countries under study, scientist-journalist interactions were not the province of a small set of scientists but, rather, were more common than anticipated (fig. S1A). Of the respondents, 30% said they had been engaged in more than five media contacts in the past 3 years, and another 39% reported one to five contacts. In all countries, epidemiologists had more contact with journalists than stem cell researchers, but there were no significant differences across countries (table S2). The primary type of media contact was the media interview; nearly two-thirds of the respondents (64%) said that they have been interviewed by journalists at least once in the past 3 years (fig. S1B). Frequency of contact with journalists was clearly associated with leadership functions and research productivity; a scientist's personal attitude was also a factor but was relatively less important (fig. S2). The amount of contact, as well as its association with leadership role, research productivity, and personal attitude, formed a pattern that is quite similar across countries (tables S2 and S5).

We posed 16 motives, both positive and negative, that could influence scientists' willingness to interact with journalists and asked respondents to rate the importance of each factor to themselves personally. Despite some differences regarding the perception of risks and benefits across countries, three main findings emerged (table S8).

Increasing the public's appreciation of science was the most important benefit mentioned by scientists as an incentive to interact with the media. More than 9 in 10 respondents (93%) indicated that achieving "a more positive public attitude toward research" was an important motivator; about as many (92%) similarly identified "a better-educated general public."

In interactions with the media, many scientists indicated they felt uncertain and perceived a lack of control. Nine in 10 respondents identified the "risk of incorrect quotation" in stories as an important disincentive, and 8 in 10 felt that the "unpredictability of journalists" was also a problem.

Norms of the scientific community committing researchers to strong peer orientation and highly precise information (delivered in a formal, impersonal style) have historically been regarded as major deterrents to scientists' interactions with journalists (*3*). However, those norms seem to be playing a more nuanced role today as only 34% of our sample identified "incompatibility with the scientific culture" as an important concern. Furthermore, the impact of scientific norms seemed to be perceived inconsistently (table S10). Although "possible critical reactions from peers" were considered important concerns for 42% of the respondents, a similar proportion (39%) found "enhanced personal reputation among peers" to be an important outcome of media contacts.

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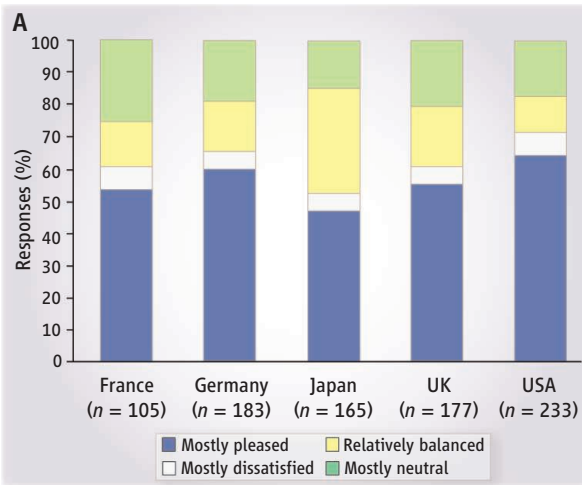
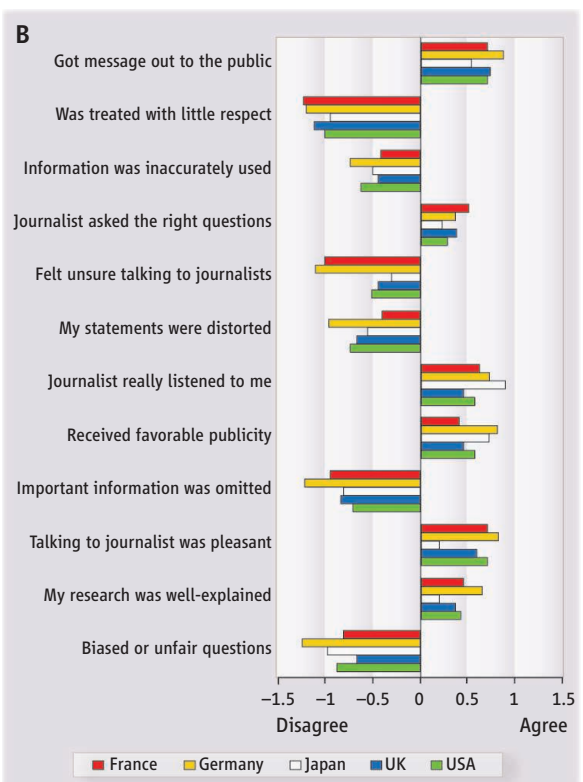
In all five countries, a plurality of scientists who had contact with the media in the past 3 years rated the impact of those contacts on their careers positively (see chart, page 204). Overall, 46% of the respondents perceived a "mostly positive" impact, whereas only 3% reflected a "mostly negative" impact. Nearly one in five felt that the positive and negative impacts balanced each other (table S4). By cross-tabulating, we were able to check that, with one exception, concerns and perceived benefits were not significantly correlated with scientist's management roles and number of publications. The one exception was that for researchers with lower rank (no management position, few publications) "possible critical reactions from the heads of department or organization" were somewhat more important than for researchers with higher rank [gamma test of strength of associations, $\gamma = 0.23$ and 0.29, respectively]. This is not at all surprising.

While respondents were certainly critical of journalists (see above), they assessed their personal interactions with journalism quite positively. Overall, 57% of the respondents said they were "mostly pleased" about their "latest appearance in the media," and only 6% were "mostly dissatisfied" (see chart, right, part A). When asked to evaluate their encounters with journalists over time and across a variety of characteristics, scientists in all countries agreed with positive statements about their contacts and disagreed with negative ones (see chart, right, part B).

In contrast, when assessing the quality of media coverage of scientific topics in general on four aspects (accuracy, use of credible sources, presence of a hostile tone, and comprehensiveness), scientists on average were neither clearly positive nor negative (table S9). We do not mistake scientists' ratings of science coverage as valid evaluations of its quality. Such an evaluation would have to be based on an analysis of the coverage itself and is not the subject of this paper. Rather we take the difference between assessment of one's own contacts and assessment of media science coverage in general as a cue that scientists apply different criteria when assessing journalistic performance with respect to coverage of their own research relative to research in general.





Scientists' assessment of media contacts by country. Only respondents reporting media contact(s) in the past 3 years are included in the graphs. (**A**) Distribution of answers to the question: "Think back to the latest occasion when you were mentioned, quoted, or interviewed by the media. [...] What was your own general response to that latest appearance in the media?" (**B**) Average agreement or disagreement of respondents with six positive and six negative statements about their encounters with journalists, measured on five-step answering scales ranging from −2 ("strongly disagree") to +2 ("strongly agree"). The question was: "Scientists have a variety of experiences when serving as media sources. What are your typical reactions to encounters you have had with journalists in the past 3 years?" Labels in (B) are abbreviated. See table S7 for the exact item wording and for a breakdown of responses by country and research field.

The data did illuminate minor country differences. Japanese researchers reported being slightly less "pleased" with their latest appearance in the media than their Western colleagues were, and researchers from the USA and Germany were slightly more "pleased" than British and French scientists (see figure this page, top). These country differences were statistically significant (table S6). Assessments of media coverage of science in general also varied modestly but significantly by country (table S9). German and French researchers rated the quality of science coverage most positively, British researchers perceived it most negatively, and U.S. and Japanese researchers took middle positions.

Our analysis shows that interactions between scientists and journalists are more frequent and smooth than previously thought. This five-country survey also suggests that the scientists most involved in these interactions tend to be scientifically productive, have leadership roles, and—although they consider concerns as well as perceived benefits—that they perceive the interactions to have more positive than negative outcomes. Despite minor variations in the assessment of media contacts across the five countries, the basic patterns are surprisingly similar. The functional necessity of public science communication may be a global phenomenon in democratic knowledge societies.

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GENETICS

Celebrating Spuds

Genetic studies are shedding light on the origins and domestication of potatoes and are helping to improve the properties of cultivated species.

Sandra Knapp

After the cereal grains rice, wheat, and maize, the potato (*Solanum tuberosum*) is the fourth most important source of carbohydrates in the human diet. The United Nations Food and Agriculture Organization (FAO) has declared 2008 the International Year of the Potato to raise awareness of the tuber's importance. Recent genetic studies have shed light on questions such as where the potato was first cultivated and how its resistance to blight and its nutritional content can be increased. Many more insights are expected from the potato genome, due to be completed by the end of 2010.

The cultivated potato is 1 of about 1500 species of the genus *Solanum* (Solanaceae), 1 of the 10 most species-rich genera of flowering plants that also include pepper, eggplant, and tomato (*1*). Potatoes and their wild relatives form one of the 13 major clades of *Solanum* (*2*) and are most closely related to the tomatoes (*3*). All ~190 wild potato species are found in the montane regions of the American tropics (*4*). Wild potato taxonomy is complicated by polyploidy (different species can have from two to six sets of chromosomes) (*5*), suspected rampant hybridization (*6*), and extensive morphological variation. Cultivated potatoes have variously been treated as 21 different species or 1 hugely variable species, but a recent study (*7*) suggests that they can be reliably classified into four taxa with different ploidy levels.

Potato is one of several tuber crops from the Andes (*8*) and was the staple of the Inca Empire when the Spanish first arrived in the New World. The origins of *S. tuberosum* have been controversial, but a recent genotyping study of hundreds of traditional varieties (landraces) has shown unambiguously that the species cultivated today had its origins in populations of the diploid group of wild species related to *S. brevicaule* in southern Peru and northern Bolivia (*9*). *S. tuberosum* is tetraploid (having four sets of chromosomes). It has recently been confirmed (*5*) that this property arose through simple chromosome doubling rather than through the merging of two different chromosome sets from different progenitors. The latter process is responsible for the polyploidy of many wild species of *Solanum* section *Petota* in the Andes and in the mountains of Mexico and Central America. Comparisons of gene trees based on nuclear and plastid DNA sequence data has suggested maternal and paternal progenitors for many of these naturally occurring polyploids (*5*); it will be very interesting to see how these new data can be applied to breeding programs to improve the cultivated species (*10*).

Wild tubers. Tubers of potato landraces in Peru are highly variable in color, size, and shape. Potato genetic variation is conserved at CIP (Centro Internacional de la Papa/International Potato Center) in Peru. The potatoes shown are all edible.

S. tuberosum was brought to Europe by the Spanish, where it quickly became a key foodstuff for the poor. By 1573, just decades after the "conquest" of Peru, the potato was recorded as a staple in the Hospital de Sangre (the poorhouse) of Seville (*11*). It was long thought that the first genotypes of *S. tuberosum* brought to Europe were from the equatorial Andes, and that Chilean genotypes were brought in to replenish stocks after the 19th-century blight epidemics; almost all potatoes grown today in Europe are Chilean genotypes. However, the story is more complicated. A 241–base pair deletion in the plastid genome differentiates clearly between Chilean and equatorial Andean landraces of *S. tuberosum*. Ames and Spooner (*12*) have assayed for this in carefully preserved historical herbarium specimens, some from as far back as 1680, and have shown that both Andean and Chilean genotypes were present in Europe before and after the epidemics, with Chilean landraces arriving as early as 1700.

The ease with which potato is vegetatively propagated and the dependence of poor, rural populations on it profoundly affected world history. The potato famine of the mid-19th century was due to a strain of late blight (*Phytophthora infestans*) that swept though the small potato fields cultivated by the rural Irish poor. The complete failure of the one staple crop caused a death toll in the millions, long-lasting bitter feelings in the British Isles, and a huge wave of immigration that changed the nature of the United States.

Late blight is still a huge problem in potato cultivation worldwide. Most popular and accepted potato varieties are several decades old, due to the difficulties and conservatism of potato breeding. Breeding efforts have concentrated on introducing blight resistance genes from wild species into cultivated potatoes, but the mold soon evolves ways to overcome resistance (*13*). It may be much easier and more efficient to introduce useful genes transgenically into an existing accepted variety than to recover ideal trait combinations after outcrossing and traditional methods. Recent attempts to genetically engineer blight resistance with genes from wild species have been controversial but effective (*14*).

Both traditional breeding programs and genetic engineering can also be used to improve other traits, such as nutritional content. For example, although packed with nutrition, the potato is poor in vitamin A. In 2007, the potato cultivar "Mayan Gold" was released in the United Kingdom with increased levels of

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carotenoid pigments that are the precursors of this essential nutrient. Genetically engineered potatoes have been produced that have fourfold increases in vitamin A precursor levels—a true "golden potato" (*15*, *16*). All three important carotenoid genes are necessary for attaining maximal levels of tuber carotenoids in potato, unlike in the more famous golden rice or canola, where a single gene is more important than the rest.

The complexity of potato genetics and variation is the subject of intense study by genome scientists. An international consortium has begun to sequence the potato genome (*17*) using a diploid line. Coupled with the emerging tomato genome sequence (*18*), the potato genome will provide new data for not only plant breeding, but also for evolutionary studies. The fact that gene order is similar across several crop species in the Solanaceae (such as pepper, eggplant, and petunia) (*19*) should enable insightful comparative studies.

The humble spud has come a long way from its origins in the high Andes. As research uniting genomics, breeding, and biodiversity accelerates, the potato looks set to play an even more important role not only in aiding our understanding of genetics, but in feeding a still-growing human population. It is fitting that we celebrate a plant that has given our species so much, and still has so much to offer.

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ECONOMICS

Homo experimentalis Evolves

Economists are increasingly using field experiments to explore economic behaviors.

John A. List

The fundamental challenge in the social sciences is how to go beyond correlational analysis to provide insights on causation. Economists have long used precise models and econometric techniques to answer causal questions using variations in naturally occurring data (*1*). Increasingly, insights on causation are also gained through the use of controlled experimentation. In this approach, causation is usually identified through randomization, much like controlled experiments used in drug trials.

CREDIT: PETER HOEY

In economics, laboratory experiments have been used to meaningfully test theories, lend important qualitative insights, and provide a first glimpse at what can happen in an economic system. To complement lab and naturally occurring economic data, studies that gather data via field experiments have become more frequent during the past decade. Such experiments are a useful marriage between laboratory and naturally occurring data in that they represent a mixture of control and realism usually not achieved in the lab or with naturally occurring data.

Economists use three main types of field experiments to sample various populations and situations (*2*). Artifactual field experiments share many of the qualities of conventional lab experiments but use a subject pool from the population of interest. Framed field experiments account for important situational features of the market of interest by embedding decisions in their natural context, and therefore making decisions much less abstract. Yet the subjects remain aware that they are part of an experimental study. Framed field experiments are a cousin of social experiments of the 20th century such as employment programs and housing allowances (*3*). Finally, in a natural field experiment, the analyst manipulates experimental conditions in a natural manner, whereby the experimental subjects are unaware that they are participating in an experiment. This approach combines the most attractive elements of the laboratory and of naturally occurring data: randomization and realism.

Each of these field experiment types is a means of collecting data. In the sciences, data are generally collected for three purposes: to provide enough facts to help construct a theory, to test the predictions of a theory, and to measure key parameters. Field experiments in economics can also be a useful tool for each of these data purposes.

For example, Anderson and co-authors (*4*) have used a natural field experiment to collect facts useful for constructing a theory about consumer reactions to advertisements. Working with a retail catalog merchant, the authors manipulated the frequency of catalog advertising sent to randomly selected customer samples. Over an 8-month period, one set of consumers received 17 catalogs, whereas another set received 12 catalogs. The increased frequency positively influenced sales among the consumers who purchase infrequently, but the effect on the company's highly valued consumers was negative in the long run. The results pinpoint important roles for both brand-switching and how advertisements affect consumers balancing current consumption against future consumption.

In another recent natural field experiment, Reiley and Katkar (*5*) used Internet-based auctions to test the theory of reserve prices in auctions. The authors designed a field experi-

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ment to compare outcomes in auctions with secret versus public reserve prices, two common approaches used to auction goods on the Internet. They auctioned 50 matched pairs of Pokemon trading cards on eBay: one with a minimum bid of 30% of the card's book value, and one with a minimum bid of $0.05 and a secret reserve price equal to 30% of the card's book value. Keeping the reserve price secret reduced the probability of selling any card, the number of serious bidders in an auction, and the amount of the winning bid. Thus, contrary to the beliefs of many eBay sellers and to the predictions of models of rational bidder behavior, using secret reserve prices instead of public reserve prices actually lowers a seller's expected returns.

An example of a natural field experiment designed to measure key parameters of a theory is (*6*), where parameters associated with why people give to charities are estimated. In this study, Karlan and I worked with a private charity to explore the effects of different matching rates on charitable giving by soliciting contributions from more than 50,000 supporters. In one group, solicitees were informed that for every dollar contributed, an outside donor would match the contribution 1:1. A control group received no match, and other groups received more generous matching rates (such as 2:1 or 3:1). Simply announcing that a match is available increases the revenue per solicitation by 19%. In addition, the match offer increases the probability that an individual donates by 22%. These estimates shed light on a key parameter for fundraisers: how sensitive contributions are to the "price" of giving.

In the examples above, I have focused on natural field experiments; similar examples can be found for artifactual and framed field experiments. The various field experimental approaches, lab experiments, and econometric methods using naturally occurring data should be thought of as strong complements—much like theory and empiricism. Combining insights gained from each methodology will permit scholars to develop a deeper scientific understanding. For example, economists have shown that there is much to be gained from gathering data from a variety of settings, both controlled and uncontrolled. In those cases where behaviors are robust, the advice to policy-makers can be unequivocal. In other instances, behaviors might differ systematically, and developing theory to explain such discrepancies deepens our economic understanding. Similar gains can accrue within the sciences more broadly.

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GENETICS

Insights into the Pathogenesis of Autism

Genetic analysis of inbred families reveals genes associated with susceptibility to autism.

James S. Sutcliffe

Autism is a common developmental disorder that profoundly impairs the emergence of social behaviors and communication in children before 3 years of age. Repetitive, stereotyped, and obsessive-compulsive–like behaviors are also prominent features of the disorder (*1*), and are often accompanied by cognitive impairment, seizures or epilepsy, gastrointestinal complaints, disordered sleep, and other problems. Identifying risk factors for autism has become a high priority of scientists, lay groups, and parents of autistic children. On page 218 of this issue, Morrow *et al*. (*2*) add several more genes to a growing number of genetic abnormalities that correlate with susceptibility to autism (see the figure).

Twin and family studies demonstrate that the etiology of autism has a substantial genetic component. Current estimates of sibling recurrence risk—the likelihood that a younger sibling of an autistic child will also have autism—is greater than 15% (*3*–*5*). Comparing this to population rates of approximately 1 per 500 children for narrowly defined autism or 1 per 150 children for the more broadly defined autism spectrum disorders indicates a high degree of heritability in families.

Determining specific genetic changes that increase the risk of developing disorders like autism is extraordinarily complex (*6*) due to heterogeneity—different kinds of variation at many underlying genes are involved. One type of variation consists of rare disease-causing or highly penetrant mutations, and these have implicated specific biological processes. Similarly, common variation—usually discrete changes in DNA sequence—has been identified in autism, but only a few specific findings have been replicated. Other important clues to genetic factors in autism include abnormalities such as chromosomal translocations, inversions, and large deletions or duplications, which are more frequent in individuals that present clinically with dysmorphic features and severe cognitive impairment. Geneticists have long hypothesized that genes disrupted by chromosomal abnormalities in isolated cases may play a role in susceptibility to autism more broadly and have pursued experiments toward this end.

Recent advances in DNA microarray technologies have revealed a substantial etiological role for small losses and gains of DNA—so-called copy number variation—in autism (*7*–*12*). All individuals harbor this common form of genetic variation, which can be inherited from a parent or can arise as a sporadic event de novo. However, a large and growing number of deletions and duplications of DNA have been found in people with autism. As comparisons to control samples identify which variants are unique, more frequent, or equal in autism versus control cases, we will be better able to interpret the observed copy number variation.

Much discussion has focused on whether a copy number variant is inherited or arises de novo, with greater interpretive weight vis-à-vis disease association given to the latter. As with large chromosomal abnormalities, it may be that the disruption or dysregulation of gene expression underlies the risk or causal effect for a given copy number variant. Genes may be lost or an extra copy may be present on a given chromosome; genes flanking a DNA

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PUTATIVE AND KNOWN AUTISM-RELATED GENES	
Glutamatergic synapse function and/or neuronal cell adhesion	
FMR1[A,B]	Fragile X mental retardation 1
NLGN3[B]	Neuroligin 3
NLGN4[B]	Neuroligin 4
NRXN1[B,C]	Neurexin 1
SHANK3[B,C]	SH3 and mulitple ankyrin repeat domains 3
CNTNAP2[B,C,D]	Contactin-associated protein-like 2
PCDH10[C]	**Protocadherin 10**
CNTN3[C]	**Contactin 3**
Endosomal trafficking	
NHE9 (SLC9A9)[B,C]	**Na^+/H^+ exchanger isoform 9**
NHE6 (SLC9A6)[B]	Na^+/H^+ exchanger isoform 6
***DIA1* (c3orf58)**[C]	**Deleted in autism 1**
A2BP1[C]	Ataxin 2-binding protein 1
Neuronal activity regulation	
FMR1[A,B]	Fragile X mental retardation 1
MECP2[B,C]	Methyl CpG binding protein 2
***DIA1* (c3orf58)**[C]	**Deleted in autism 1**
PCDH10[C]	**Protocadherin 10**
NHE9 (SLC9A9)[B,C]	**Na^+/H^+ exchanger isoform 9**
A2BP1[C]	Ataxin 2-binding protein 1
UBE3A[B,C]	Ubiquitin protein ligase E3A
Implicated in related disorders	
FMR1[A,B]	Fragile X mental retardation 1
MECP2[B,C]	Methyl CpG binding protein 2
NHE6 (SLC9A6)[B]	Na^+/H^+ exchanger isoform 6
A2BP1[C]	Ataxin 2-binding protein 1
UBE3A[B,C]	Ubiquitin protein ligase E3A
Other functions	
EN2[D]	Engrailed homeobox 2
SLC6A4[B,D]	Serotonin transporter (SERT, 5-HTT)
MET[D]	Met proto-oncogene (c-Met, HGFR)
SCN7A[C]	**Na^+ channel, voltage-gated, type VII**
RNF8[C]	**Ring finger protein 8**

Genes implicated in autism pathogenesis. Genes have been implicated in autism (*1*, *2*) on the basis of different functions and forms of genetic variation, and also on their association with disorders that show features of autism. They share common or related pathways, as shown. A, genes showing triplet repeat expansion; B, genes with rare mutations or coding variants; C, genes with copy number variation or chromosomal abnormality; D, association of common alleles. Genes implicated from (*2*) are shown in bold.

deletion or duplication may be subject to dysregulation because of altered local chromatin structure or separation from key enhancer elements (which regulate gene expression). Thus, copy number variation is a major category of genetic risk for autism spectrum disorders, and is implicated in 10 to 20% (or more) of cases (*7*–*12*). The genetic heterogeneity of autism, however, greatly complicates the task of identifying genes that increase susceptibility to the disorder.

Morrow *et al.* use the powerful genetic technique of homozygosity mapping to identify autism genes. Geneticists have long taken advantage of the statistical power afforded by genetic analysis of families in which parents of affected individuals share a common ancestry (e.g., first cousins). Such consanguineous families, more common in the Middle East, are at substantially increased risk for autosomal recessive conditions [traits that are expressed when an individual is homozygous (has two identical copies) for a particular gene]. There is a growing recognition that inbred families are also useful in identifying genes for complex disorders, such as autism.

Morrow *et al*. use DNA microarrays to study numerous consanguineous families from the Middle East. By analyzing the inheritance of DNA throughout the genome in these pedigrees, they identify chromosomal regions that are inherited in common by the affected individuals who share the same two copies of these regions. These homozygous segments, which are heterozygous in the related parents, are likely to represent a causal or risk factor. In several of these families, the regions linked to the autism spectrum disorder and inherited "identical by descent" contained deletions. Thus, the affected individuals were completely deficient for the genes (or potential regulatory DNA) that lie within the deleted intervals. By extension, the absence of those gene products, and/or the possible altered expression of genes in the immediate vicinity of the deletion, is predicted to cause the autism spectrum disorder in that family.

An important question is whether a gene identified as causing disease in a single inbred family has any relevance to autism in nonconsanguineous families. In addition, establishing which gene (or genes) lies within or near a deleted interval—the disruption of which is causing the disorder—is not trivial. Here, a nice story is developed for one such region on chromosome 3q containing a large (~886 kilobase) deletion. A gene called *DIA1* (*deleted in autism1*; also known as *c3orf58*) encoding an uncharacterized protein is completely removed, whereas *NHE9* (*Na^+/H^+ exchanger 9*), a nearby gene encoding a membrane protein that exchanges intracellular H^+ for extracellular Na^+, remains intact but could be dysregulated. To assess the broader relevance of these genes in autism, Morrow *et al.* sequenced the coding regions of *NHE9* in affected subjects from nonconsanguineous U.S. families and found a loss-of-function mutation in one family. Similar mutations cause an epilepsy phenotype in mice, and for the related *NHE6* gene, they cause a phenotype with autistic symptoms and epilepsy. In addition, other variation is implicated, because a focus on autism families with epilepsy led the authors to observe a much greater number of coding variants in cases compared with controls. Taken together, these findings support dysregulation of *NHE9* as a contributing or causal factor in that family.

The most provocative observations from this study point to an important functional class of genes involved in autism susceptibility. The authors show that several of the genes identified in or likely affected by homozygous deletions are regulated by neuronal activity—that is, their expression changes in response to stimulation of neuronal activity. Because autism is a neurodevelopmental disorder, emphasis has been placed on prenatal development, which is guided by intrinsic gene-expression patterns. The brain continues to develop long after birth, however, and experience and environmental input play an important role in subsequent development. Synapses (connections between neurons) mature partly as a function of experience-dependent neuronal activity and of the gene-expression changes that accompany it. But if those genes are disrupted by mutation or copy number variation, that could suggest that the process of activity-regulated synaptic development itself is disrupted in some way. Indeed, this is the authors' hypothesis.

Dysregulation of synaptic development is an established idea in autism research. Although it is conceptually a big step, and the authors are cautious in their conclusions, the possibility that dysregulation of these genes results in disruption of synaptic development in response to early-life environment and experiences is an intriguing proposal, whose validity must await the results of further research.

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ENGINEERING

Phase-Change Materials for Electronic Memories

The materials used in rewritable CDs and DVDs also show promise as electronic memories systems.

Greg Atwood

Cell phones, digital music players, and digital cameras have become pervasive and almost essential components of everyday life. The next wave of digital devices, such as ultramobile PCs and internet-connected personal assistants, promise to further influence the way we live and work. All these devices have been enabled by rapid increases in the storage density of solid-state nonvolatile memory, called flash memory, which operates by injecting and storing a charge for long periods of time in an insulated, floating gate structure. Encouraged by the success of flash, alternatives are being explored that may be faster, smaller, and offer higher levels of functionality. One approach aims to exploit the phase-change materials used today in rewritable CDs and DVDs.

Flash memory is used to store not only music, pictures, and other data on portable devices but also the operating system and application programs that enable the devices to work. Information is stored by placing or removing electrons on an electrically isolated capacitor that is integrated into a transistor. The presence or absence of electrons changes the behavior of the transistor which can be associated with a binary data bit (a "0" or a "1"). The distinctive feature value of flash versus other solid-state memory is nonvolatility. Stored data will remain even in the absence of power. A single flash memory device today can store 10's of billions of bits of information, but as new portable device applications continue to emerge, demands are constantly being made to increase storage density. However, some of the physical limits for the storage mechanism are being approached, which has stimulated interest in the study of new storage physics.

State-of-the-art flash uses features of ~45 nm today, but serious issues begin to arise for feature sizes below about 20 nm due to retention of electrons. Of the new nonvolatile memory alternatives, phase-change memory is rapidly gaining favor as the leading candidate to succeed flash, but also because it offers the possibility of new usage models for nonvolatile memory.

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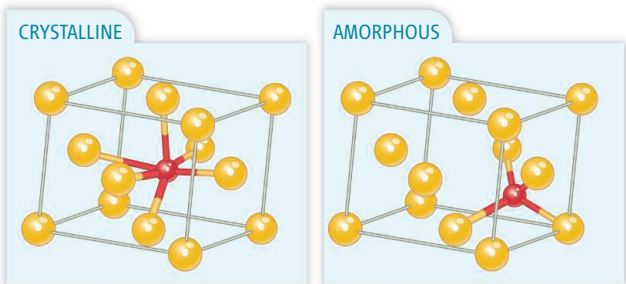


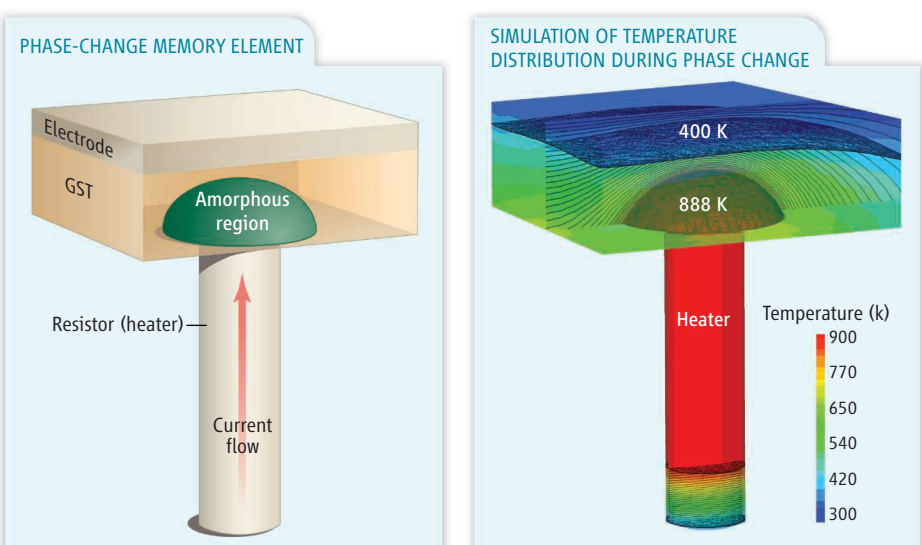
Crystal versus amorphous phase. A germanium atom in the face-centered cubic structure formed by tellurium atoms. The germanium atoms occupy the octahedral and tetrahedral symmetry positions in the crystalline and amorphous states, respectively. [Adapted from (*1*)]

Phase-change memory uses a different storage mechanism than does flash memory. Data are stored not as charge but as a physical structural difference in the material. These materials undergo a stable, rapid, and reversible transition between an ordered crystalline and a disordered amorphous atomic structure. The two phases have different reflectivities, which have been exploited in optical memories, but also different conductivities, which can be exploited in electronic memories.

The materials are generally called chalcogenides, because they contain one of the group 6 periodic elements. One of the most widely studied materials is GST, a glassy alloy of germanium, antimony, and tellurium.

The transition between the two phases for chalcogenides is achieved by applying heat to "melt" the material, putting it in a disordered state, and subsequently cooling it, which will "freeze" the material in the disordered phase or allow crystals to grow and result in a locally ordered phase; the final phase is determined by the rate of cooling. The transition between these two phases for GST can be quite fast: about 1 ns to form the disordered phase and about 100 ns to form the ordered phase.

The transition is rapid because only a small change in atomic structure is required to alter its phase. In GST [see the first figure and (*1*)], the ordered (crystalline) phase is believed to be a distorted rock salt structure, with the germanium atoms in an octahedral coordination; the disordered (amorphous) phase bonds the germanium atoms to a tetrahedral coordination. This structural change takes place locally, does not require relaxation of the complete structure,



Making resistive memories. (Left) Phase-change memory element. **(Right)** Simulation of temperature distribution during phase change.

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and does not require diffusion. The change in electronic state accounts for the large change in optical and electrical properties.

Exploitation of the phase-change behavior of chalcogenide materials for storage is accomplished by one of two means: optical or electrical. In the amorphous phase, the material has a low reflectance and high resistance; in the crystalline phase, it has a high reflectance and low resistance. For optical storage, the heat required to change the phase is provided by a laser, and the reading of the stored data is done using a lower-power laser. However, high-power, slow, and large rotating optical drives are not suitable for the small devices that use flash.

Recent research (*2*) has focused on exploiting the electrical-resistive change behavior of phase-change materials for the development of a solid-state memory device. For these devices, the heat required to invoke phase change (and hence resistance change) is provided by passing a current through a resistor, or heating element, in contact and in series with the chalcogenide material (see the second figure). The stored data is read by passing a lower current through the chalcogenide and sensing its resistance. The power required to invoke a phase change is low because the volume of melted material is small, with a cross section of about 50 nm by 5 nm, making it viable for the development of high-density, high-performance memories.

Electrical phase-change memories are interesting for two primary reasons. The first is enhanced functionality. Phase-change memory can be altered at the bit level and can be written more than 1 million times, whereas flash must be altered in large blocks of bits and can be written only tens of thousand of times. The merger of some of the properties of today's flash and dynamic random-access memory (DRAM) provides a new level of functionality that can result in not only replacing flash but also replacing some usages of DRAM, such as storing frequently used operating code and high-performance disk caching.

The second reason for interest in phase-change memory is the small size of its memory element and its scalability. The phase-change physics shows promise to be scalable to dimensions of below 5 nm (*2*), providing the opportunity to continue the rate of cost reduction and density increase established by flash memory well into the next decade.

The ability to store more than one bit of information in a single phase-change memory element has been demonstrated (*3*), opening the possibility for further density increases. Multiple-bit storage exploits the fact that the resistance can be set to one of many discrete values through the proper combination of amorphous and crystalline properties in the memory element. For example, four resistance levels in a single memory element effectively store two bits of information.

Phase-change memory is emerging as a leading contender for replacing flash memory and expanding the capabilities of nonvolatile memory into the next decade. High-density, 128 Mb, phase-change memory prototypes have been demonstrated (*4*) at 90 nm, showing good performance and reliability. These prototypes are initiating the first steps of this technology into the marketplace.

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PALEONTOLOGY

New Tricks with Old Bones

Paleogenomic researchers are finding new ways to solve the problems of sample rarity and contamination.

Rachel Mackelprang[1,2] and Edward M. Rubin[1,2]

Progress in the field of ancient-organism genomics is prying open doors into the tombs of the long-deceased relatives of humans and other mammals. Advances in high-throughput DNA sequencing have provided initial glimpses of the nuclear genomes of ancestral organisms—cave bears, mammoths, and our closest relative, Neandertals, which diverged from a common ancestor with humans approximately 700,000 years ago (*1*–*4*). New discoveries have answered long-standing questions and opened new areas of debate, particularly regarding human evolution. Despite these developments, the preciousness of ancient remains, age-associated DNA damage, and sample contamination persist as barriers to progress, and the field of ancient-organism genomics lags far behind that of extant organisms. A series of promising new technologies and approaches for advancing the field—including novel ways to maximize DNA recovery, targeted capture of specific genomic regions, and analysis strategies for addressing the important issue of result contamination—are poised to contribute to insights into the genomes of extinct organisms.

Material for ancient DNA studies is restricted by preciousness of the specimens (particularly in the case of hominids) and the destructive processes required for the extraction of DNA from samples. Whole-genome amplification techniques begin to address this problem, but the small fragment sizes (often fewer than 100 base pairs) and damage characteristics of ancient DNA tend to result in the preferential amplification of undamaged modern and environmental contaminating DNA (*5*). Emulsion polymerase chain reaction (emPCR), a recently developed technique in which DNA molecules are spatially segregated and amplified in aqueous droplets in a water-in-oil emulsion (*6*), is a promising method for increasing sample yield. Each droplet contains a single template DNA molecule, and the cycles of enzymatic reactions that replicate it occur in the isolated droplets, substantially reducing the preferential amplification of undamaged modern DNA molecules over ancient damaged ones. This approach enables an unbiased increase in the yield of authentic endogenous nuclear DNA. Recently, nanogram amounts of starting material from 50,000- to 70,000-year-old horse, wolf, and bison were amplified by a factor of 500 to 1000 to microgram quantities (*7*). Applied to precious ancient material, this technique should greatly increase the amount of DNA sequence obtained per unit mass, reducing the degree of sample destruction required to carry out genomic studies.

The introduction of new parallel sequencing platforms has dramatically increased the production of random genome sequence, but many studies require the sequencing of specific regions from an organism's genome.

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Stone Age genomics. Neandertal skull and femur fragments that are approximately 40,000 years old were isolated from sites of Neandertal habitation in the Altai region of Siberia.

PCR amplification of specific loci from ancient material has largely focused on mitochondrial DNA. This is because mitochondrial genomes are relatively abundant and present in up to thousands of copies per somatic cell, whereas there are only two copies of each chromosome in the nucleus of a somatic cell. However, access to nuclear DNA is necessary for making most functional inferences because it contains the vast majority of genes and regulatory sequences, the keys to the biology of an organism.

Very recently, traditional PCR techniques were used in attempts to target two Neandertal nuclear genes: the melanocortin 1 receptor (*Mc1r*) (*8*), involved in hair and skin pigmentation, and forkhead box P2 (*Foxp2*), implicated in speech and language (*9*). In the *Mc1r* study, two Neandertal specimens were found to have the same nucleotide substitution that resulted in decreased receptor activity when tested in vitro. This change was not observed in a large number of DNA samples from modern humans, which suggests that the substitution is genuinely Neandertal in origin and, based on the phenotype of humans with similar melanocortin 1 receptor activity, is likely associated with red hair and pale skin in Neandertals. By contrast, the *Foxp2* genomic PCR results from two Neandertals indicate that they share a form of the gene (allele) with modern humans. This finding is inconsistent with previous estimates of the timing of the acquisition of this allele, which was dated using genetic variation data from extant humans. A reanalysis of the data from this study has raised the possibility of contamination (*10*).

The susceptibility of PCR to contamination and the difficulty of interpreting results when the Neandertal sequence appears to match that of modern humans highlight the need for other methods to target specific regions in nuclear DNA. Direct selection of specific DNA fragments from a mixture of sequences through a hybridization strategy is one such method that is less prone to false results from contamination. PCR does not distinguish between the scenarios of one starting template molecule versus multiple ones. Direct selection permits such discrimination, and a variation observed on multiple independent fragments is more likely to be genuine rather than the result of damage, contamination, or errors.

Microarray-based hybridization, coupled with high-throughput sequencing of recovered DNA, has recently been used to capture thousands of targets in parallel from modern DNA samples. With these strategies, a DNA sample is directly applied to an array of specifically designed oligonucleotide probes immobilized on a chip. Complementary fragments hybridize to the probes while the remaining nonbound DNA is washed away. The hybridized DNA can then be eluted from the chip and sequenced, resulting in enrichment of targeted genomic regions (*11*). Alternatively, chip-synthesized oligonucleotide probes have been released from the chip and used to capture molecules in solution (*12*). A purely solution-based method, where sets of probes are designed against a reference genome and used as a bait to "hook" corresponding sequences from a DNA pool (*13*), has been used to recover specific regions of nuclear DNA from Neandertal and cave bear genomic sequence libraries (*1*). These various capture approaches hold promise for economically investigating the same sequence in multiple different samples as well as examining multiple independent molecules of an allele isolated from a single sample.

The discrimination of genuine ancient sequences from modern contaminating material is of great concern in Neandertal genomics. A recently developed analysis approach addresses this problem by using the characteristic damage signatures of ancient DNA molecules (*14*). DNA molecules from ancient samples exhibit short fragment length and contain a large number of nucleotide transitions (from cytosine to thymine) due to cytosine deamination, whereas contaminating modern DNA molecules are longer than ancient ones and lack damage-induced sequence changes. This results in correlations among fragment length, damage profile, and the degree of contamination of a sample. This insight was used to reanalyze data from a Neandertal study, which yielded unexpected recent human divergence-time estimates (*3*) that differed substantially from those of other genetic studies (*1*, *15*, *16*). The analysis revealed that long fragments had an estimated divergence time nearly identical to that of modern human DNA, whereas short molecules yielded much older estimates. This, coupled with a reduced cytosine deamination damage signature in the larger DNA molecules, suggests the presence of modern DNA contamination as a possible explanation for the discrepant results (*14*). Examination of correlations among fragment length, divergence time estimates, and DNA damage profiles represents an important validation strategy for future studies to improve confidence in the authenticity of ancient DNA results.

Application of emerging molecular technologies to genomic analysis of extinct organisms is anticipated to circumvent many of the challenges currently facing the field—namely limited sample availability, contamination, and result authenticity. The combination of these techniques and high-throughput sequencing has profound implications for exploring the biology of ancient organisms. The public availability of large ancient DNA datasets to individuals without access to samples has provoked a recent influx of new ideas and analysis strategies for the study of ancient genomes, a phenomenon likely to spur rapid advances in this field.

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Enzymes Without Borders: Mobilizing Substrates, Delivering Products

Federico Forneris and Andrea Mattevi*

Many cellular reactions involve both hydrophobic and hydrophilic molecules that reside within the chemically distinct environments defined by the phospholipid-based membranes and the aqueous lumens of cytoplasm and organelles. Enzymes performing this type of reaction are required to access a lipophilic substrate located in the membranes and to catalyze its reaction with a polar, water-soluble compound. Here, we explore the different binding strategies and chemical tricks that enzymes have developed to overcome this problem. These reactions can be catalyzed by integral membrane proteins that channel a hydrophilic molecule into their active site, as well as by water-soluble enzymes that are able to capture a lipophilic substrate from the phospholipid bilayer. Many chemical and biological aspects of this type of enzymology remain to be investigated and will require the integration of protein chemistry with membrane biology.

The hallmark of cell architecture is the organization in compartments and organelles that are separated by phospholipid membranes. This property intrinsically defines two main cellular environments with substantially different physical-chemical properties. Hydrophobic compounds tend to partition preferentially into lipid bilayers, whereas the aqueous environments of the cytosol and the interiors of cellular organelles create a favorable medium for polar substances (*1*). However, between the hydrophobic membranes and the aqueous intra-organelle environment, there is not a static and rigid separation. Rather, there is a continuous reshuffling of metabolites, as well as a traffic of macromolecules (*2*–*4*), and many cell processes involve both hydrophobic and hydrophilic substrates—i.e., substances that typically belong to the membrane phase and water phase of the cell, respectively.

Enzymatic reactions involving compounds that reside within organelle lumens and the membranes have peculiar mechanistic requirements. If the enzyme is located in a water-soluble environment, how is the active site made accessible to hydrophobic substrates? Conversely, for a membrane-embedded active site, what are the mechanistic requirements for admission of hydrophilic substrates? An important concept that needs to be clarified is the definition of hydrophobic and hydrophilic substances (*5*). Considering the chemical diversity of the compounds that populate the cell compartments, it is impossible to unambiguously classify each of these molecules as "hydrophobic" or "hydrophilic." In general, we will define as "hydrophilic" the water-soluble compounds that are commonly present in the aqueous lumen of cell organelles, and as "hydrophobic" the molecules that belong to the lipid bilayer, even though they may have limited solubility in water solutions.

We have conducted a survey of the three-dimensional protein structures deposited in the Protein Data Bank (*6*), which has led us to identify a set of enzymes that can provide a structural framework to the reactions involving substrates of opposite chemical nature. This survey is clearly not intended to be a comprehensive analysis, which would be beyond the scope of this review. Our aim is to illustrate the mechanistic strategies underlying this type of cellular reaction and to frame a context for further research that is likely to require the development of new methods and concepts in enzymology.

Water Reacting with Lipophilic Substrates

An elegant example of the use of a hydrophilic compound in a hydrophobic context is highlighted by integral membrane metalloproteases, which cleave the peptide bonds of membrane-bound proteins (*7*). The reaction takes place inside the lipid bilayer by lateral diffusion (i.e., diffusion in the plane of the membrane) of the transmembrane peptide substrate into the enzyme catalytic center. Proteolysis is a hydrolytic process that requires usage of a water molecule, the quintessential hydrophilic substance. In this regard, membrane proteases are unique in that they can function in an environment that does not allow water molecules to reach the active site by simple diffusion. The recent structural investigation of several members of this enzyme class reveals the presence of a hydrophilic inner cavity that connects the catalytic center to the cytosol-exposed protein surface (Fig. 1A) (*8*–*10*). Water molecules can access this cavity, enabling the enzyme to capture its hydrophilic substrate and bring it to where it is needed for catalysis.

Fatty acid amide hydrolase (FAAH) displays another type of mechanism of action. The enzyme degrades compounds of the endocannabinoid class of lipids and terminates their neural signaling activity (*11*, *12*). FAAH is a functionally dimeric monotopic membrane protein with a large solvent-exposed globular body (Fig. 1B) (*13*). Its catalytic site is formed by a cavity that is located in the water-soluble globular region of the protein. The question then arises as to how the hydrophobic substrate is captured from the membrane and hydrolyzed by the enzyme. The three-dimensional structure of FAAH features an internal hydrophobic tunnel that connects the membrane-binding region to the active site (Fig. 1B). The endocannabinoid lipid substrate is apparently "sucked" by means of this channel, being thereby admitted into the binding site. A second channel, located on the cytoplasmic side of the protein, allows water molecules to approach the substrate, affording its hydrolysis. FAAH and intramembrane proteases exhibit two different catalytic strategies for hydrolyzing a lipophilic molecule (Fig. 1, A and B): desorption versus lateral diffusion. FAAH desorbs the substrate from the membrane and binds it inside the cytosol-exposed main body of the protein, whereas intramembrane proteases channel water molecules into the membrane-embedded active site, which can be accessed by the hydrophobic substrate through lateral diffusion.

Certain lipophile-processing hydrolytic enzymes are not membrane-associated. Therefore, unlike FAAH, they cannot use a direct desorption mechanism for recruiting their hydrophobic substrate(s). For their activity, these enzymes rely on auxiliary carrier proteins that can extract a lipophilic molecule from the membrane. Such a peculiar functionality is illustrated by saposins (*14*), whose crystal structures feature large and flexible hydrophobic pockets for binding different glycosphingolipids (*15*, *16*). These proteins carry out the dual roles of recruiting the ligands from the membrane and presenting them to water-soluble hydrolytic enzymes such as arylsulfatase A or glucosylceramide–β-glucosidase in the proper orientation for efficient catalysis (*14*). In these enzymatic systems, the solution adopted to solve the problem of hydrolyzing a hydrophobic substrate is to uncouple the catalytic reaction from the capturing of the lipophile from the membrane, by making use of helper proteins that desorb and encapsulate the substrate and make it available to hydrolytic enzymes.

Bringing Together Bulky Substrates

What happens when the hydrophobic and hydrophilic substrates are both relatively large, bulky molecules? This situation is exemplified by the hydroxysteroid dehydrogenases (HSDs), enzymes that use NADPH (nicotinamide adenine dinucleotide phosphate, reduced) to catalyze the reduction of the keto group of glucocorticoid steroids, generating important metabolites such as cortisol, a nuclear-receptor ligand (*17*, *18*). Members of this enzyme class are often anchored to the phospholipid bilayer through an integral N-terminal transmembrane helix (*19*) (Fig. 1C).

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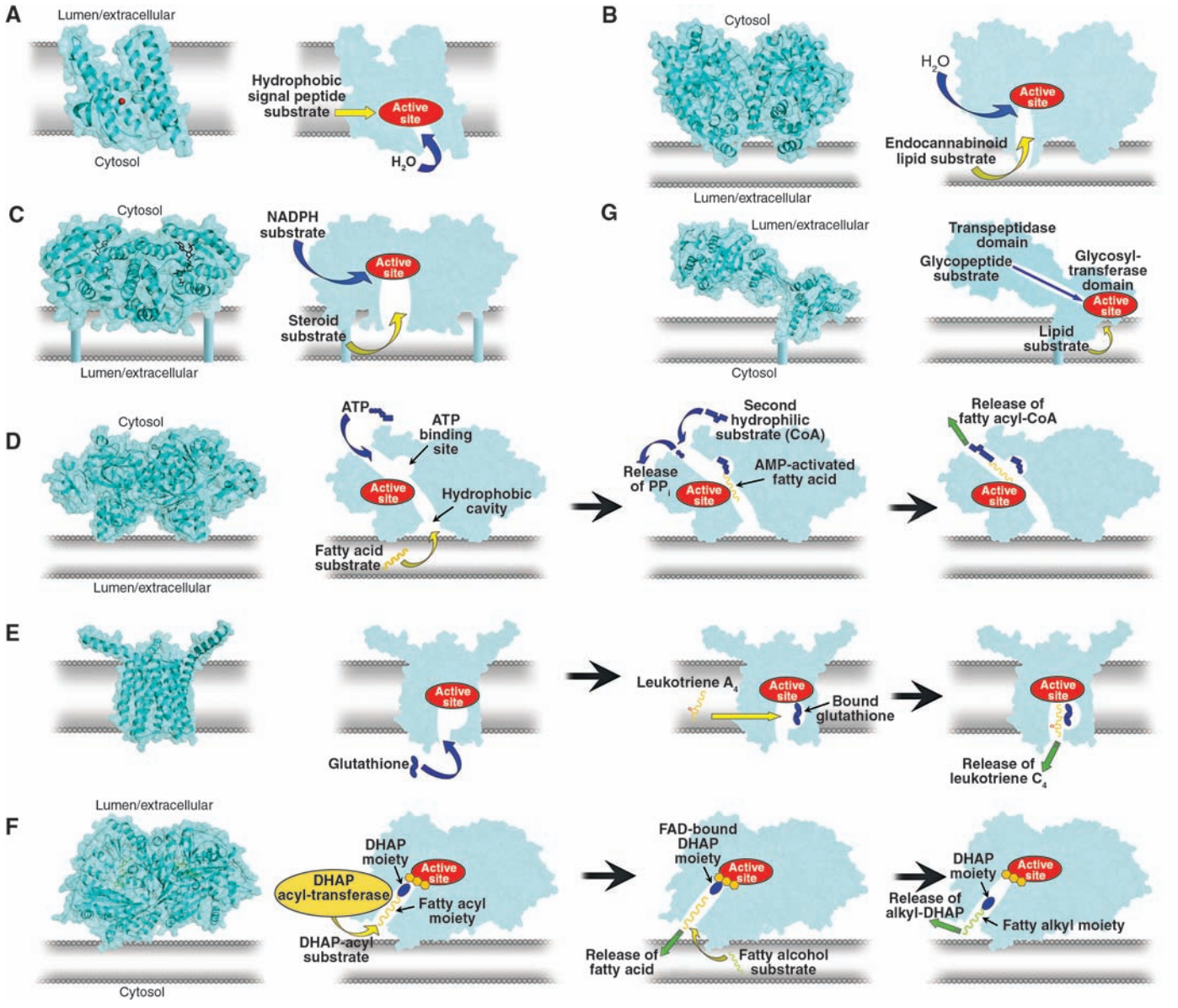

Fig. 1. Mechanisms of enzymatic reactions simultaneously involving hydrophobic and hydrophilic substrates. For each enzyme, the left panel shows the ribbon diagram and the molecular surface of the protein, whereas the right panel presents a schematic analysis of the structural basis of their mechanism of action. For the sake of simplicity, only one active site is shown for multimeric enzymes. Proteins are colored in cyan, the membrane bilayer is schematically drawn in gray, and the location of the active site is outlined in red. Transmembrane helices not visible in the crystal structures are represented by a vertical rectangle. (**A**) The catalytic center of bacterial site-2 metalloprotease is located in the bilayer and is accessible to membrane-embedded hydrophobic peptide substrates by lateral diffusion. Water, which is needed for the hydrolytic peptide cleavage, gains access through a tunnel. The red circle on the left indicates the position of the active-site Zn^{2+} atom. (**B**) The structure of dimeric membrane-anchored FAAH features a cavity open to the membrane bilayer. This cavity allows the partitioning of the lipid substrate from the membrane to the active site, which is located in the main body of the enzyme and can be readily accessed by water molecules. (**C**) The membrane association of dimeric HSD enzymes enables them to desorb the hydrophobic substrate from the membrane and reduce it by making use of a water-soluble electron donor such as NADPH (shown as black sticks in the left panel). (**D**) FACS conjugates fatty acids to coenzyme A to direct them into the cytosolic metabolism. Fatty acids (yellow) are proposed to access the catalytic cavity directly from the membrane, whereas the ATP and coenzyme A substrates (blue) reach the active site from the solvent-exposed side of the dimeric protein. (**E**) LTC_4S illustrates another type of mechanism: the sequential binding in the same pocket of a hydrophilic substrate followed by a hydrophobic molecule. Only after glutathione (blue) binding does the catalytic cavity become suitable to bind the hydrophobic leukotriene A_4 (yellow, with reactive epoxide bond in red) substrate by lateral diffusion from the membrane phase. In the current literature, there is a contradiction regarding the orientation of LTC_4S in the membrane and, therefore, we do not specifically indicate the cytosolic and extracellular sides of the membrane. (**F**) ADPS illustrates a reaction in which a hydrophilic compound (dihydroxyacetonephosphate; DHAP, shown in blue) is encapsulated by a membrane-associated enzyme that acts on long-chain aliphatic substrates. In the first part of the catalytic cycle, this FAD-dependent enzyme (FAD is shown as a three-membered yellow ring) binds acyl-DHAP, forms a covalent bond with DHAP, and releases the acyl product (yellow) to the lipid bilayer. The reaction is completed by binding a membrane-extracted fatty alcohol (green) to generate the final alkyl-DHAP product. (**G**) The active site of peptidoglycan transglycosylase is localized at the membrane surface. This feature allows the protein to cross-link a hydrophilic glycopeptide to a membrane-embedded lipid without extracting the substrates from the aqueous and membrane phases, respectively. Another well-known and already extensively reviewed example of interfacial catalysis is provided by phospholipases that catalyze hydrolysis of phospholipids at the membrane interface (*50*, *51*). The drawings were generated with the use of Protein Data Bank entries 3b4r (*8*), 1mt5 (*13*), 1xse (*20*), 1v26 (*25*), 2uui (*31*), 2uuu (*34*), and 2olu (*36*).

For our analysis, we will refer to the guinea pig 11β-hydroxysteroid dehydrogenase, a representative protein of this widely studied dehydrogenase class (*19*, *20*). Inspection of the crystal structure shows that the catalytic center is accessible both from the membrane, through a so-called steroid channel perpendicular to the membrane surface, and from the cytosol, to permit binding of the hydrophilic NADPH (*20*). Such architecture allows the two substrates to directly contact each other within the active site in order to facilitate the direct transfer of a hydride anion from NADPH to the steroid. The enzyme desorbs the lipophilic substrate from the lipid bilayer, catalyzes its chemical modification (a reduction) by making use of a water-soluble electron donor, and then releases the modified lipophile back to the membrane environment. A similar "desorb-and-modify" catalytic strategy underlies the reactions of several other well-known enzymes such as cholesterol oxidase (*21*), estrone sulphatase (*22*), prostaglandin synthase (*23*), and squalene cyclase (*24*).

A more elaborated mechanism of action can be identified in enzymes that catalyze the conjugation of hydrophobic and hydrophilic molecules, as in the case of bacterial fatty acyl–CoA synthetase (FACS) (*4*, *25*, *26*). This enzyme catalyzes the build-up of fatty acyl–CoA esters in the cytosol. The complicated multistep reaction of FACS shows how a catalytic site can be simultaneously accessed by three substrates coming from the cytosol and the membrane. The fatty acid is desorbed from the lipid bilayer to which the enzyme is associated and binds in a hydrophobic cavity at the interface between the two monomers of the dimeric enzyme (Fig. 1D). The elongated conformation of the fatty acid precisely positions its carboxylate group in direct contact with the binding sites for the two hydrophilic substrates, adenosine 5′-triphosphate (ATP) and coenzyme A. These two molecules bind sequentially through a hydrophilic opening exposed to the cytosolic face of the protein. The fatty acid is first activated by formation of an acyl–adenosine 5′-monophosphate intermediate and then reacts with coenzyme A to generate the final acyl-CoA product, which is eventually released to the cytosol through the hydrophilic opening. FACS exemplifies the case of an enzyme that enhances the water solubility of a lipophile by conjugation with a hydrophilic molecule (coenzyme A) in order to make it available to the cytosolic reactions.

In the above-described reactions, the common theme is the exploitation of distinct channels and binding sites for hydrophobic and hydrophilic substrates. This is not always the case, and in certain enzymes both substrates reach the same catalytic cavity in two consecutive events. The family of membrane-associated proteins in eicosanoid and glutathione metabolism represents a good example of this reaction mechanism (*27*–*29*). A prominent family member is leukotriene C_4 synthase (LTC_4S), which catalyzes the conjugation of glutathione to leukotriene A_4, an unstable hydrophobic compound generated by arachidonic acid metabolism (*30*). The product of this conjugation reaction is cystenyl leukotriene or leukotriene C_4, a key mediator involved in acute and chronic inflammatory diseases of the cardiovascular and respiratory systems. The catalytic cavity of the enzyme is located at the interface between two monomers of the biologically active LTC_4S trimer, and is buried in the lipid bilayer (Fig. 1E). As highlighted by the recently solved crystal structures, the shape and charge distribution of the active site are such that only an ordered stepwise mechanism of binding is possible (*31*, *32*) (Fig. 1E). In the proposed catalytic scheme, glutathione first enters the binding pocket through a solvent-exposed opening, leading to a partial conformational change as well as a redistribution of the charges inside the binding site. This reorganization promotes the admission of the hydrophobic leukotriene A_4 substrate by lateral diffusion from the membrane. At this stage, the reaction can take place, generating the leukotriene C_4 product that is finally released from the enzyme. LTC_4S is unusual in that binding of the hydrophilic glutathione appears to be a prerequisite for the admission of the hydrophobic substrate. The unstable leukotriene A_4 is thus never in danger of being exposed to water molecules that would promptly react with it. Rather, the sequential binding ensures that the substrate remains protected in its membrane environment and enters the active site only when the proper "conjugating partner" (glutathione) is already present.

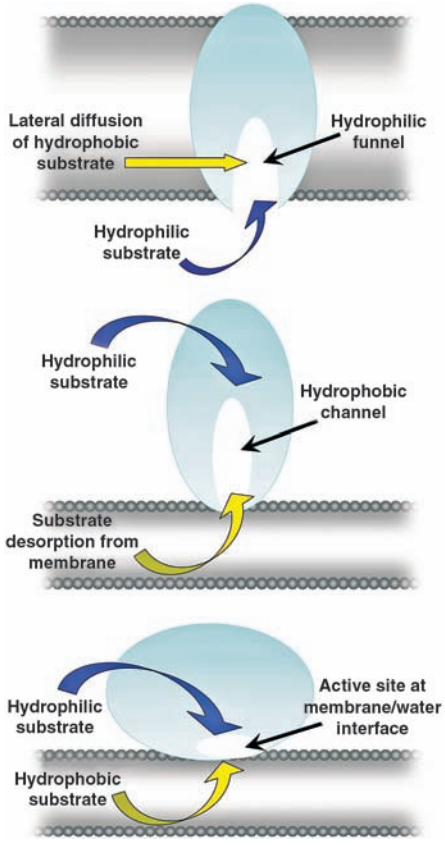

Fig. 2. Schematic view of the catalytic strategies used by enzymes working with hydrophobic and hydrophilic substrates; lateral diffusion and hydrophilic funneling (top), desorb-and-modify (middle), and working at the interface (bottom). The color coding is the same as in Fig. 1.

Hydrophilic Intermediates and Hydrophobic Substrates

A variation on the theme is provided by alkyldihydroxyacetonephosphate synthase (ADPS), an enzyme involved in the biosynthesis of ether phospholipids (*33*). ADPS transfers the hydrophilic dihydroxyacetonephosphate (DHAP) moiety of acyl-DHAP to a highly hydrophobic long-chain fatty alcohol. From a mechanistic standpoint, the enzyme is peculiar because it uses a typical redox cofactor such as flavin adenine dinucleotide (FAD) for a nonredox reaction. ADPS is thought to be associated to the membrane surface, and its three-dimensional structure exhibits a narrow hydrophobic tunnel that runs from the membrane-protein interface to the active site (Fig. 1F) (*34*). The acyl-DHAP substrate, which is most likely directly donated by the preceding acyltransferase enzyme of the biosynthetic pathway, binds with its acyl chain extending into this tunnel toward the membrane surface. This binding mode promotes the reaction of its DHAP group, which forms a covalent bond with the FAD cofactor. Trapping of DHAP enables the enzyme to release the acyl product and then bind the fatty alcohol to finally generate the alkyl-DHAP compound. Thus, ADPS features the outstanding property of being able to sequester a hydrophilic intermediate (DHAP) while its lipophilic binding tunnel exchanges long-chain aliphatic ligands by direct diffusion to and from the membrane bilayer.

Working at the Membrane Surface

Intuitively, the easiest way of combining hydrophobic and hydrophilic substrates is to make them react at the membrane-water interface (Fig. 1G). A beautiful example is given by peptidoglycan transglycosylase, an enzyme involved in the biosynthesis of the bacterial cell wall (*35*). It is composed of two functional domains that catalyze the conjugation of a membrane-embedded lipid molecule with a polypeptide and a polysaccharide. The transpeptidase domain is responsible for the formation of a soluble glycopeptide that is then processed by the glycosyltransferase domain to generate the final product. Recent evidence from the structural analysis of this bifunctional enzyme (*36*) indicates that the glycosyltransferase domain is partially submerged in the lipid bilayer (Fig. 1G). Notably, its catalytic site is located exactly at the interface between the membrane and the cytosol, allowing both substrates to approach each other without being removed from their original environment. The lipid is recognized by the protein transmembrane region and placed in a productive orientation for catalysis, whereas the water-soluble glycopeptide,

deriving from the transpeptidase domain, is cross-linked to the hydrophobic substrate without entering the lipid bilayer. The final polymeric product is released, retaining its lipid moiety inside the membrane and its hydrophilic peptidoglycan head protruding from the membrane surface, fully exposed to solvent.

Challenges for the Future

The study of enzyme function remains an exciting research field because of the diverse and often unpredictable solutions that proteins develop to perform even seemingly impossible tasks. Consistently, the main concept emerging from our analysis is that there is not a unifying mechanism or working scheme that underpins the ability of enzymes to deal with a combination of hydrophobic and hydrophilic substrates. The hallmark of such diversity is the fact that these reactions can be catalyzed by integral membrane proteins, as well as by water-soluble enzymes. In this perspective, a striking observation is that there are no characteristic folding topologies that identify enzymes acting on hydrophobic-hydrophilic substrates. On the contrary, these enzymes are structurally unrelated and usually show a clear homology and evolutionary relationship with proteins that do not feature this type of enzymatic property. However, bearing in mind such a mechanistic and structural diversity, our analysis suggests a few general functional schemes illustrating how enzymes overcome the problem of acting on substrates of opposite chemical nature that typically reside within different cell compartments and environments (Fig. 2):

1) Lateral diffusion and hydrophilic funneling. When the active site is located in the lipid bilayer, the hydrophobic substrate laterally diffuses from the membrane. Hydrophilic substrates are funneled into the transmembrane portion of the enzyme across water-exposed hydrophilic channels. A fundamental feature of these membrane-embedded enzymatic systems is their topology, because the biological action exerted by the reaction product will depend on from which side of the membrane the product is released (*37*).

2) Desorb-and-modify. Enzymes that do not possess a membrane-embedded active site often extract their lipophilic substrates from the bilayer by direct association to the membrane surface. This process typically occurs by desorption of the lipophile by means of a tunnel or cavity that leads from the protein-membrane interface to the active site. The protein regions that mediate association to the membrane are often involved in this binding process. Alternatively, water-soluble enzymes can be assisted by carrier proteins that desorb lipophilic molecules from the membrane and make them available to the enzyme active site.

3) Working at the interface. Enzymes can have their catalytic center precisely located at the membrane surface. In this way, both hydrophobic and hydrophilic substrates may stay in their original environment during catalysis.

Clearly, there are several open questions that concern the function of these enzymes, especially with regard to the dynamics of both proteins and membranes and the energetics of substrate binding. Possibly, the most intriguing aspect is the desorption of a substrate from the membrane and its binding to the protein. The dynamics of this process is dictated by the association and mutual influence between the protein and the membrane phospholipids, but the biophysical bases of these phenomena remain poorly understood. New molecular dynamics methods are being developed to improve the efficacy of simulations on membrane proteins (*38*). Preliminary insight has been provided by simulations on saposin B (*39*) and on proteins that bind cholesterol [cholesterol oxidase (*40*) and Osh4, an oxysterol-binding protein (*41*)]. In these systems, the association between the membrane phospholipids and the protein is proposed to affect the conformation of a gating loop that is partially submerged into the bilayer and controls the access to the active site. The recent advances in various spectroscopic and microscopy techniques [solution and solid-state nuclear magnetic resonance (*42*), small-angle x-ray scattering (*43*), and atomic force microscopy (*44*)] will have a tremendous impact in this area because they will enable researchers to directly probe and visualize the conformational changes and the dynamical events underlying the processes of substrate binding at the membrane-protein interfaces.

In this context, a fascinating problem is the dependence of the substrate desorption process on the physical properties of the membranes, including their thickness and curvature, the packing of the phospholipids, and the physical state of the bilayer (i.e., whether solid-like or liquid-like). Indeed, alterations in the membrane physical properties have been shown to affect enzyme catalytic efficiency, as demonstrated for cholesterol oxidase (*45*). Likewise, it will be interesting to see whether the membrane-lipid composition, which differs among cell compartments, plays a role in controlling the binding affinities and catalytic efficiency, effectively acting as a modulator of these enzymatic systems. A very promising approach will be the use of nanodiscs, which provide a membrane-like environment allowing the formation of stable structures that can be investigated by different biophysical approaches (*46*, *47*).

An in-depth analysis of substrate desorption and lateral diffusion processes must await the development of a mature model for membrane dynamics (*48*). Equally relevant will be the quantitative understanding of the hydrophobic effect (*49*) and of the energetics that favors the partitioning of a molecule from the membrane into the active site (and vice versa for product release). Investigation of the enzymatic reactions that overcome the phospholipid/water boundaries will rely on the use of emerging new techniques and on the integration of protein chemistry with membrane biology. We suspect that these studies will lead to the development of new concepts in enzymology.

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Source Analysis of the Crandall Canyon, Utah, Mine Collapse

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On 6 August 2007, a magnitude 3.9 seismic event was associated with the tragic collapse of a Utah coal mine, which ultimately killed six miners and three rescue workers. The event was recorded on the local network of the University of Utah Seismograph Stations and the Advanced National Seismic System (ANSS) operated by the U.S. Geological Survey. In addition, the National Science Foundation Earthscope USArray stations had recently been installed in the region (*1*). These stations provided good coverage (Fig. 1A) enabling seismic source analysis of the recorded signals, which revealed an unusually shallow depth and anomalous radiation pattern, both contrary to the expectation for a tectonic earthquake.

First-motion polarities from vertical-component records of the seismic event are down, or dilatational, indicative of an implosional source (*2*). Consistent with this observation, the moment tensor inversion of complete, three-component, low-frequency (0.02 to 0.10 Hz) ground displacement recovered a mechanism that also satisfies the observed first motions and is agreeable with the gravity-driven vertical collapse of a horizontally oriented underground cavity at a shallow depth (<1 km), consistent with the mine workings (Fig. 1B). The total seismic moment of this mechanism was 1.91×10^{15} N m ($M_W = 4.1$). However, a closing horizontal crack theoretically has no Love wave excitation, and in order to explain the large-amplitude Love waves observed on the tangential component (Fig. 1C) the mechanism must contain a secondary noncrack component that is 24% of the dominant vertical collapse moment (1.71×10^{15} N m). The secondary source excitation of the moment tensor can be represented in multiple ways because the moment tensor decomposition is nonunique (*3*). Plausible interpretations of the secondary source include vertical dip-slip faulting, horizontal shear, nonuniform crack closure, and elastic relaxation in response to the mine collapse.

The source-type diagram (*4*) in Fig. 1B illustrates the deviation from a pure earthquake double-couple (DC) source at the center in terms of a volumetric component (explosion or implosion) on the ordinate and a deviatoric component in terms of a volume compensated linear vector dipole (CLVD) on the abscissa. The moment tensor solution for the 6 August 2007 event plots in the region of a negative or closing crack. The diagram shows that, despite the secondary source component, the seismic waveforms are best fit by a model that is primarily composed of a closing horizontal crack, or underground collapse, and is similar to solutions obtained for other mine and Nevada Test Site (NTS) cavity collapses (*5*). In contrast, NTS nuclear explosions modeled with the same method (*6*) plot squarely in the explosion region of the diagram. Both the explosions and collapses are significantly separated from the population of earthquakes, which locate in the center of the diagram. Deviation from pure DC mechanisms in the earthquake population can be a result of several factors, including complex faulting, noise, and the effect of approximate Earth structure models on the basis Green's functions used in the inversion. Despite the scatter within the three source populations, there is clear separation between each, indicating that regional distance seismic moment tensor methods are capable of source-type discrimination.

Our findings show that the seismic waveforms associated with the mine collapse primarily reflect the collapse; however, the seismic source process was more complex than those observed in other collapse events (*5*) with a secondary source generating strong Love waves. This application of seismic moment tensor analysis demonstrates the feasibility of continuous monitoring of regional distance seismic wavefields for source-type identification, including nuclear explosion monitoring and given rapid access to the seismic waveform data, for emergency response applications.

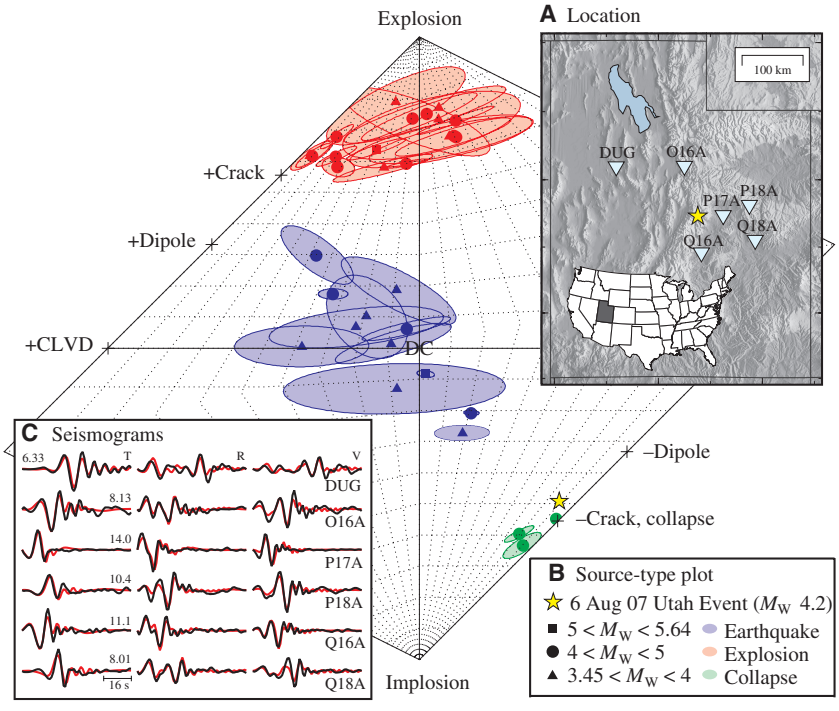

Fig. 1. (**A**) Locations of the 6 August 2007 event and six of the closest USArray and ANSS stations. (**B**) Source-type plot from the method of (*4*) shows separation of populations of earthquakes, explosions, and collapses. The yellow star shows the solution for the 6 August 2007 seismic event. (**C**) Observed seismograms (black) are compared to synthetics (red) for the non-dc solution, which is dominated by a horizontal closing crack (B). The maximum displacement (10^{-7} m) of each set of tangential (T), radial (R), and vertical (V) observations is given.

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Supporting Online Material

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Identifying Autism Loci and Genes by Tracing Recent Shared Ancestry

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To find inherited causes of autism-spectrum disorders, we studied families in which parents share ancestors, enhancing the role of inherited factors. We mapped several loci, some containing large, inherited, homozygous deletions that are likely mutations. The largest deletions implicated genes, including *PCDH10* (*protocadherin 10*) and *DIA1* (*deleted in autism1*, or *c3orf58)*, whose level of expression changes in response to neuronal activity, a marker of genes involved in synaptic changes that underlie learning. A subset of genes, including *NHE9* (*Na^+/H^+ exchanger 9*), showed additional potential mutations in patients with unrelated parents. Our findings highlight the utility of "homozygosity mapping" in heterogeneous disorders like autism but also suggest that defective regulation of gene expression after neural activity may be a mechanism common to seemingly diverse autism mutations.

Autism is a severe neuropsychiatric disorder characterized by impaired social interaction and communication and by repetitive and stereotyped interests and behavior. Autism includes mental retardation in up to 70% (*1*) and seizures in 20 to 25% of cases (*2*). Although autism spectrum disorders (ASDs) are highly heritable, they exhibit wide clinical variability and heterogeneous genetic architecture, which have hindered gene identification (*3*, *4*).

The great majority of identified ASD genes have high rates of de novo mutation. Large, de novo, microscopically evident chromosomal anomalies have been reported in 1 to 2% of cases of autism (*5*), and recent work has identified submicroscopic deletions and duplications, collectively called copy number variants (CNVs), affecting many loci, in 10% or more of sporadic cases (*4*, *6*–*9*). Mutations in *FMR1*, *TSC1*, *TSC2*, *NF1*, *UBE3A*, and *MECP2* that cause monogenic neurological disorders also cause syndromic autism and are all associated with high rates of de novo or recent mutation. The extreme genetic heterogeneity of autism, and the high de novo mutation rate, have hindered linkage studies of inherited autism susceptibility loci (*3*, *4*). The accumulating number of distinct, individually rare genetic causes in autism (*5*, *10*, *11*) suggests that the genetic architecture of autism resembles that of mental retardation and epilepsy, with many syndromes, each individually rare, as well as other cases potentially reflecting complex interactions between inherited changes (*12*).

"Homozygosity mapping" (*13*, *14*) in pedigrees with shared ancestry has been a successful methodology to discover autosomal recessive disease genes for many genetically heterogeneous neurodevelopmental conditions, such as brain malformations and mental retardation (*15*–*17*). Because of the large amount of genetic information that can be obtained from pedigrees in which parents share a recent common ancestor, the need to pool information from multiple families is reduced. Homozygosity mapping has been suggested as potentially useful for mapping complex traits as well (*18*), but this hypothesis has not been tested, other than one study of patent ductus arteriosus (generally considered to be a multifactorial condition) (*19*). Although segregation analyses have supported a role for autosomal recessive genes in ASD (*20*), homozygosity mapping has not been applied to autism to date. Here, we show that homozygosity mapping can be useful for identifying loci and genes in ASDs in consanguineous populations.

Ascertainment of pedigrees with autism and recent shared ancestry. The Homozygosity Mapping Collaborative for Autism (HMCA) (*21*) has recruited 104 families (79 simplex and 25 multiplex) from the Arabic Middle East, Turkey, and Pakistan (table S1 and fig. S1), of which 88 pedigrees (69 simplex and 19 multiplex) have cousin marriages (i.e., parental consanguinity). To establish thorough research diagnoses, international participating clinicians received training in accepted autism research scales. When research scales were not available in the language of their country, these clinicians enrolled patients and family members based on DSM-IV-TR diagnoses that were informed by these clinicians' experience with validated research scales. Additional direct assessments of patients were conducted by clinical members of the Boston team, which included developmental psychologists (J.W., E.L., R.M.J.), pediatric neurologists (G.M., A.P.), a clinical geneticist (W.H.T.), and a neuropsychiatrist (E.M.M.). Reliability between clinician assessments was high; a description of clinical methods is available in (*22*).

Marriage between first cousins increases the prevalence of neurological birth defects by about 100%, with this excess attributable to increased autosomal recessive causes (*23*, *24*), and with de novo chromosome anomalies representing a correspondingly reduced portion of the total (*24*). Although comparable epidemiological data for autism are not available, we reasoned that a prominent involvement of autosomal recessive genes in autism would be signaled by differences in the male-to-female (M/F) ratio of affected children in consanguineous (related) versus nonconsanguineous marriages (although recessive causes of autism may still retain some gender-specific difference in penetrance). Across the HMCA, the M/F ratio of affected individuals was typical, at 4.8:1 (115 males: 24 females). However, in consanguineous, multiplex pedigrees, the M/F ratio was 2.6:1 (34 males: 13 females) (fig. S1), compared to 7.4:1 (81 males: 11 females) for the other categories of families (i.e., nonconsanguineous and consanguineous simplex) (chi-square = 5.37, df = 1, $P = 0.02$). The M/F ratio of 2.6:1 is close to what would be predicted if the prevalence of autism were doubled in these families, with the excess attributable to recessive causes (*23*, *24*).

An increased role for inherited factors in autism families with shared ancestry was also suggested by a low rate of de novo CNVs that segregated with disease, despite the use of two sensitive methods for detecting them: the Affymetrix GeneChip Human Mapping 500K single-nucleotide polymorphism (SNP) array, as well as bacterial artificial chromosome (BAC) comparative ge-

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nomic hybridization (CGH) microarrays [principally an extensively validated, commercial microarray from Signature Genomics (see *22*)]. Whereas rates of inherited CNVs (some potentially causative) were high in both the SNP and BAC arrays, ranging in size from 1.4 kb to 3.9 Mb (tables S2 and S3), overall rates of de novo CNVs that segregated with ASD were 0% in consanguineous multiplex (0 of 42 patients) and 1.9% in consanguineous simplex families (1 of 52 patients), which were considerably lower than reported for non-consanguineous families: 1.28% in the HMCA overall versus 7.1% using representational oligonucleotide microarray analysis in autism (*6*) (chi-square = 4.438, df = 1, $P < 0.02$), or versus 27.5% (*7*) (chi-square = 17.733, df = 1, $P < 0.01$) using another BAC array in syndromic autism. A large study, using identical BAC arrays run in the same lab as our study, found 5.6% (84 of 1500) of patients referred to Signature Genomics with de novo or pathogenic CNVs (chi-square = 3.052, df = 1, $P < 0.05$) (*25*). The HMCA rate of de novo CNVs was similar to previously reported rates in multiplex pedigrees with autism [1.28% in the HMCA versus 2.6%, or 2 of 77, in multiplex autism (*6*), chi-square = 0.557, df = 1, $P = 0.22$] and in controls [1.28% HMCA versus 1.0%, or 2 of 196, in control subjects (*6*), chi-square = 0.001, df = 1, $P = 0.49$], despite the fact that the 500K platform used here has significantly higher coverage. The single large de novo CNV discovered was a 3-Mb deletion at 22q11.21, the velocardiofacial syndrome (VCFS) locus (encompassing all SNPs between rs432770 and rs1014626), which has been previously reported in autistic patients (*26*). The relatively reduced M/F ratio of affected children and the reduced rate of linked de novo CNVs in the consanguineous sample (not significantly different from rates in control) both suggest that consanguineous pedigrees with autism are enriched for autosomal recessive causes similar to other congenital neurological disorders in consanguineous populations (*23*, *24*).

Homozygosity mapping implicates heterogeneous loci and genes. Homozygosity mapping in consanguineous autism pedigrees suggested considerable genetic heterogeneity, implicating several genetic loci, with limited overlap between pedigrees. Using the Affymetrix Gene-Chip Human Mapping 500K SNP arrays, a locus-exclusionary approach was taken assuming a model of autosomal recessive inheritance and high penetrance. Several single families showed one or two loci with strong support for linkage [multipoint logarithm of the odds ratio for linkage (lod) scores ranging from 2.4 to 2.96] (table S4), corroborated by microsatellite analysis. Potentially linked loci were generally nonoverlapping between families, consistent with genetic heterogeneity, although two families shared linkage to an overlapping region of chromosome 2q (AU-4500, lod = 2.41, and AU-4200, lod = 1.81) that has been previously implicated in other autism linkage studies (*27*). The higher lod scores from single families, although not achieving genome-wide signifi-

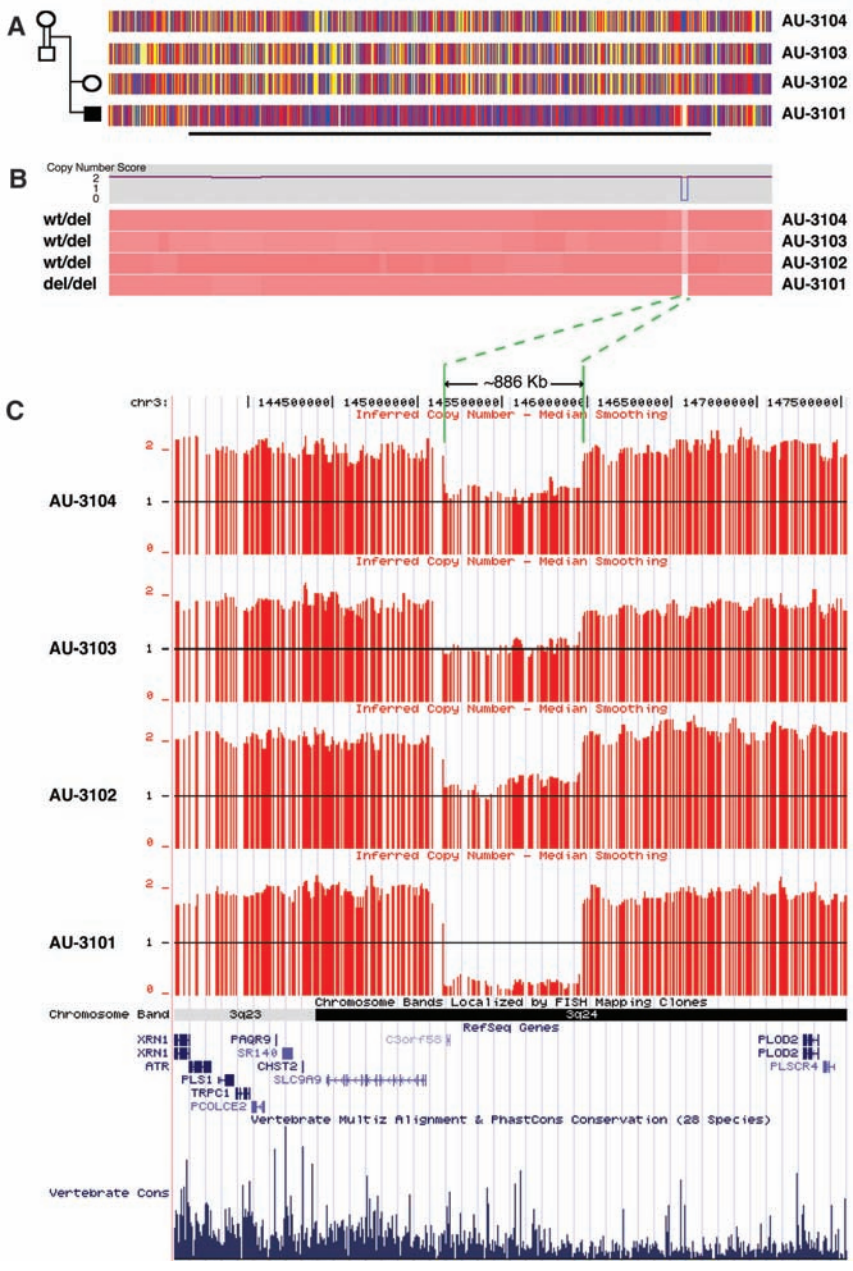

Fig. 1. Homozygosity mapping in pedigree AU-3100 reveals an ~886-kb inherited homozygous deletion at 3q24 within a 74 cM block of IBD in patient AU-3101, who has autism with seizures. (**A**) SNP genotypes for each subject in the pedigree using the 500K SNP microarray along chromosome 3q. The four horizontal tracks represent SNP genotyping data along 3q from centromere to telomere moving left to right, aligned with each individual in the pedigree. Red and blue vertical hatches represent homozygous SNPs, and yellow hatches indicate heterozygosity. The horizontal black line demarcates the 74-cM region of IBD in patient 3101 that is not found in an unaffected sibling or parents. (**B**) Copy number data using the 500K SNP microarray and *dCHIP* (*45*) hidden Markov model inferred methodology aligned with the genotyping SNPs from (A). The top panel indicates copy number (CN) score for AU-3101, and the lower panel shows pink tracks corresponding to CN data for all corresponding SNPs along 3q24 above. Dark pink shade indicates CN = 2 for the majority of this region. A white area in AU-3101 represents the homozygous deletion. The light pink equivalent region in AU-3102, AU-3103, and AU-3104 represents CN = 1 or carrier status of the wild-type deletion (wt/del). (**C**) Mapping of inferred CN data SNP-by-SNP on the University of California Santa Cruz (UCSC) genome browser demonstrates the deletion of *c3orf58* and an extensive genomic (likely regulatory) region 5′ to the transcriptional start of *SLC9A9* (*NHE9*). Horizontal red lines indicate each SNP with copy number of 0, 1, or 2. Green lines and arrows demarcate the extent of the deletion. Alignment of annotated genes in the National Center for Biotechnology Information RefSeq database are shown, as well as a representation of vertebrate conservation using multiz and related tools in the UCSC/Penn State Bioinformatics comparative genomic alignment pipeline.

cance, are comparable to the highest lod scores achieved by pooling hundreds of nonconsanguineous pedigrees (*3*, *4*, *27*).

Although the large size of linked loci precluded systematic gene sequencing in most cases, we were surprised to see that several consanguineous pedigrees showed large, rare, inherited homozygous deletions within linked regions, some of which are very likely causative mutations (Figs. 1 and 2 and table S5). Such deletions were present in 5 of 78 consanguineous pedigrees (6.4%) and ranged in size from 18 thousand base pairs (kbp) to > 880 kbp. For example, patient AU-3101, a boy diagnosed with autistic disorder and seizures, demonstrated a 74-cM segment of identity by descent (IBD) on chromosome 3q (lod score = 1.45) (Fig. 1), with an ~886-kbp homozygous deletion within 3q24. The deletion is hemizygous in both parents and an unaffected sibling (hence inherited from a common grandparent or more distant ancestor) but was not present in any of the other 393 samples from our autism pedigrees, nor in 184 Middle Eastern control chromosomes, nor in 2200 samples from the Autism Genetic Resource Exchange (AGRE) repository (www.agre.org). This deletion was confirmed using Agilent oligo arrays (fig. S2) and polymerase chain reaction (PCR) (fig. S3). The deletion completely removes *c3orf58*, which encodes an uncharacterized protein with a signal peptide that localizes to the Golgi (*28*). Moreover, the deletion is near the 5′ region of *NHE9*, such that only 60 to 85 kbp upstream of the transcription initiation site is spared. *NHE9* (also known as *SLC9A9*) encodes a $(Na^+, K^+)/H^+$ exchanger previously reported to have been disrupted in a pedigree with a developmental neuropsychiatric disorder and mild mental retardation (*29*). Of note, SNPs within *NHE9* and less than 100 kb from *c3orf58* were among the top 21 regions ($P < 0.00001$) in the human genome showing adaptive selection (i.e., evidence for recent evolutionary selection) in a recent genome-wide analysis (*30*). A second >300 kbp, linked, homozygous deletion (again not present in >2000 individuals other than this family) is closest to *PCDH10* on 4q28 (Fig. 2 and table S5), which encodes a cadherin superfamily protein essential for normal forebrain axon outgrowth (*31*). Smaller deletions (also unique to the individual family) (table S5) were closest to *CNTN3*, encoding BIG-1, an immunogloglobulin superfamily protein that stimulates axon outgrowth (*32*); *RNF8*, encoding a RING finger protein that acts as a ubiquitin ligase and transcriptional coactivator (*33*); and *SCN7A* (amid a cluster of voltage-gated sodium channels that also includes *SCN1A*, *SCN2A*, *SCN3A*, and *SCN9A*) on 2q. Homozygous deletions were confirmed by PCR (*22*). Of note, all of the implicated genes have high levels of expression in brain. Although without further data it is not known that all of these "private" homozygous deletions are causative, some are very likely to be, with larger deletions also more commonly pathogenic than smaller ones (*4*, *6*).

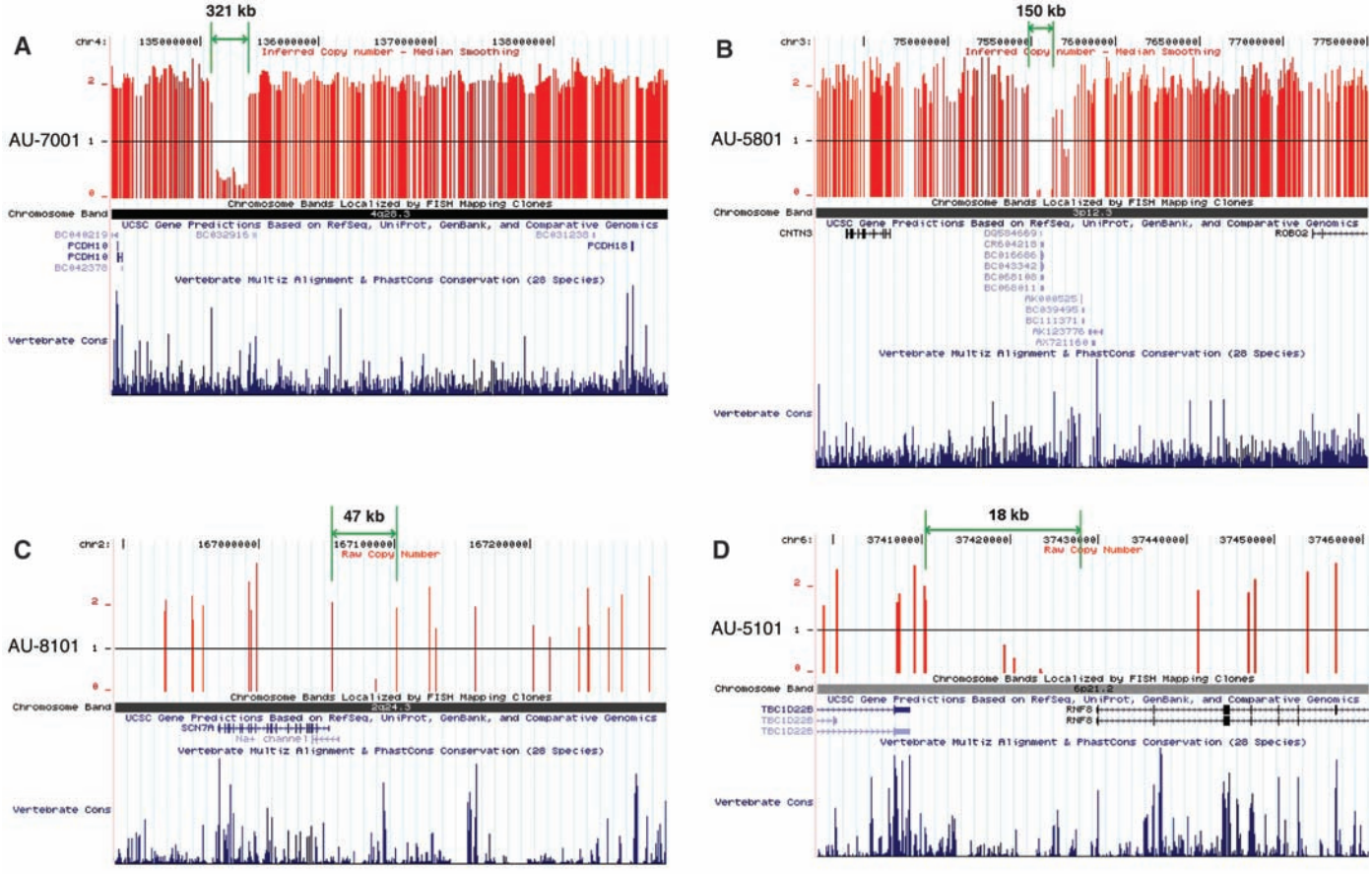

Fig. 2. Homozygous deletions within regions of IBD that segregate with disease were identified using the Affymetrix 500K microarray and are represented as schematic diagrams using the UCSC genome browser. Vertical red lines indicate each SNP with copy number of 0, 1, or 2. The green lines and arrows indicate the distance between the two SNPs with copy number equal to or greater than 1 flanking each deletion. Chromosomal bands containing deletions, genes in the vicinity of deletions, and vertebrate conservation using multiz and related tools in the UCSC/Penn State Bioinformatics comparative genomic alignment pipeline are also shown. A second large deletion: (**A**) Homozygous deletion in AU-7001 within a protocadherin cluster proximal to *PCDH10*. Smaller deletions: (**B**) Homozygous deletion in AU-5801 encompasses 5′ noncoding region of *CNTN3*. (**C**) Homozygous deletion in AU-8101 contains 5′ noncoding regions of *SCN7A* and a related sodium channel isoform. (**D**) Homozygous deletion in AU-5101 removes 5′ region of *RNF8* and 3′ noncoding region of *TBC1D228*, an uncharacterized Rab guanosine triphosphatase. This deletion was fine-mapped using PCR to demonstrate that the deletion excludes the first exon of RNF8. (See also table S5.)

Autism-associated genes are regulated by neuronal activity. Unexpectedly, the three genes within or closest to the two largest deletions (*c3orf58*, *NHE9*, and *PCDH10*) were all independently identified by unbiased screens looking for genes regulated by neuronal activity or for targets of transcription factors induced by activity. Neuronal activity induces a set of transcription factors (including *MEF2*, *NPAS4*, *CREB*, *EGR*, *SRF*, and others) with time courses of minutes to hours, and these transcription factors induce or repress specific target genes that mediate synaptic development and plasticity (*34*). This activity-based gene expression results in protein changes that selectively enhance or repress synapses, likely forming a part of learning paradigms (*35*). In microarray screens using cultured rat hippocampal neurons (performed blind to the genetic study), 1005 complementary DNAs (cDNAs) (of 22,407 nonredundant genes tested, i.e., ≈5% of the transcriptome) were identified as altered in expression after neuronal membrane depolarization by elevated KCl (*21*). Among these "neural activity–regulated" genes, *c3orf58* (deleted in patient AU-3101) was robustly increased within 6 hours of membrane depolarization (Fig. 3A). *c3orf58* contained several evolutionarily conserved binding sites for *MEF2*, *CREB*, and *SRF* (Fig. 3B), and depolarization-dependent transcription of *c3orf58* was strongly inhibited by RNA interference (RNAi) knock-down of the *MEF2* transcription factor (Fig. 3A), which suggests that *c3orf58* may be a direct or indirect MEF2 target. We propose renaming this gene *DIA1* (*deleted in autism-1*). In the same forward screen, transcription of *PCDH10* (the gene closest to the second-largest, >300-kbp, homozygous deletion in patient AU-7001) (Fig. 2A) was strongly up-regulated in hippocampal neurons in response to membrane depolarization (Fig. 3C). Although *PCDH10* was not greatly affected by MEF2 RNAi, *PCDH10* was robustly induced by a MEF2-VP16 fusion protein, reaching 1.31 ± 0.09 fold-induction of transcription and 1.94 ± 0.23 fold-induction at 1 hour and 2.5 hours, respectively. This transcriptional activation also suggests strongly that *PCDH10* is a transcriptional target of MEF2. Because the KCl depolarization assay identified fewer than 5% of the transcriptome as altered in expression, the identification in this assay of two of three genes associated with the two largest deletions is quite unlikely by chance alone (binomial test, $P < 0.006$ for two or more genes in the "altered expression" category). Enrichment of genes induced by membrane depolarization remains significant even when genes closest to all of the five homozygous deletions are considered (two of six transcripts found on the array, binomial test, $P < 0.027$). Moreover, a separate screen using RNAi knock-down of *NPAS4,* a transcription factor activated in response to depolarization, showed that *NHE9* was one of 292 out of 22,407 cDNAs (1.3%) whose transcription was significantly altered (in this case, increased) (Fig. 3D) (*22*), although *NHE9* expression was not detectably altered by membrane depolarization alone. These transcriptional effects suggest that the homozygous deletions in autism patients may preferentially involve activity-regulated genes, either mutating their coding sequence (e.g., *DIA1*) or potentially affecting conserved DNA sequences (*NHE9*, *PCDH10*) that may be critical for proper transcriptional regulation.

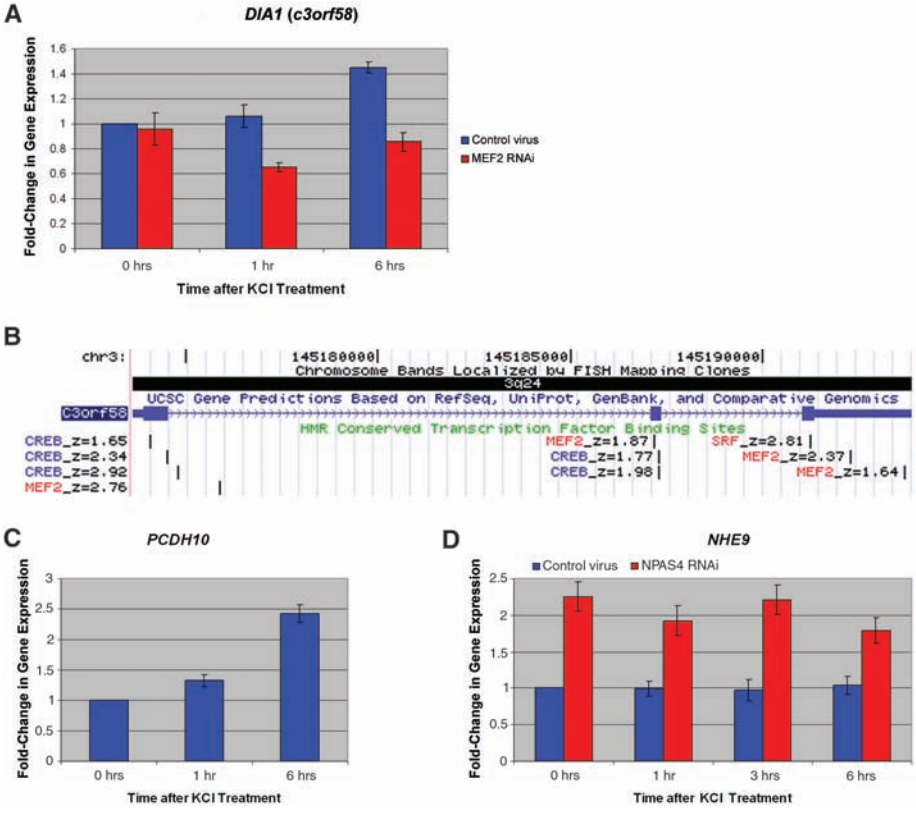

Fig. 3. Genes within or juxtaposed to homozygous deletions show activity-dependent gene regulation or are targets of transcription factors regulated by neuronal activity. (**A**) Activation of *DIA1* (*c3orf58*) gene expression in rat hippocampal cultures (0, 1, and 6 hours) after membrane depolarization with KCl. Control lentivirus shown in blue, and cultures transduced with *MEF2A and MEF2D* RNAi lentivirus shown in red. (**B**) The genomic structure of *DIA1* (*c3orf58*), also showing highly conserved transcription factor binding sites based on meeting computation thresholds of conservation in human/mouse/rat alignment with the Transfac Matrix Database (v7.0) (www.gene-regulation.com). Prominent activity-regulated transcription factor sites, namely *MEF2/SRF* (red) and *CREB* (blue), are shown. *Z*-scores of evolutionary conservation are also shown, *z*-score > 1.64 corresponding to $P < 0.05$, and *z*-score > 2.33 corresponding to $P < 0.01$. (**C**) Activation of *PCDH10* gene expression in rat hippocampal cultures (0, 1, and 6 hours) after membrane depolarization. (**D**) Activation of *NHE9* gene expression in hippocampal cultures (0, 1, 3, and 6 hours) after membrane depolarization with KCl in control lentivirus cultures (blue) and *NPAS4* RNAi lentivirus (red).

A subset of genes identified in HMCA samples shows potential mutations in nonconsanguineous pedigrees. Further analysis of *NHE9* demonstrated deleterious sequence variants associated with similiar autistic phenotypes in patients whose parents were not related. Because the proband AU-3101 with the deletion juxtaposed to *NHE9* showed autism as well as epilepsy, we sequenced *NHE9* in other patients with autism and epilepsy. A heterozygous CGA to TGA transition, changing arginine 423 residue to a stop codon, was found, and it creates a predicted protein truncation in the final extracellular loop of this multispanning transmembrane protein (Fig. 4, A to C). This nonsense change occurs within two amino acids of a similar nonsense mutation in *Nhe1* that causes slow-wave epilepsy in mice (*36*) (Fig. 4B). The *swe* mouse mutation results in a gene dosage-dependent reduction of protein levels and loss of function in brain (*36*). A similar nonsense mutation in the final extracellular loop has recently been found in the related *NHE6* gene in a patient with an Angelman-like syndrome, which involves both autism symptoms and epilepsy (*37*). The *NHE9* nonsense change here is carried by two male siblings with autistic disorder as well as their mother, who was reported to have had childhood language delay based on a parental language questionnaire (blind to genotype). One autistic son has electroencephalogram-confirmed epilepsy, and the second autistic son had two probable seizures but does not have known epilepsy. This nonsense change was not found in greater than 3800 control chromosomes. Complete resequencing of all exons and exon-intron boundaries in a greater than fivefold excess (480) of controls revealed no

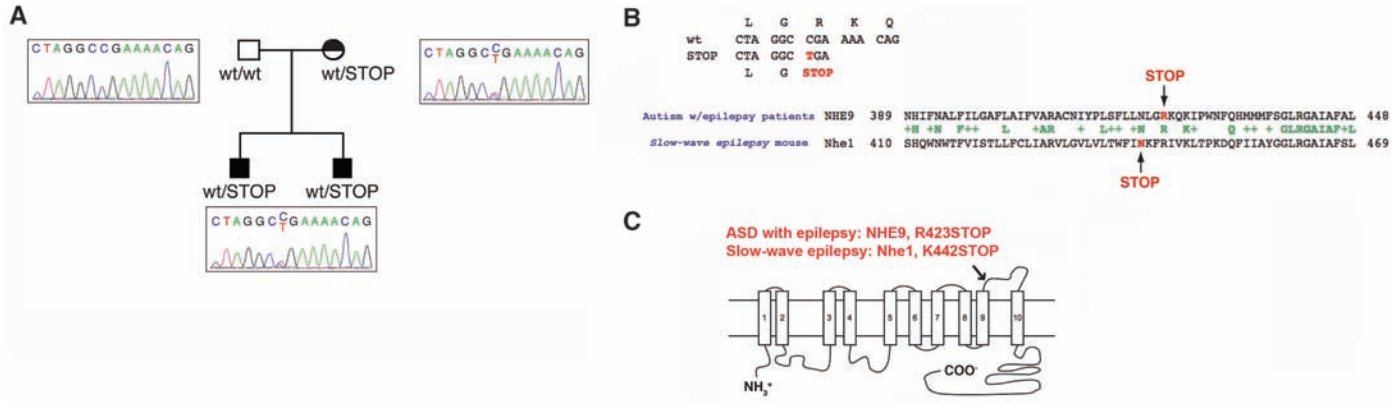

Fig. 4. Mutational analysis of *NHE9* in nonconsanguineous pedigrees with comorbid autism and epilepsy. (**A**) Mutational analysis of *NHE9* in an AGRE pedigree reveals a nonsense change highly similar to the nonsense mutation in *Nhe1* in the slow-wave epilepsy mouse. The AGRE pedigree structure includes two sons with autistic disorder. Patient 1 has comorbid epilepsy, and patient 2 had potential seizures at a younger age but does not currently carry the diagnosis of epilepsy. The mother does not have autism but is reported to have had a speech delay as a child (represented by a half-shading). Both patients and the mother carry the nonsense change. Sequence traces demonstrate the heterozygous C→T transition in Exon 11. This transition occurs at a CpG position consistent with a mutation at a methylated CpG. (**B**) Sequence trace indicating the position and consequences of the C→T transition. The CGA→TGA transition results in a change from an arginine residue at position 423 to a stop codon. The lower trace demonstrates that the position of this nonsense change in *NHE9* occurs in a similar position as the causative, null missense mutation in *Nhe1* in the slow-wave epilepsy mouse. (**C**) Nonsense mutations in the last extracellular loop of NHE proteins: NHE9 in patients with comorbid autism and epilepsy, and Nhe1 in the slow-wave epilepsy mouse. The human nonsense change was not found in greater than 3800 control chromosomes. Complete resequencing of all exons and exon-intron boundaries in a fivefold excess (480) of controls revealed no nonsense changes (table S6).

nonsense changes (table S6). Rare, nonconservative coding changes were more common in patients with autism with epilepsy compared with control subjects (5.95% versus 0.63%, Fisher's exact test, $P = 0.005$ for total changes), although autistic patients without seizures did not differ significantly from controls in the rate of nonconservative changes (1.14% autism without seizures versus 0.63% controls). The heterozygous changes, in particular the nonsense mutation, likely affect gene dosage and suggest that the study of consanguineous pedigrees may identify genes of importance in nonconsanguineous populations as well.

Discussion. Our copy number analysis, linkage, and resequencing together support other recent studies (*3*, *4*, *6*) suggesting that autism is highly heterogeneous genetically, but our data further suggest that homozygosity mapping provides an important approach to dissect this heterogeneity. We show that individuals with related parents are more likely to have inherited causes of disease, likely autosomal and recessive, and that these pedigrees allow mapping of loci from small numbers of families. Such families can also provide linkage evidence to support the identification of mutations, both coding and noncoding. Genes that act in a recessive manner may make good candidates for future analysis in association or resequencing studies, because they may show interactions with other nonlinked mutations (*38*). Our data implicating noncoding elements in patients with shared ancestry, as well as the heterozygous nonsense changes in patients without shared ancestry, suggest that loss of proper regulation of gene dosage may be an important genetic mechanism in autism. This possibility is also strongly supported by the numerous heterozygous CNVs found in patients from nonconsanguineous pedigrees (*4*, *6*).

Our data add to accumulating evidence for numerous individually rare loci in autism. These include *FMR1*, *MECP2*, *NLGN3*, *NLGN4* (*9*), *SHANK3* (*10*), *CNTNAP2* (*39*–*41*), *A2BP1* (*41*), *NRXN1* (*4*) (also implicated by inherited CNVs in our study, table S2), and now candidate genes such as *PCDH10*, *DIA1* (*c3orf58*), *NHE9, CNTN3*, *SCN7A*, and *RNF8*, in addition to chromosomal and CNV anomalies (*4*, *6*–*8*, *26*). Whereas genes involved in glutamatergic transmission seem to be important in autism (*4*), data from our study and others (*6*, *42*) implicate other biological mechanisms as well. Potential disease mechanisms include failures in neuronal cell adhesion molecules such as *NLGN3*, *NLGN4*, and *NRXN1*; *PCDH10* and *CNTN3,* identified as potential candidates here, may have similar roles. Endosomal trafficking and protein turnover is another potential mechanism implicated by *NHE9*, which itself is localized to endosomes (*43*). Further, mutations in *NHE6* (which encodes a protein highly related to that encoded by *NHE9*) were found in a series of patients with an Angelman syndrome–like phenotype, with epilepsy and autism-like symptoms in some patients (*37*). *DIA1* (*c3orf58*) appears to encode a protein localized to the Golgi apparatus (*28*), and so may also relate to protein trafficking.

The regulation of expression of some autism candidate genes by neuronal membrane depolarization suggests the appealing hypothesis that neural activity–dependent regulation of synapse development may be a mechanism common to several autism mutations. Early brain development is driven largely by intrinsic patterns of gene expression that do not depend on experience-driven synaptic activity (*44*). Mutations in the genes active in early development can lead to brain malformations or severe mental retardation. In contrast, postnatal brain development requires input from the environment that triggers the release of neurotransmitter and promotes critical aspects of synaptic maturation. During this process, neural activity alters the expression of hundreds of genes, each with a defined temporal course that may be particularly vulnerable to gene dosage changes. The connection between experience-dependent neural activity and gene expression in the postnatal period forms the basis of learning and memory, and autism symptoms typically emerge during these later stages of development. Our finding that deletions of genes regulated by neuronal activity or regions potentially involved in regulation of gene expression in autism suggests that defects in activity-dependent gene expression may be a cause of cognitive deficits in patients with autism. Therefore, disruption of activity-regulated synaptic development may be one mechanism common to at least a subset of seemingly heterogeneous autism-associated mutations.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5886/218/DC1
Methods
Figs. S1 to S3
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REPORTS

Supernova Shock Breakout from a Red Supergiant

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Massive stars undergo a violent death when the supply of nuclear fuel in their cores is exhausted, resulting in a catastrophic "core-collapse" supernova. Such events are usually only detected at least a few days after the star has exploded. Observations of the supernova SNLS-04D2dc with the Galaxy Evolution Explorer space telescope reveal a radiative precursor from the supernova shock before the shock reached the surface of the star and show the initial expansion of the star at the beginning of the explosion. Theoretical models of the ultraviolet light curve confirm that the progenitor was a red supergiant, as expected for this type of supernova. These observations provide a way to probe the physics of core-collapse supernovae and the internal structures of their progenitor stars.

The explosive deaths of massive stars are dramatic events that seed the Universe with heavy elements (*1*, *2*), produce black holes, pulsars, and the most energetic gamma-ray bursts (GRBs) (*3*). Their energy input can regulate the growth of galaxies (*4*). Even though a large amount of theoretical effort has been expended on trying to explain how the terminal collapse of a star's core leads to a luminous supernova, we do not fully understand the process by which the collapse of the core produces an outward-moving shock that leads to the ejection of the envelope (*5*–*7*). This shock heats and accelerates the stellar envelope as it passes through it. By the time the shock dissipates at the surface of the star, several solar masses of previously static envelope material are expanding at a few percent of the speed of light. At the time of core collapse, a nearby external observer equipped with a detector of neutrinos or of gravitational waves might receive a brief warning of the future explosion, but for most of the passage of the shock through the star, that observer would notice no further change. Only when the shock

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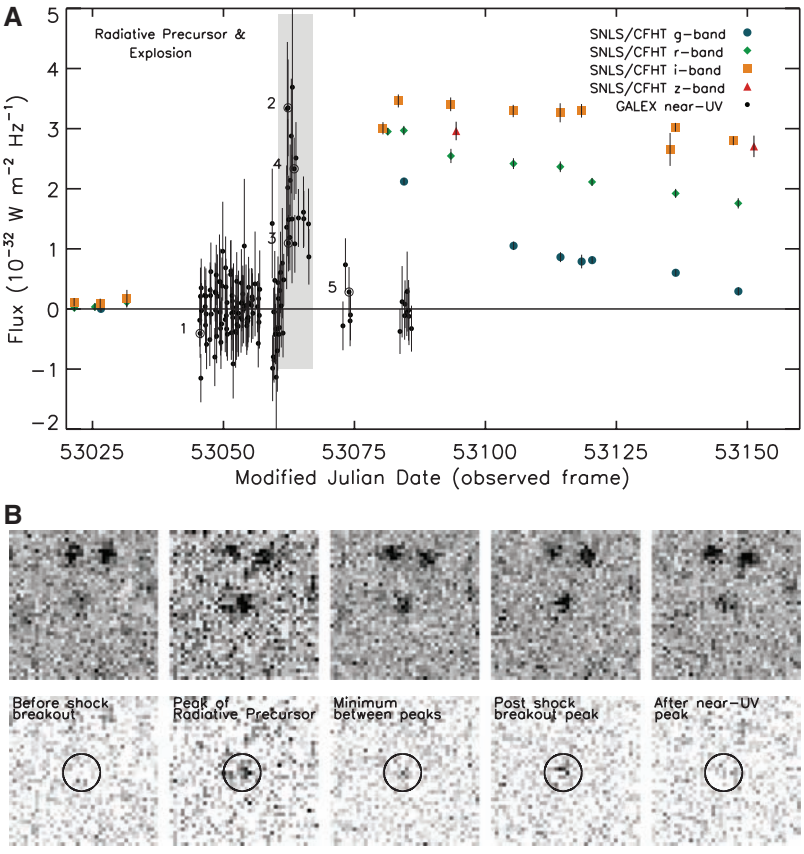

Fig. 1. (**A**) Composite of the optical SNLS and the UV GALEX light curves, or observed fluxes as a function of modified Julian date. All fluxes are host galaxy subtracted and are not corrected for internal extinction. The gray box indicates the time of the radiative precursor. The points highlighted by circles indicate five phases of the radiative precursor in the UV, as observed by GALEX. (**B**) These five phases are illustrated by a time sequence of original near-UV images (upper row, 1 × 1 arcmin) and difference images (lower row, with a pre-supernova image subtracted) to emphasize the transient source. Note the drop from point 2 to the minimum at 3 and the rise to 4, clearly visible in the GALEX images. The lack of optical data during the UV event is due to both poor weather conditions and technical problems. Both GALEX and SNLS light curves are available as tables in (*25*).

approaches the surface does radiation diffuse far enough ahead of the shock wave to raise the temperature of the stellar photosphere. This phase is sometimes referred to as "shock breakout," although the associated radiation is from the "radiative precursor" of the shock, long before the shock actually reaches the surface. This radiative precursor raises the temperature of the star to ~10^5 K before the surface expands dramatically (*8*).

Shock breakouts have been inferred for a few relatively local GRBs and x-ray flashes, which may involve shocks traveling through dense winds outside compact blue stars, including the recent SN 2008D (*9*–*14*). Here, we describe the brightening of a red supergiant due to the theoretically predicted radiative precursor before the supernova shock reaches the surface of the star. Such observations provide information about the density profile inside the progenitor star (*15*) and the physics of radiative shocks, and knowledge of the spectrum of the associated ultraviolet (UV) flash has implications for the ionization of the circumstellar medium (*16*, *17*).

Although core-collapse supernovae are expected to be most luminous around the time of shock breakout, most of this energy emerges as extreme UV or soft x-ray radiation. Hence, core-collapse supernovae are typically only discovered several days after the supernova explosion near the peak of their optical light curve; observations of early light curves are rare (*18*, *19*). To circumvent this problem, we exploit two complementary data sets: an optical survey to locate supernovae and UV data to search for serendipitously associated shock breakouts. The first is the Supernova Legacy Survey (SNLS) (*20*), which studies distant supernovae using data taken every 4 days at the 3.6-m Canada-France-

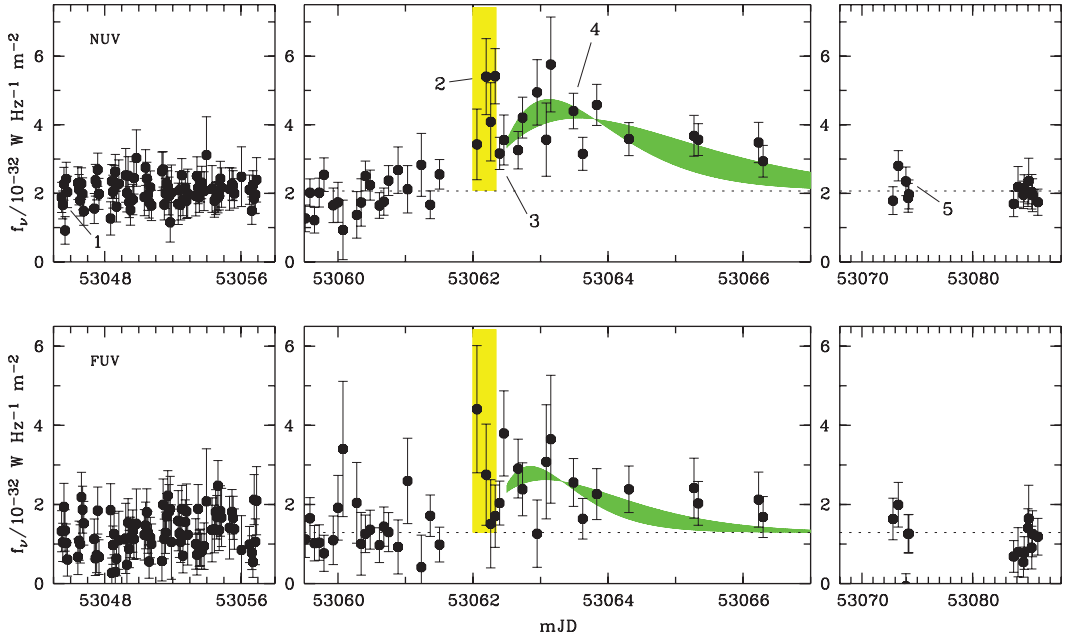

Fig. 2. The GALEX near-UV and far-UV flux against time (modified Julian date in days). This is a zoomed-in version of the shaded time range of Fig. 1 and we mark the same five data points. The background levels are shown before and after the supernova (left and right panels), and the central panels show the event itself. The radiative precursor is highlighted in yellow. Models for the post-explosion expansion are shaded in green; these models assume an initial photospheric radius of 500 to 1000 $R_\odot$. The width of the green band is due to the range of assumed expansion velocities (1 to 2 × 10^7 m s^{-1}). These models assume adiabatic free expansion of a radiation-dominated plasma and black-body emission from a well-defined photosphere. The models were only fitted to the near-UV data but are also consistent with the far-UV data.

Hawaii Telescope (CFHT). The second is from the Galaxy Evolution Explorer (GALEX) UV space telescope (*21*, *22*), which took a deep 100-hour combined exposure coincident with the early-2004 SNLS data in the Cosmological Evolution Survey (COSMOS) field (*23*, *24*). The Galaxy Evolution Explorer (GALEX) data were taken using subexposures of 15 to 30 min over several weeks, providing data with the time resolution necessary to resolve UV-luminous events occurring before the SNLS supernovae.

One SNLS event, designated SNLS-04D2dc and confirmed as a Type II supernova from the hydrogen lines in an optical spectrum taken at the European Southern Observatory (ESO) Very Large Telescope (VLT) [see supporting online material S1 (SOM text S1) (*25*)], shows a dramatic brightening in the GALEX near-UV images about 2 weeks before the discovery by the SNLS, consistent with shock breakout. The host galaxy appears to be a normal star-forming spiral galaxy at a redshift of $z = 0.1854$. The supernova spectrum, Gemini host galaxy spectrum, and Hubble Space Telescope image of the host are presented in (*25*). The optical light curve has a plateau that identifies the explosion as a Type IIP supernova (Fig. 1), suggesting a red-supergiant progenitor (*26*, *27*). Because of bad weather and technical problems with the CFHT camera, there are no optical data concurrent with the UV data; however, GALEX observed the entire radiative precursor (Fig. 2).

The GALEX light curve probes the arrival of the supernova shock at the surface of the star. We can interpret the two peaks in this light curve (Fig. 2) in terms of distinct physical processes. The first peak in the UV light curve is due to radiation traveling ahead of the shock wave. This heats the surface of the star before it begins to explode. The near-UV light curve samples the brightening caused by this precursor over ~6 hours. We can compare the duration of the observed precursor with theoretical expectations by equating the photon diffusion time scale with the time scale for the shock to escape from the envelope. If v is the shock speed and the density of the hydrogen-dominated atmosphere is ρ, we find $d \approx 2.5 \times 10^{11}$ m $(10^{-8}$ kg m$^{-3}/\rho)$ $(10^{7}$ m s$^{-1}/v)$ for the depth of the shock d (from the surface of the star) at the time when the radiative precursor becomes visible at the surface (SOM text 3 and 4). This value for d leads to a prediction for the duration of the shock precursor of $d/v = 2.5 \times 10^{4}$ s for the parameters above, that is, almost 7 hours, consistent with our observations of the precursor. This indicates that the progenitor was a large star, that is, a red supergiant, as expected for the progenitor of a type IIP supernova (*26*, *27*), whereas previous calculations indicated that radiative precursors from blue supergiant stars would last for minutes rather than hours (*8*). To model the radiative precursor, we solved simplified radiation-hydrodynamics equations for an outward-moving shock inside a stellar envelope. Figure 3 shows representative models that are consistent with the data; they require radii and envelope densities appropriate for a red-supergiant star. These models also indicate that only the initial ~4 hours of the first UV peak occur before the shock reaches the surface of the star.

The peak in the total luminosity of the source occurs at the time of the first UV peak, and the total luminosity monotonically decreases after this point. The temperature behind the shock is lower than the temperature at the shock front itself, which leads to a rapid drop in the luminosity of the star after shock breakout (*8*). The near-UV light curve in Fig. 3 shows this dip in brightness after the shock has escaped from the star.

Although the radiative precursor does cause some expansion of the star, there is little change in the stellar radius until the shock reaches the surface. Behind the shock, the radiation-dominated plasma expands at almost constant velocity and cools rapidly as a result of adiabatic expansion (*1*). The UV light curve is now governed by the expansion of the photospheric radius (and concomitant increase in radiating surface area), the adiabatic cooling of the surface, and the shift of the spectral energy distribution toward longer wavelengths, causing the second peak in the UV light curve. In the adiabatic cooling phase, the photospheric temperature T is approximately inversely proportional to the photospheric radius R. Because for a black body this drop in T causes a more rapid decrease in the luminosity ($L \propto T^4 \propto R^{-4}$) than the increase due to the growing surface area ($L \propto R^2$), the total luminosity of the supernova continues to decrease. However, in the Rayleigh-Jeans portion of the spectrum, the increase in the surface area of the photosphere is more important than the decrease in emission per unit area, and the luminosity at those wavelengths increases (SOM text S4.3). The observed UV luminosity rises until the peak of the black-body spectral energy distribution nears the UV waveband. Thereafter, the UV luminosity decreases with continued adiabatic expansion and cooling. The model curves in Fig. 2 show that this simple physical description reproduces the GALEX data with parameters as expected for a red supergiant progenitor. Initial photospheric radii of 500 to 1000 solar radii ($R_\odot$), expansion velocities of 1 to 2 × 10^7 m s^{-1}, and initial temperatures of ~10^5 K match the observed fluxes well. The biggest uncertainty arises from the adopted extinction (SOM text S1); any increase in the near-UV extinction would increase the range of preferred initial radii. Measuring precise radii of supernova progenitor stars would be a valuable constraint of the late stages in the evolution of massive stars; this requires higher time resolution and more accurate temperature determinations, for example, from observing the full spectral energy distribution from x-ray to optical. In addition, detailed light curves of radiative precursors probe the energetics of supernova shocks and the structures of the stellar envelopes through which they travel.

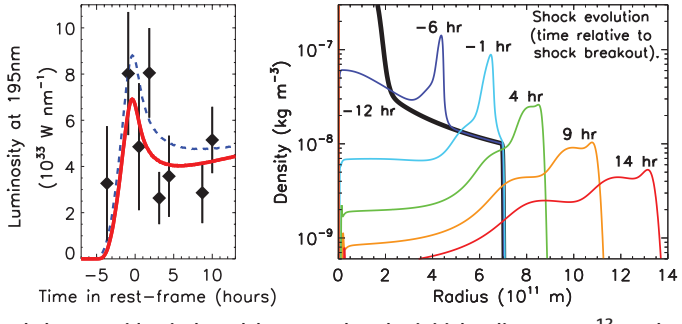

Fig. 3. (**Left**) Representative model precursor light curves compared with the precursor data (diamonds with error bars). The solid red curve represents the light curve produced from a model with an initial radius of 7×10^{11} m (≈1000 $R_\odot$) and an initial density distribution $\propto 1/r$. The dashed curve in the left panel shows an identical model, except that the initial radius was 10^{12} m; the absolute normalization of these luminosities is uncertain to factors of order unity (*25*). The zero point of the time axis is approximately at shock breakout. (**Right**) The time evolution of the internal density profile for the model producing the solid light curve in the left panel. About 4 hours before the shock has reached the surface, the UV luminosity has already begun to rise.

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SOM Text
Figs. S1 to S4
Tables S1 to S4
References

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High-Efficiency Organic Solar Concentrators for Photovoltaics

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The cost of photovoltaic power can be reduced with organic solar concentrators. These are planar waveguides with a thin-film organic coating on the face and inorganic solar cells attached to the edges. Light is absorbed by the coating and reemitted into waveguide modes for collection by the solar cells. We report single- and tandem-waveguide organic solar concentrators with quantum efficiencies exceeding 50% and projected power conversion efficiencies as high as 6.8%. The exploitation of near-field energy transfer, solid-state solvation, and phosphorescence enables 10-fold increases in the power obtained from photovoltaic cells, without the need for solar tracking.

Photovoltaic (PV) concentrators aim to increase the electrical power obtained from solar cells. Conventional solar concentrators track the Sun to generate high optical intensities, often by using large mobile mirrors that are expensive to deploy and maintain. Solar cells at the focal point of the mirrors must be cooled, and the entire assembly wastes space around the perimeter to avoid shadowing neighboring concentrators.

High optical concentration without excess heating in a stationary system can be achieved with a luminescent solar concentrator (LSC) (*1*–*5*). LSCs consist of a dye dispersed in a transparent waveguide. Incident light is absorbed by the dye and then reemitted into a waveguide mode. The energy difference between absorption and emission prevents reabsorption of light by the dye, isolating the concentrated photon population in the waveguide. In this way, LSCs can achieve high optical concentrations without solar tracking (*6*). Unfortunately, the performance of LSCs has been limited by self-absorption losses that restrict the maximum possible concentration factor. Here we describe an efficient variant of an LSC that mimics a four-level laser design and exhibits optical concentrations suitable for practical applications.

Typically, LSC dye molecules are cast into a transparent plastic sheet; however, we deposited a thin film of organic dye molecules onto glass. Our devices were fabricated with thermal evaporation, but solution processing could also be used. Precise control over the film composition allowed us to apply the recent advances of organic optoelectronics to LSCs, including Förster energy transfer (*7*), solid state solvation (*8*), and phosphorescence (*9*). We term the resulting devices organic solar concentrators (OSCs).

To obtain the highest power efficiencies, we constructed tandem OSCs (*2*). Incident solar radiation first encounters an OSC employing a short-wavelength dye. Longer wavelengths are transmitted through the first OSC and absorbed by a longer-wavelength dye in a second OSC (Fig. 1). Alternatively, solar radiation transmitted through the top OSC can be gathered by a bottom PV cell or used to heat water in a hybrid PV thermal system (*2*).

We quantify self-absorption losses in OSCs using the self-absorption ratio S, defined as the ratio of the absorption coefficients at the absorption and emission maxima. We examined two emissive dyes: 4-(dicyanomethylene)-2-*t-butyl*-6-(1,1,7,7-tetramethyljulolidyl-9-enyl)-4*H*-pyran (DCJTB) (*10*) and platinum tetraphenyltetrabenzoporphyrin [Pt(TPBP)] (*11*). As shown in Fig. 2A, S for a DCJTB-based OSC is ~80. DCJTB belongs to the dicyanomethylene (DCM) class of laser dyes and is characterized by large Stokes shifts and red emission with near-unity quantum efficiency. Batchelder *et al.* selected this class of dyes for solar-concentrator applications partly because of its high self-absorption ratio (*3*, *4*).

To reduce concentration quenching, DCJTB was doped (2% v/v) into the host material tris(8-hydroxyquinoline) aluminum (AlQ_3), which forms stable amorphous films. The resulting AlQ_3:DCJTB (2%) film was 5.7 μm thick with an absorbance of 1.1 absorbance units (au) at the DCJTB absorption peak. S is enhanced when AlQ_3 is used as the host. AlQ_3 provides a polar environment that stabilizes the highly polar DCJTB excited state. The effect is known as solid-state solvation, and it red-shifts the DCJTB photoluminescence (PL) (*8*).

Förster energy transfer was used to reduce the required concentration and hence the self-absorption of the emissive dye. For example, in the rubrene-based OSC of Fig. 2A, we used rubrene and DCJTB in a 30:1 ratio, the maximum possible without incurring significant concentration quenching in rubrene or incomplete Förster transfer to DCJTB. The resulting AlQ_3:rubrene (30%):DCJTB (1%) film was 1.6 μm thick with an absorbance of 1.2 au at the rubrene absorption peak. Förster energy transfer from rubrene to DCJTB increases the self-

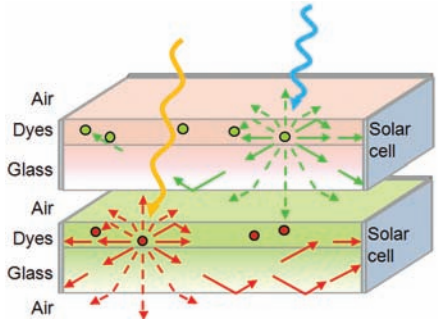

Fig. 1. Physical configuration of OSCs. (**Top**) OSCs consist of a thin film of organic dyes deposited on high-refractive-index glass substrates. The dyes absorb incident solar radiation and reemit it at a lower energy. Approximately 80% of the reemitted photons are trapped within the waveguide by total internal reflection for ultimate collection by a PV device mounted on the substrate edges. Photon loss (dashed lines) occurs because of nontrapped emission or absorption by other dyes. Blue arrow, high-energy incident visible light; green circles, dye molecules. (**Bottom**) Light transmitted through the first OSC can be captured and collected by a second OSC, whose dyes absorb and emit light at lower energies, for electrical conversion at a second, lower-bandgap PV device. Alternatively, the bottom OSC can be replaced by a low-cost PV cell or used to heat water in a hybrid PV thermal system. Yellow arrow, low-energy incident visible light; red circles, dye molecules.

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absorption ratio relative to the DCJTB-based OSC at the expense of narrower spectral coverage. However, rubrene is nonpolar and, together with a slight reduction in the DCJTB concentration, the DCJTB PL shifts ~20 nm back toward the blue.

Unlike DCJTB, which is fluorescent, Pt(TPBP) is phosphorescent. It emits from a weakly allowed triplet-state relaxation at wavelength (λ) = 770 nm with a PL efficiency of ~50% (*12*). As compared with conventional fluorescent dyes, an advantage of phosphorescent dyes is that the emissive state is only weakly absorptive. Thus, phosphors typically exhibit large Stokes shifts, eliminating the need for Förster transfer to a longer-wavelength emissive dye. *S* for the Pt(TPBP)-based OSC is ~500 (Fig. 2B). To fill the gap in the Pt(TPBP) absorption spectrum between λ = 430 and 610 nm, we added DCJTB, which efficiently transfers energy to Pt(TPBP). The resulting AlQ_3:DCJTB (2%):Pt(TPBP) (4%) film was 5.8 μm thick with an absorbance of 2.1 au at the Pt(TPBP) absorption peak. Förster energy transfer and phosphorescence are illustrated schematically in Fig. 2, C and D, respectively.

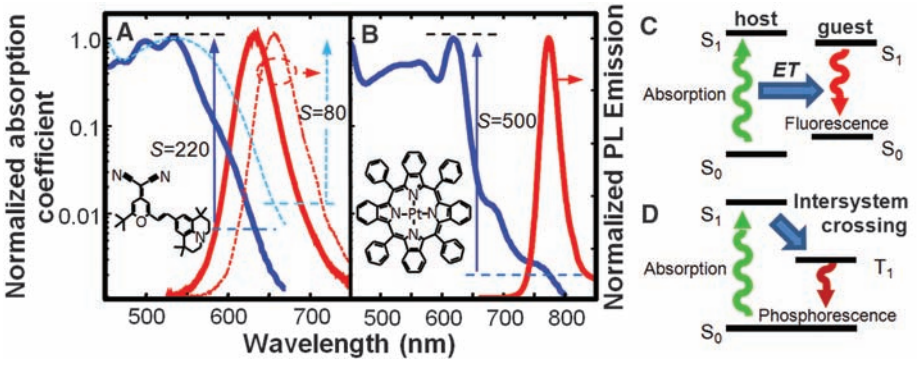

Fig. 2. Normalized absorption and emission spectra of OSC films. (**A**) The ratio between the peak absorption coefficient and the absorption coefficient at the emission wavelength provides an estimate of the self-absorption in an OSC film. In a DCJTB-based OSC, S = 80 (dotted lines). A larger ratio of S = 220 is obtained in a rubrene-based OSC (solid lines). S increases because the amount of DCJTB is reduced by a factor of 3. Its absorption is replaced by rubrene, which then transfers energy to DCJTB (C). The inset at left shows the DCJTB chemical structure. (**B**) Phosphorescence is another method to reduce self-absorption (D). In a Pt(TPBP)-based OSC, S = 500. The inset at left shows the Pt(TPBP) chemical structure. (**C**) Near-field dipole-dipole coupling known as Förster energy transfer can efficiently transfer energy between the dye donor and acceptor molecules. The concentration of guest molecules can be less than 1%, significantly reducing self-absorption. (**D**) Spin-orbit coupling in a phosphor increases the PL efficiency of the triplet state and the rate of intersystem crossing from singlet to triplet manifolds. The exchange splitting between singlet and triplet states is typically about 0.7 eV, significantly reducing self-absorption.

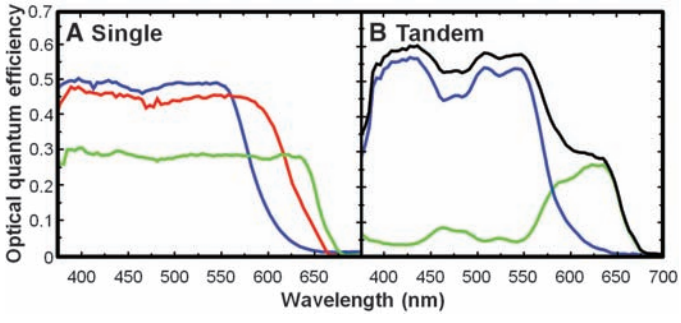

Fig. 3. OQE spectra at G = 3. The OQE is the fraction of incident photons that are emitted from the edges of the substrate. (**A**) The OQE spectra of the DCJTB- (red), rubrene- (blue), and Pt(TPBP)-based (green) single-waveguide OSCs. The DCJTB-based OSC is a 5.7-μm-thick film of 2% DCJTB in AlQ_3. The rubrene-based OSC is a 1.6-μm-thick film of 30% rubrene and 1% DCJTB in AlQ_3. The Pt(TPBP)-based OSC is a 5.9-μm-thick film of 2% DCJTB and 4% Pt(TPBP) in AlQ_3. (**B**) In the tandem configuration, light is incident first on the rubrene-based OSC (blue). This filters the incident light on the second, mirror-backed, Pt(TPBP)-based OSC (green). The composite OQE is shown in black.

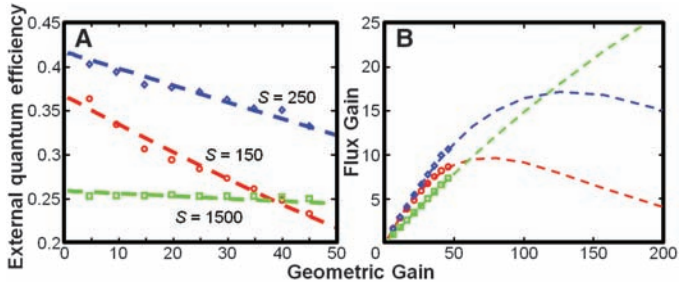

Fig. 4. OSC efficiency and flux gain as a function of geometric gain. (**A**) With increasing G, photons must take a longer path to the edge-attached PV, increasing the probability of self-absorption losses. The fit lines apply theory described in the SOM (*13*). (**B**) The flux gain is estimated based on the measured EQE (*13*). Flux gain increases with G but reaches a maximum when the benefit of additional G is cancelled by self-absorption losses. Near-field energy transfer (blue) and phosphorescence (green) substantially improve the flux gain relative to the DCJTB-based OSC (red). The dashed lines give the theoretical prediction for G > 50 based on the data for G < 50 (*13*).

The optical quantum efficiency (OQE), defined as the fraction of incident photons emitted from the edges of the OSC waveguides, was determined within an integrating sphere. The OQE is fundamentally limited by the product of the PL efficiency of the terminal dye and the fraction of photons that are emitted into waveguide modes (*13*). For an organic film refractive index of n = 1.7 au, and assuming photons are reemitted isotropically, ~80% of the photons are emitted into waveguide modes in the organic film or glass substrate (*2*). Waveguided photons not lost to self-absorption or scattering emerge from the edges of the OSC and are coupled to a PV cell. The remaining photons are emitted into the air through the top and bottom faces of the OSC. We distinguished between edge and facial emission by selectively blocking edge emission with ink and tape.

The ratio of the area of the concentrator to the area of the PV cell is the geometric gain G, also known as the geometric concentration factor. The OQEs of the single-waveguide OSCs at low geometric gain (G = 3, glass dimensions 25 by 25 by 2 mm, and n = 1.8 au) are compared in Fig. 3A. Higher-efficiency tandem OSCs were used with a rubrene-based OSC on top to collect blue and green light and the Pt(TPBP)-based OSC on the bottom to collect red light. Together, this tandem OSC combines higher-efficiency collection in the blue and green with lower-efficiency performance further into the red, as shown in Fig. 3B. At G = 3, the self-absorption is negligible, and the ratio of OQE between the DCJTB- and Pt(TPBP)-based devices approximately matches the ratio of their PL efficiencies (*13*).

The external quantum efficiency (EQE) is the number of harvested electrons per incident photon and includes all optical losses as well as the coupling losses at the PV interface and the quantum efficiency of the PV. The OSC films are evaporated onto a 100 by 100 by 1 mm SF10 glass substrate with a crystalline silicon PV attached along a single edge with optical epoxy. We measured EQE(G) by sweeping a point excitation normal to the detection edge while

monitoring the photocurrent (*13*). Figure 4A shows EQE(G) for each of the films, measured at λ = 534 nm for the fluorescent systems and λ = 620 nm for the phosphorescent system. The DCJTB-based OSC showed the strongest self-absorption. The self-absorption was lower in the rubrene-based OSC, which is consistent with the spectroscopic data in Fig. 2A. Finally, the Pt(TPBP)-based OSC showed no observable self-absorption loss for G < 50. The data matches the theoretical performance (*3*, *4*), assuming S = 150, S = 250, and S = 1500, for DCJTB-, rubrene-, and Pt(TPBP)-based OSCs, respectively (*13*).

Power-conversion efficiencies were estimated by integrating the product of the OQE, the AM1.5G spectrum (the standard spectrum of sunlight at Earth's surface), and solar-cell EQE weighted by the emission spectrum of each film (*13*). OSCs with emission from DCJTB were paired with GaInP solar cells (*14*); those with emission from Pt(TPBP) were paired with GaAs (*15*). The resulting power-conversion efficiencies are listed in Table 1. The estimated efficiency of the tandem OSC peaks at 6.8%.

We also calculated the power efficiency of tandem systems consisting of a top rubrene-based OSC whose transmission is incident on a CdTe or Cu(In,Ga)Se_2 (CIGS) PV cell (*16*, *17*). The OSC is predicted to increase the efficiency of CdTe and CIGS cells from 9.6 and 13.1% to 11.9 and 14.5%, respectively. There is a substantial opportunity to improve OSC efficiencies. The PL efficiency of the emissive dye can be increased, solar cells can be optimized for monochromatic and bifacial excitation, and the absorption spectrum should be expanded into the near infrared. With these advances, the power efficiency of tandem OSCs may exceed 20% (*2*).

Device stability in early LSC demonstrations was frustrated by the absence of photo-stable dyes (*4*). Since the original LSC studies, advances in dye-molecule design and packaging have yielded substantial progress in organic light-emitting diode (OLED) stability. In accelerated OLED stress tests, DCJTB and Pt(TPBP) have demonstrated stabilities exceeding 100 and 10 years, respectively (*11*, *18*). We have conducted preliminary stability measurements on our Pt(TPBP)-based OSCs using accelerated testing under an Oriel solar simulator with irradiance of 0.78 W/cm^2. The temperature of the OSC was 60°C. We observed that the PL efficiency of a 4% Pt(TPBP)-in-AlQ_3 sample decreased by 8% as compared with a dark-aged sample after the equivalent of 3 months outside (*13*). We expect that OSC lifetimes will approach the OLED standards when packaged and protected by ultraviolet filters.

The cost of a PV concentrator measured in cost per peak watt generated, $(\$/W_p)_{conc}$, is determined by its flux gain F, which is equal to the geometric gain after being corrected for efficiency losses in the concentrator; that is, $F = G\eta_{conc}/\eta_{PV}$ and

$$(\$/W_p)_{conc} = \frac{\text{collector cost}}{\eta_{conc} L} + \frac{1}{F}(\$/W_p)_{PV} \quad (1)$$

where L is the solar intensity, $(\$/W_p)_{PV}$ is the cost of the PV cell, and the power efficiencies of the concentrator and PV are η_{conc} and η_{PV}, respectively (*3*, *4*).

To compete with conventional power generation, $(\$/W_p)_{conc}$ must be <$\$1/W_p$. At mature production scales, we estimate that GaInP PV cells attached to the OSC will cost at least $\$50/W_p$ (*13*). Thus, the cost model yields a threshold value of $F \approx 50$. Equation 1 also shows that η_{conc} should be maximized to offset the collector cost. Previous LSC demonstrations yielded F < 5 with η_{conc} = 1.3% for the DCM class of laser dyes (*4*). In Fig. 4B and Table 1, we compare F for the three OSCs coupled to bandgap-matched solar cells. Based on measurements of OQE and EQE(G), we calculated F = 11 and η_{conc} = 4.7% for the rubrene-based OSC at G = 45. We extend the theoretical fit of OQE versus G to project performance at high geometric gain and predict a peak of F = 17 ± 2 for the rubrene-based OSC and F = 46 ± 15 for the Pt(TPBP)-based OSC.

OSCs aim to exploit high-performance PV cells in low-cost, nontracking solar concentrators. By using near-field energy transfer, solid-state solvation, and phosphorescence in thin-film organic coatings, we report OSCs that reduce the effective cost of inorganic solar cells by at least an order of magnitude. Combined with the potential for low-cost solution processing, the high flux gains and power efficiencies realized here should reduce the cost of solar power.

Table 1. Calculated power efficiency and flux gain of OSCs. The OSC parameters of OQE and spectral coverage were measured as a function of G. To project the performance of OSCs in combination with various solar cells, we calculated estimates of the power conversion efficiency of the combined systems (*13*). These calculations may underestimate the actual efficiencies by ignoring the benefits of optical concentration on the solar-cell open-circuit voltage and the ability to tailor solar-cell performance to narrow-band excitation. OSCs using energy transfer or phosphorescence best preserve power efficiency at high optical concentration (high G). However, both processes yield slightly lower performance at low G. Energy transfer decreases spectral coverage, and phosphorescence decreases the potential open-circuit voltage in the attached solar cell. The highest efficiencies are obtained from tandem structures: either combinations of rubrene- and Pt(TPBP)-based OSCs, or combinations of the rubrene-based OSC with CdTe or CIGS PV cells. The baseline efficiencies of the production CdTe and CIGS cells are 9.6 and 13.1%, respectively (*16*, *17*). SSS, solid state solvation; ET, energy transfer; Phos, phosphorescence; dash, not applicable.

Terminal absorber	Emitter	Processes	Power conversion efficiency at G = 3, 45	Flux gain at G = 45	Projected maximum flux gain
DCJTB	DCJTB	SSS	5.9%, 4.0%	9	12 ± 2 at G = 80
Rubrene	DCJTB	ET	5.5%, 4.7%	11	17 ± 2 at G = 125
Pt(TPBP)	Pt(TPBP)	Phos	4.1%, 4.1%	7	46 ± 15 at G = 630
Tandem: rubrene/Pt(TPBP)	DCJTB/Pt(TPBP)	ET/Phos	6.8%, 6.1%	—	—
Tandem: rubrene/CdTe PV	DCJTB	ET	11.9%, 11.1%	11	17 at G = 125
Tandem: rubrene/CIGS PV	DCJTB	ET	14.5%, 13.8%	11	17 at G = 125

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Supporting Online Material

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Control of Exciton Fluxes in an Excitonic Integrated Circuit

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Efficient signal communication uses photons. Signal processing, however, uses an optically inactive medium, electrons. Therefore, an interconnection between electronic signal processing and optical communication is required at the integrated circuit level. We demonstrated control of exciton fluxes in an excitonic integrated circuit. The circuit consists of three exciton optoelectronic transistors and performs operations with exciton fluxes, such as directional switching and merging. Photons transform into excitons at the circuit input, and the excitons transform into photons at the circuit output. The exciton flux from the input to the output is controlled by a pattern of the electrode voltages. The direct coupling of photons, used in communication, to excitons, used as the device-operation medium, may lead to the development of efficient exciton-based optoelectronic devices.

The advancement of signal processing and communication has led to the development of optoelectronic and all-optical circuits, which expand signal processing into an optically active medium (*1*, *2*). Semiconductor-based optoelectronic components are of particular interest because they can be integrated into circuits in a way similar to that of electronic integrated circuits. The advances in this direction include in particular the development of an optoelectronic transistor (*3*) and compact microring (*4*) and Mach-Zehnder (*5*) modulators. The latter devices have achieved switching speeds exceeding several gigahertz with active-region dimensions in the range of 10 to 100 μm (*4*, *5*). The development of optoelectronic devices with still smaller dimensions is attractive because this would permit a high packing density, one of the key advantages of electronic integrated circuits.

Excitons, bound pairs of electrons and holes, have been used in the development of semiconductor-based optoelectronic devices, which utilize an optically active medium. The optoelectronic devices operating with excitons include modulators (*6*), storage devices (*7*, *8*), field-gradient devices (*9*, *10*), and transistors (*11*). Yet, integration of these optoelectronic devices into circuits, a crucial requirement for signal processing, has remained an open challenge. We demonstrated an excitonic integrated circuit (EXIC), which can be used to perform operations on photonic signals such as switching and merging.

Because an exciton can be described as a hydrogen-like bosonic particle (*12*), the control of exciton fluxes opens a pathway to the study of excitons in controllable potential profiles—a bosonic counterpart of electronic mesoscopics, the field concerning electron transport in potential profiles.

Exploiting excitonic transport in devices requires the ability to control the exciton energy by an applied gate voltage, and also requires a sufficiently long exciton lifetime so that the excitons can travel over large distances exceeding the device dimensions. A regular exciton is a neutral particle without a built-in dipole moment. It is therefore only weakly sensitive to an applied electric field. Furthermore, its lifetime in a direct-gap semiconductor is typically less than a nanosecond, allowing it to travel only a small distance before it recombines. It is of particular interest to study the mesoscopics of cold excitons analogously to the mesoscopics of electrons, in which interesting physics emerges at low temperatures. However, the same rapid electron-hole recombination, which does not allow regular excitons to travel over large distances, also does not allow them to reach low temperatures within their short lifetime.

An indirect exciton is composed of an electron and a hole, which are confined in spatially separated layers and can be formed in coupled quantum wells (CQWs) (Fig. 1A). The indirect excitons in CQWs are dipoles, with the dipole moment close to the distance between the QW centers (d). Therefore, an electric field F_z perpendicular to the QW plane results in the exciton energy shift $\delta E = eF_z d$ (Fig. 1B). The laterally modulated electrode voltage $V(x,y)$ creates a laterally modulated electric field $F_z(x,y)$ and, in turn, a lateral relief of the exciton energy $\delta E(x,y) = eF_z(x,y)d \propto V(x,y)d$. Shaping of $V(x,y)$ by an appropriate patterning of electrodes allows the creation of virtually any required in-plane potential profiles for excitons. Particular cases include potential gradients (*9*, *10*), one-dimensional (*13*–*16*) and two-dimensional (*17*) lateral lattices, and traps (*17*–*19*). Furthermore, changing the applied electrode voltage $V(x,y)$ allows the in situ control of the in-plane potential on a time scale much shorter than the exciton lifetime (*11*).

Because of the spatial separation between the electron and hole layers, the lifetime of the indirect excitons exceeds by orders of magnitude the lifetime of regular excitons and varies typically from 10 ns to 10 μs. The indirect excitons can travel over large distances of tens and hundreds of micrometers within their lifetime (*9*–*11*, *20*–*24*), distances for which devices can be readily patterned.

Furthermore, because of their long lifetime and high cooling rate (*25*), the indirect excitons can cool to low temperatures close to the lattice temperature. Previous studies (*26*) have shown that the temperature of indirect excitons (T_X), exceeds the lattice temperature (T_L) by only a few

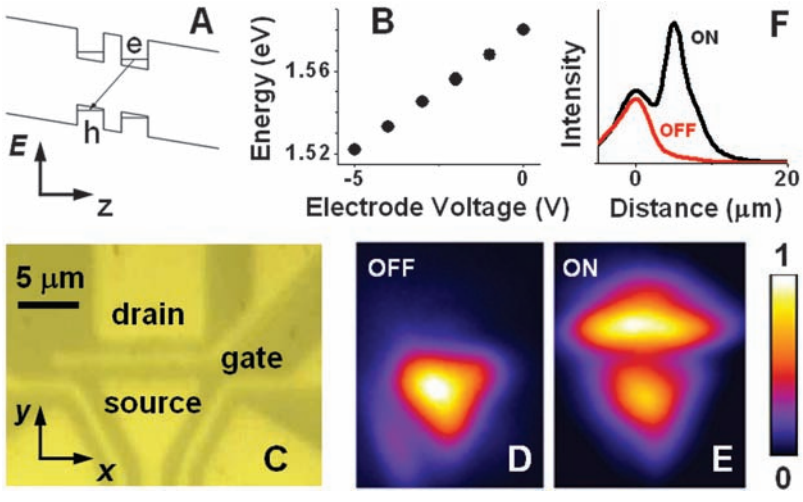

Fig. 1. Principle and operation of the EXOT. (**A**) Energy-band diagram of the CQW structure. e, electron; h, hole. (**B**) Control of the energy of the indirect excitons by gate voltage. (**C** to **E**) Realization of the EXOT. (C) Electrode pattern. [(D) and (E)] Emission of the EXOT in off (D) and on (E) states. The excitons are excited in the source electrode. The energy gradient for the indirect excitons from the source toward the drain is created by the electrode voltages $V_{source} = -1.5$ V and $V_{drain} = -2.5$ V. The exciton flux is controlled by the gate electrode: $V_{gate} = 0$ for the off state, and $V_{gate} = -3$ V for the on state. (**F**) Emission intensity along the exciton flux for off (red line) and on (black line) regimes [which correspond to the false-color images in (D) and (E)].

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kelvin in the area of the laser excitation. In this work, the experimental data were taken at T_L = 1.4 K. For this lattice temperature, $T_X \sim 3$ K in the laser excitation area (*26*). In the course of exciton transport to a few micrometers away from the laser excitation area, the indirect excitons can cool down even further and essentially reach the lattice temperature (*26*). In the temperature range of a few kelvin, the exciton thermal de Broglie wavelength $\lambda_{dB} = \sqrt{2\pi\hbar^2/(mk_BT_X)} \sim 0.1$ μm (where $\hbar^2$ is the Planck constant, m is the exciton effective mass, and k_B is the Boltzmann constant) and the exciton coherence length approaches a micrometer (*27*), length scales that are not much smaller than the device size (Figs. 1 to 3). This relation between the lengths is typical for mesoscopic devices. Although the ability of indirect excitons to cool to low temperatures is not necessary for optoelectronic applications, it makes them useful for studying the mesoscopics of bosons.

Devices based on excitons can only be operational at temperatures in which the excitons exist. This temperature range is determined to be roughly below E_X/k_B, where E_X is the exciton-binding energy (*28*). For indirect excitons formed in a $Al_{0.33}Ga_{0.67}As/GaAs$ CQW structure with d = 12 nm, E_X/k_B is on the order of 40 K (*29*). However, E_X can be varied by choosing different semiconductor materials and different structure parameters. For instance, in wide-bandgap semiconductor materials, E_X/k_B approaches the room temperature. The operation temperature is also limited by the possibility of spatial separation of electrons and holes by the applied electric field: The energy eF_zd should be higher than k_BT [in the sample described in this paper, eF_zd reached 50 meV; that is, $eF_Zd/k_B \sim 600$ K (Fig. 1B)]. Efficient device operation requires high-quality samples with a low, nonradiative recombination rate.

We built an EXIC that consisted of three exciton optoelectronic transistors (EXOTs). The EXIC was realized in a $Al_{0.33}Ga_{0.67}As/GaAs$ CQW structure (*30*). Both the operation principle and geometry of the EXOT mirror those of an electronic field-effect transistor (FET). Both the FET and EXOT are three-terminal devices in which the electron (FET) or exciton (EXOT) flux between two electrodes is controlled by the voltage applied to the third electrode. A transistor can operate in the modulation (switching) mode or amplification mode, depending on the loading scheme. In our circuit, the EXOTs operate in the former mode. The excitons are excited in the source electrode and travel from the input (source) to the output (drain) because of the potential energy gradient $\delta E \sim e(F_{zd} - F_{zs})d \propto (V_d - V_s)d$ created by the difference in the source voltage V_s and drain voltage V_d. The exciton flux from the source to the drain is controlled by a gate voltage V_g, which controls an energy barrier for the indirect excitons in the region of the gate electrode. The exciton emission rate can be controlled by F_z, and therefore the optical readout can be driven by applying a voltage pulse to the output electrode; however, this was not used in the demonstration described here. The emission image for one of the EXOTs in both the off state and on state is shown in Fig. 1, D and E, respectively. Figure 1F shows that the on/off ratio of the signal integrated over the output exceeds 30. The distance between the source and drain for the EXOT is 3 μm (limited by the resolution of the lithography used for the sample processing). The EXOT spatial dimensions may be able to be reduced below 1 μm, thereby permitting a high

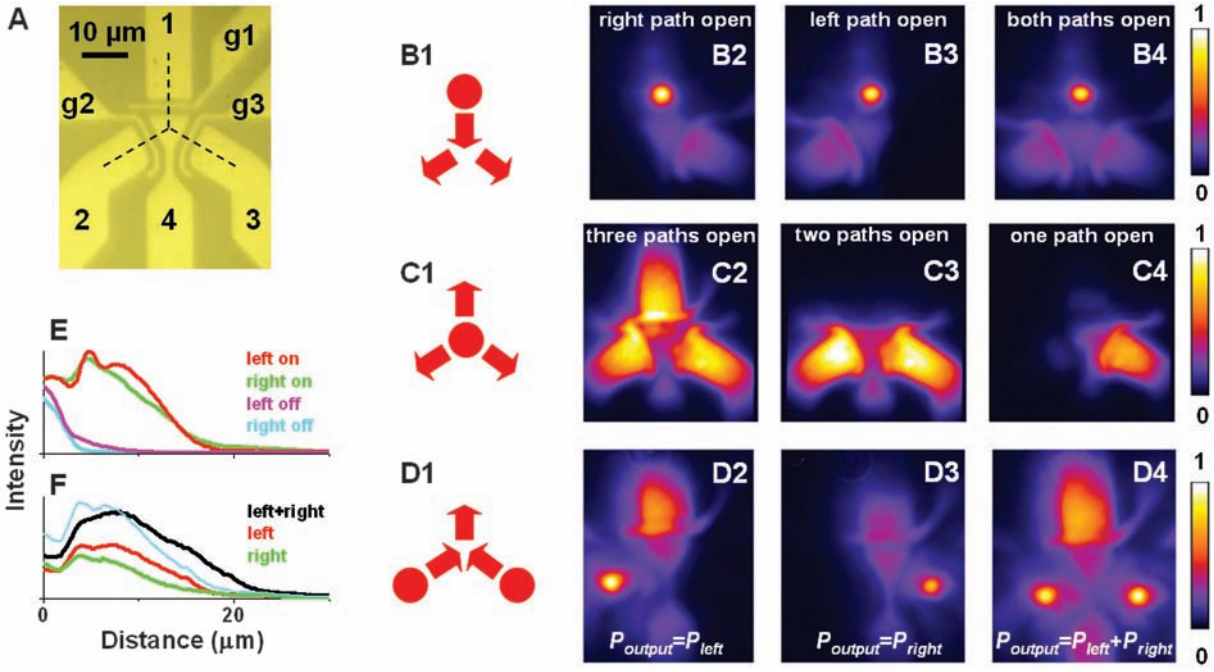

Fig. 2. Operation of the excitonic integrated circuit. (**A**) Electrode pattern. (**B1**, **C1**, and **D1**) Schematics for photoexcitation spots (points) and fluxes (arrows) of indirect excitons in the circuit. (**B2** to **B4**) Demonstration of the directional switch. The excitons are photoexcited at electrode 1. The energy gradient for the indirect excitons from electrode 1 toward electrodes 2 and 3 is created by the electrode voltages $V_1 = -0.5$ V, $V_4 = -1.5$ V, $V_2 = V_3 = -2.5$ V, and $V_{g1} = -2$ V. The exciton fluxes are directed by electrodes g2 and g3: (B2), $V_{g2} = 0$, $V_{g3} = -3$ V; (B3), $V_{g2} = -3$ V, $V_{g3} = 0$; and (B4), $V_{g2} = -3$ V, $V_{g3} = -3$ V. (**C2** to **C4**) Demonstration of the star switch. The excitons are photoexcited at the center. The energy gradient for the indirect excitons from electrode 4 toward electrodes 1 to 3 is created by the electrode voltages $V_1 = V_2 = V_3 = -1.5$ V and $V_4 = -0.5$ V. The exciton fluxes are directed by electrodes g1 to g3: (C2), $V_{g1} = V_{g2} = V_{g3} = -2$ V; (C3), $V_{g1} = 0$ V, $V_{g2} = V_{g3} = -2$ V; and (C4), $V_{g1} = V_{g2} = 0$, $V_{g3} = -2$ V. (**D2** to **D4**) Demonstration of flux merging for indirect excitons. The excitons are photoexcited at electrodes 2 (D2), 3 (D3), or 2 and 3 (D4). The energy gradient for the indirect excitons from electrodes 2 and 3 toward electrode 1 is created by the electrode voltages $V_1 = -2.5$ V, $V_2 = V_3 = -0.5$ V, $V_4 = -1.5$ V, $V_{g1} = -3$ V, and $V_{g2} = V_{g3} = -2$ V. (**E**) Emission intensity along the exciton fluxes [lower dashed lines in (A)] for the left and right paths of the directional switch in on and off regimes [which correspond to the false-color images in (B)]. (**F**) Emission intensity along the exciton flux [top dashed line in (A)] for the left, right, and both paths open of the flux merger [which correspond to the false-color images in (D)]. The experimental combined signal integrated over the output is within 5% of the sum of the signals from the left and right paths (thin blue line).

packing density, one of the key advantages of EXICs.

The device geometry allows the construction of EXICs. For instance, a drain of one EXOT can serve as a source of another EXOT. Figure 2 describes the integrated circuit, in which three EXOTs have a common electrode and form the geometry of a three-beam star. This excitonic integrated circuit can perform several operations with the exciton fluxes, which are determined by the patterns of the exciton photoexcitation and the electrode voltage.

The first scheme in Fig. 2 demonstrates the directional switching of the exciton flux. The excitons are photoexcited at the input of the top EXOT (electrode 1) and travel to the left and right paths (electrodes 2 and 3, respectively) because of the potential energy gradient, which is created by the voltages on electrodes 1 to 4. The exciton flux is controlled by the voltages on the gate electrodes V_{g1}, V_{g2}, and V_{g3}, as demonstrated in Fig. 2B. Opening the right gate and closing the left gate directs the exciton flux to the right path, whereas exchanging V_{g2} and V_{g3} switches the flux direction. The exciton fluxes are visualized by the exciton emission. Figure 2E shows that the on/off ratio of the excitonic directional switch exceeds 50. The typical transported exciton density, estimated from the exciton energy shift [as in (*26*)], is $n \sim 5 \times 10^{10}$ cm^{-2} when the device is open. In turn, the corresponding number of transported excitons $N = nS \sim 10^5$, where S is the area of the exciton cloud at the region of the output electrode.

The second scheme in Fig. 2 demonstrates the directional switching of the exciton flux in a star geometry (Fig. 2C). The excitons are photoexcited at the common electrode at the center of the circuit and travel to the top, left, and right paths because of the potential energy gradient, which is created by the voltages on electrodes 1 to 4. The exciton flux is controlled by V_{g1}, V_{g2}, and V_{g3}, which open or close the corresponding path. Figure 2 demonstrates the directing of the exciton fluxes to three paths (Fig. 2, C2), two paths (Fig. 2, C3), and one path (Fig. 2, C4).

The third scheme in Fig. 2 demonstrates the merging of the exciton fluxes (Fig. 2D). The excitons are photoexcited at electrodes 2 and 3 and travel toward the top electrode because of the potential energy gradient, which is created by the voltages on electrodes 1 to 4. The exciton fluxes from the left and right arms are controlled by V_{g2} and V_{g3}, respectively. The two fluxes flow to the top separately when only one of the paths is open (Fig. 2, D2 and D3) and are combined when both paths are open (Fig. 2, D4). When both paths are open, the circuit implements the optoelectronic sum operation for the exciton fluxes (Fig. 2, D2 to D4). Figure 2F shows that the excitonic flux merger performs the sum operation with a high accuracy: The experimental combined signal integrated over the output is within 5% of the sum of the signals from the left and right paths. The circuit can also implement the all-optical logic AND gate with a one set at a higher level, which is achieved by the combined input signals from both paths ($P_{output\text{-}1} \approx P_{left} + P_{right}$), and a zero set at a lower level, which cannot be achieved by the left or right input signal separately ($P_{output\text{-}0} < P_{left} + P_{right}$).

The exciton energy as a function of coordinate is presented in Fig. 3. The images demonstrate the excitons' drift down the potential energy gradient created by the pattern of electrode voltages. Previous studies have shown that, in this temperature range and in the presence of a potential energy gradient, exciton transport in a CQW structure can be described by drift and diffusion (*26*).

The circuits perform electronic operations on excitons, which can also be viewed as electronic operations on photons using excitons as intermediate media. Our device operates as a directional switch, star switch, and flux merger. The direct coupling of photons, used in communication, to excitons, used as the device operation medium, may lead to the development of efficient exciton-based optoelectronic devices. The demonstrated control of exciton fluxes opens the possibility of studying excitons in controllable potential profiles. Virtually any in-plane potential relief can be created for excitons by the appropriately designed voltage pattern, including, for instance, traps, quantum point contacts, conveyers, and lattices.

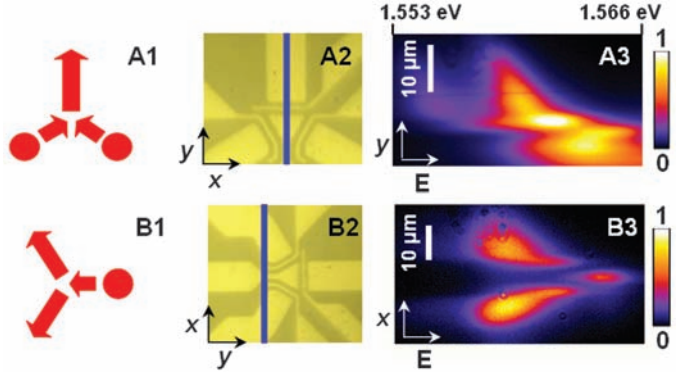

Fig. 3. Indirect exciton flux follows the energy gradient. (**A1**) Schematic for indirect exciton fluxes in the flux merger. (**A2**) *x*-*y* image of the excitonic integrated circuit. (**A3**) Energy–*y*-coordinate image of the indirect exciton flux. The exciton energy was dispersed by the spectrometer; the spectrometer slit position is shown by the blue line in (A2). Applied voltages and excitation spot positions are the same as in Fig. 2, D4. (**B1**) Schematic for indirect exciton fluxes in the directional switch. (**B2**) *y*-*x* image of the excitonic integrated circuit. (**B3**) Energy–*x*-coordinate image of the indirect exciton flux. The spectrometer slit position is shown by the blue line in B2. Applied voltages and excitation spot position are the same as in Fig. 2, B4.

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Optical Pumping and Vibrational Cooling of Molecules

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The methods producing cold molecules from cold atoms tend to leave molecular ensembles with substantial residual internal energy. For instance, cesium molecules initially formed via photoassociation of cold cesium atoms are in several vibrational levels v of the electronic ground state. We applied a broadband femtosecond laser that redistributes the vibrational population in the ground state via a few electronic excitation/spontaneous emission cycles. The laser pulses are shaped to remove the excitation frequency band of the $v = 0$ level, preventing re-excitation from that state. We observed a fast and efficient accumulation (~70% of the initially detected molecules) in the lowest vibrational level, $v = 0$, of the singlet electronic state. The validity of this incoherent depopulation pumping method is very general and opens exciting prospects for laser cooling and manipulation of molecules.

Over the past 20 years, the field of atomic physics has made enormous strides, with laser cooling and the achievement of atomic Bose-Einstein condensation. Similar advances are expected with cold molecules, involving applications, for instance, in molecular clocks, tests on fundamental physical constants, or quantum computing. Thus, the preparation of dense molecular samples in the ground state at low temperatures offers exciting prospects in both physics and chemistry (*1*–*3*).

An important step in the field of cold molecules has been the demonstration of a method for producing translationally cold samples of ground-state Cs_2 molecules via photoassociation of cold Cs atoms (*4*). This result has been quickly followed by the elaboration of various methods to prepare cold molecular samples. Methods that start with pre-formed molecules, usually in the lowest vibrational level, access translational temperatures down to a few millikelvins (*5*–*9*). Accessing temperatures even lower than these presents a major challenge. Cold molecules in the micro- or nanokelvin temperature range can only be achieved starting with cold atoms using collisional processes such as photoassociation in a thermal atomic cloud (*4*), Feshbach magnetoassociation in atomic Bose-Einstein condensates (*10*), or three-body collisions in an atomic Fermi sea to prepare molecular Bose-Einstein condensates (*11*). However, these methods of producing (translationally) cold molecules from cold atoms lead to the production of vibrationally excited molecules; that is, those with residual internal energy. For additional applications of cold molecules (*1*–*3*), the challenge is therefore to prepare and control molecules in the ground vibrational and rotational state.

Various experimental schemes can favor the formation of cold molecules in their lowest vibrational level. In a quantum gas, the adiabatic transfer of population [stimulated Raman adiabatic passage (STIRAP)] from a high ro-vibrational level toward a lower one has been achieved recently for molecules formed by magnetoassociation (*12*). In a cold thermal gas, a fraction of cold ground state Rb-Cs molecules, initially formed by photoassociation, has been prepared into the lowest vibrational level, $v = 0$, with a rate of ~500 s^{-1} by transferring ~6% of the population of a given high vibrational level into $v = 0$ (*13*). A few $v = 0$ cold ground-state potassium dimers have also been observed with the use of a two-photon process for photoassociation (*14*), but several other vibrational levels are populated as well. For further applications, what is needed is a molecular analog of optical pumping of atoms to realize vibrational laser cooling, which would transfer all the populations of the different vibrational levels into the lowest one.

Several theoretical approaches have been proposed to favor spontaneous emission toward the lowest ro-vibrational level: for instance, the use of an external cavity (*15*) or controlled interplay of coherent laser fields and spontaneous emission through quantum interferences between different transitions (*16*–*18*). As in these latter coherent control propositions, our approach uses a shaped pulsed laser but is based on an incoherent process of depopulation pumping with a train of several identical weak femtosecond laser pulses. More closely related to our work is the proposition of using a tailored incoherent broadband light source for the rotational cooling of molecular ions (*19*, *20*).

Here, we report the transfer of populations from an ensemble of vibrational levels of cold Cs_2 molecules, prepared in the electronic ground-state via photoassociation, into $v = 0$. The main idea is to use a broadband laser tuned to the transitions between the different vibrational levels (labeled v_X and v_B) of the singlet-ground-state X and an electronically excited state B. The absorption/spontaneous emission cycles lead, through optical pumping, to a redistribution of the vibrational population into the ground state (Eq. 1)

$$Cs_2(v_X) + h\nu \longrightarrow Cs_2(v_B) \xrightarrow{\text{decay}} Cs_2(v'_X) \quad (1)$$

where ideally $v'_X < v_X$ to realize vibrational cooling. The broadband character of the laser permits repetition of the pumping process from multiple vibrational states. By removing the laser frequencies corresponding to the excitation of the $v_X = 0$ level, we make it impossible to pump molecules out of this level, thus making $v_X = 0$ a dark state. As time progresses, the absorption/spontaneous emission cycle described by Eq.

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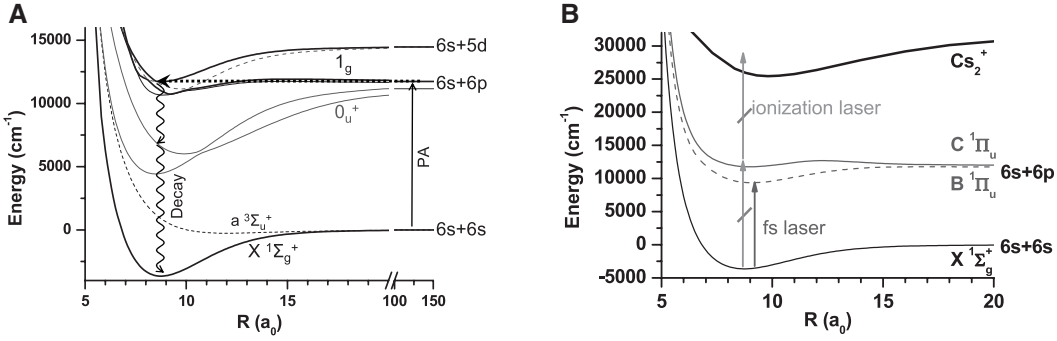

Fig. 1. Relevant schematic molecular potential curves of the Cs dimer, converging toward the dissociation limits 6s+6s, 6s+6p, and 6s+5d (for clarity, the fine structure is not labeled). (**A**) Photoassociation of cold atoms and formation of cold molecules. The cw laser (PA) is tuned ~1 cm^{-1} below the atomic transition $6s_{1/2} \longrightarrow 6p_{3/2}$. For the potentials of 1_g symmetry, long-range radial wave function is coupled to short range radial wave function by internal coupling of the potentials (*26*). The ground-state molecules, $X^1\Sigma_g^+$, are formed by a spontaneous emission cascade via the 0_u^+ potentials. (**B**) REMPI ionization process via the $C^1\Pi_u$ state by the pulsed dye laser, and electronic transition $X^1\Sigma_g^+$ toward $B^1\Pi_u$ induced by the femtosecond laser. a_0 is the Bohr radius.

1 leads to an accumulation of the molecules in the $v_X = 0$ level. We thereby realize vibrational laser cooling.

In our experiment, the formation of cold molecules is achieved in a Cs vapor-loaded magneto-optical trap (MOT) via photoassociation (*4*). Two colliding cold atoms resonantly absorb a photon with a frequency tuned slightly (~1 cm^{-1}) below the atomic $6s_{1/2} - 6p_{3/2}$ transition to create a molecule in an excited electronic state. The photoassociated molecules decay by spontaneous emission into stable vibrational levels of the molecular ground state $X^1\Sigma_g^+$ (Fig. 1A). They are then detected by resonance enhanced multiphoton ionization (REMPI). In contrast with previous studies (*4*), the REMPI frequency is tuned to ionize deeply bound vibrational levels of the X state through the excited $C^1\Pi_u$ molecular state (Fig. 1B). The complete mechanism for the formation of cold molecules in the singlet ground state is currently being studied, but the most probable scenario is shown in Fig. 1A. Photoassociation is achieved using a cw Titanium:Sapphire laser (intensity = 300 W cm^{-2}) pumped by an Argon-ion laser. The REMPI detection uses a pulsed dye laser (wave number ~ 16000 cm^{-1}, spectral bandwidth = 0.3 cm^{-1}) pumped by the second harmonic of a pulsed Nd:YAG (Nd–yttrium-aluminum-garnet) laser (repetition rate = 10 Hz, duration = 7 ns). The formed Cs_2^+ ions are detected with a pair of microchannel plates through a time-of-flight mass spectrometer. In the experimental spectrum obtained by scanning the REMPI laser wavelength (Fig. 2A), we assigned the observed lines to known transitions from the ground state levels $v_X = 1$ to 7 to various levels of the C state (*21*). In this first step, no molecules in the vibrational level $v_X = 0$ are detected. The present low REMPI resolution does not provide the capability of analyzing the rotational population of the molecules.

To achieve vibrational cooling, we applied a broadband femtosecond mode-locked laser (repetition rate = 80 MHz, pulse duration = 100 fs, SD-Gaussian bandwidth = 54 cm^{-1}, average intensity = 50 mW/cm^2, and central wavelength = 773 nm or wave number = 12,940 cm^{-1}) tuned to the electronic transitions from $X^1\Sigma_g^+(v_X)$ to $B^1\Pi_u(v_B)$ (Fig. 1B). Without shaping the femtosecond laser pulses, we observe a modification of the vibrational distribution, which we interpret as a transfer of population between vibrational levels as indicated by Eq. 1. The relative strengths of the transitions between the vibrational levels of the X-B states are given by the Franck-Condon factors (Fig. 3B). If we consider, for instance, a molecule in $v_X = 4$, the most probable excitation is to $v_B = 1$, which decays as in Eq. 1 with a partitioning ratio of ~30% to $v'_X = 0$ and 70% distributed essentially among $v'_X = 3$, 4, and 5. To control the optical pumping of the molecules, we shaped the femtosecond laser pulses by suppressing the frequencies above 13,030 cm^{-1} that could induce electronic excitation from $v_X = 0$ (Fig. 3A and hatched area in Fig. 3B). We used a home-built shaper with a diffraction grating (1800 lines/mm) after which high frequencies of the laser beam are screened out (lower part of Fig. 3A). After a few cycles of absorption of laser light and spontaneous emission, considering the populations in the observed vibrational levels ($v_X = 0 - 10$), a large fraction (65 ± 10%) of the molecules are accumulated in the lowest vibrational level ($v_X = 0$).

On application of the shaped laser pulses, the resonance lines corresponding to transition from $v_X = 0$ to $v_C = 0$ to 3 emerged strongly in the

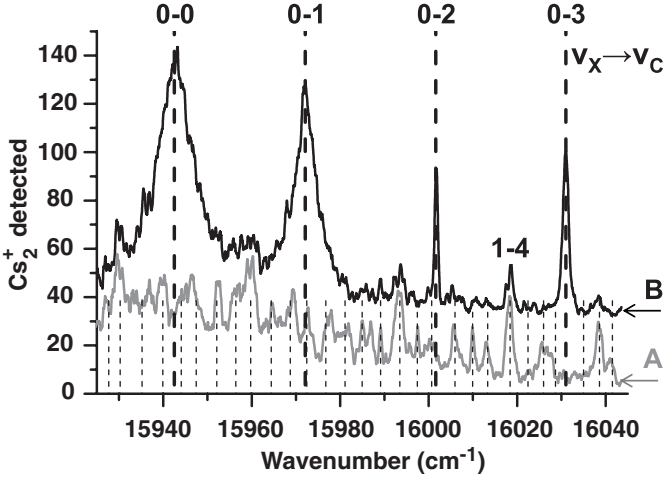

Fig. 2. Cs_2^+ ion spectra. (**A**) Spectrum without the shaped laser pulse. The spectrum has a background due to other REMPI processes that do not mask the resonance lines. Vertical dashed lines indicate the positions of all the resonances for vibrational transitions between the ground state, $X^1\Sigma_g^+$ ($v_X = 0$ to 7) and the electronically excited $C^1\Pi_u$ state (v_C). (**B**) Spectrum with the shaped laser pulse applied continuously, offset by 40 ions for higher visibility. The observed transitions from $v_X = 0$ correspond to $v_C = 0$, 1, 2, and 3. Their broadening corresponds to the saturation of the resonance in the REMPI process. Most of the lines present in spectrum (A) are greatly reduced, whereas the $v_X = 0$ lines grow more intense. The resonance labeled 1-4 indicates imperfect depopulation of $v_X = 1$ because of the roughness of the shaping.

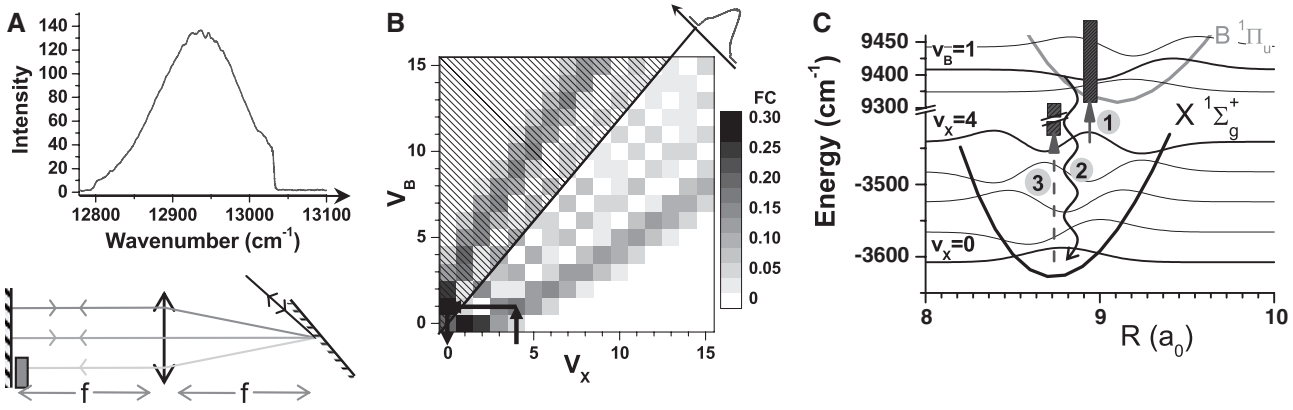

Fig. 3. Optical pumping scheme with a shaped femtosecond laser pulse. (**A**) (Top) Line gives the laser spectrum profile with shaping [also shown in (B)], close to a Gaussian profile sharply cut off at 13,030 cm^{-1}. (Bottom) 4-*f* shaping arrangement (from right to left: grating, cylindrical lens $f = 500$ mm, blocker, mirror). (**B**) Condon parabola indicating the importance (level of gray) of the Franck-Condon (FC) factors (square of the wave function overlap), corresponding to the relative transition probabilities from v_X to v_B. The diagonal line corresponds to the shaped laser cutoff frequency (13,030 cm^{-1}). The hatched area cannot be accessed in the presence of the blocker. (**C**) Optical pumping scheme and vibrational wave functions. The vertical black boxes indicate the spectral bandwidth of the laser. In (B) and (C), arrows indicate the optical pumping for $v_X = 4$ molecules. The most probable optical pumping scheme is to reach $v_X = 0$ through excitation into $v_B = 1$. Step 1, excitation toward $v_B = 1$; step 2, spontaneous decay to $v_X = 0$; and step 3, molecules in $v_X = 0$ are trapped. The incoherent dark state formed by the laser pulse shaping does not allow the excitation from $v_X = 0$ to any v_B level.

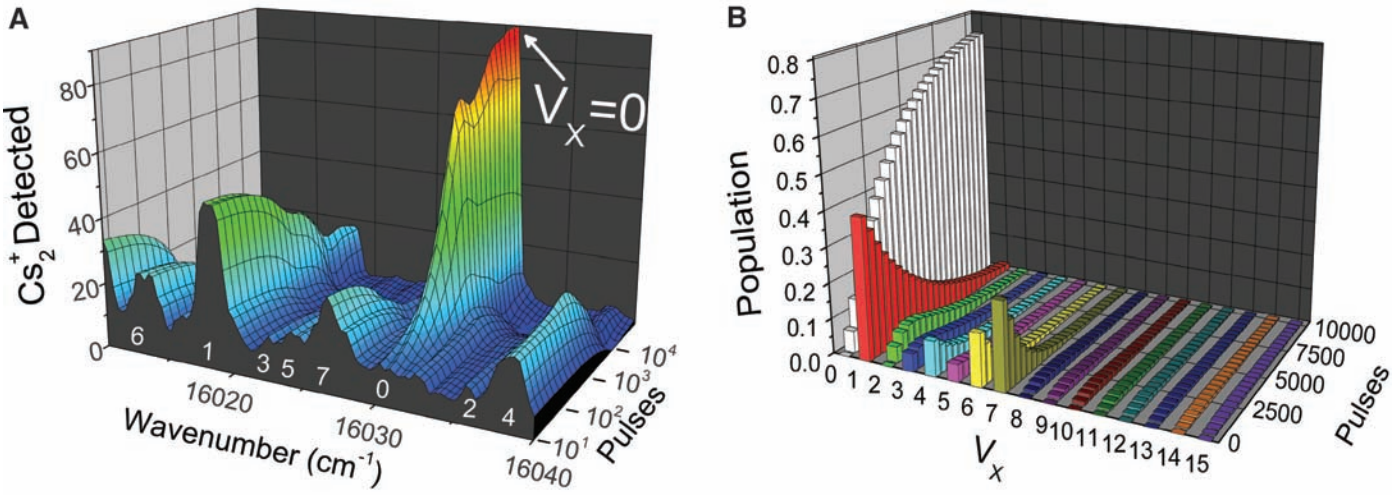

Fig. 4. Temporal evolution (pulse separation = 12.5 ns) of the populations in the different vibrational levels of the ground state. Because of our weak laser intensity, the excitation probability is only 0.1% for a single pulse. (**A**) Experimental population versus the number of applied femtosecond pulses, smoothed from five spectra (similar to Fig. 2 but taken after a controlled number of pulses). The frequencies correspond to transitions from $v_X = 0 - 7$ to v_C levels (white label). (**B**) Theoretical simulation where we represent the temporal evolution of $v_X = 0$ to 15 starting with initial conditions close to the experimental ones.

REMPI spectra (Fig. 2B). The intensity of the lines indicates efficient transfer of the molecules into the lowest vibrational level $v_X = 0$. By controlling the number of femtosecond laser pulses with an acousto-optic modulator, we analyzed the time dependence of the optical pumping scheme (Fig. 4A). At the weak laser intensities applied here, the transfer of population into the $v_X = 0$ level is almost completed after an exposure of the sample to 5000 pulses over 60 μs. Taking into account the efficiency of the detection, the detected ion signal corresponds to about 1000 molecules in the $v_X = 0$ level in the MOT area and thus to a formation rate of $v_X = 0$ molecules of more than 10^5 per second, which represent roughly 1% of the atomic loading flux in the MOT.

We have modeled the optical pumping process using the experimentally known $X^1\Sigma_g^+$ and $B^1\Pi_u$ potential curves (*22*, *23*). In our perturbative regime, the excitation probabilities are proportional to the laser spectral density at the transition frequencies. The lifetime of the electronically excited state B (~15 ns) is close to the 12.5-ns repetition period of the femtosecond laser, leaving negligible accumulation of coherence in the sample from pulse to pulse (*24*). We then assumed in our rate equation model an instantaneous spontaneous decay. The model shows that the vibrational population (v_X) proceeds by random walk, mostly through low vibrational levels, until reaching the $v_X = 0$ level. More than 70% of the total population is transferred into the $v_X = 0$ level (Fig. 4B) when we start from a distribution of vibrational levels close to the experimental one. The simulation shows that the limitation of the efficiency of the mechanism is in the optical pumping toward higher vibrational levels. Nevertheless, the simulation demonstrates that, for instance, increasing the bandwidth of the laser would reduce this detrimental pumping and would increase the population in $v_X = 0$. The theoretical model agrees well with the data in Fig. 4A. Furthermore, it indicates that only about five absorption/spontaneous emission cycles, corresponding to ~5000 laser pulses, are necessary for a molecule to be transferred into the $v_X = 0$ level. This small number of cycles does not substantially modify the temperature of the molecular sample. The theoretical simulation takes into account the rotational levels and demonstrates, for the experiment, the possibility to achieve rotational cooling for an adapted shaping, accurate enough to resolve the rotational structure.

The method—optical pumping of diatomic molecules using a shaped broadband source—is expected to be generally applicable to most molecular sample experiments that present a distribution of population of the low vibrational levels in the ground state. The efficiency will depend on the transition strengths between the different vibrational levels of the considered electronic states, but it could be optimized with a suitable shaping. The optical pumping should not be limited to cold samples of molecules prepared via photoassociation of cold atoms but should also be applicable to other cases, such as molecules in a molecular beam. Broadband shaped optical pumping could also be used as a repumping laser in laser manipulation of atoms and molecules, opening prospects in laser cooling of new species (*25*).

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A Positive Test of East Antarctica–Laurentia Juxtaposition Within the Rodinia Supercontinent

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The positions of Laurentia and other landmasses in the Precambrian supercontinent of Rodinia are controversial. Although geological and isotopic data support an East Antarctic fit with western Laurentia, alternative reconstructions favor the juxtaposition of Australia, Siberia, or South China. New geologic, age, and isotopic data provide a positive test of the juxtaposition with East Antarctica: Neodymium isotopes of Neoproterozoic rift-margin strata are similar; hafnium isotopes of ~1.4-billion-year-old Antarctic-margin detrital zircons match those in Laurentian granites of similar age; and a glacial clast of A-type granite has a uraniun-lead zircon age of ~1440 million years, an epsilon-hafnium initial value of +7, and an epsilon-neodymium initial value of +4. These tracers indicate the presence of granites in East Antarctica having the same age, geochemical properties, and isotopic signatures as the distinctive granites in Laurentia.

The supercontinent Rodinia is thought to have existed about 1 billion years ago (Ga) (*1*–*3*). Its breakup formed the nucleus of supercontinents that existed during the Phanerozoic and thus represents the starting point for understanding their subsequent evolution. The breakup of Rodinia also coincided generally with primary changes in seawater composition, the emergence of macroscopic biota, and the occurrence of Proterozoic glacial cycles, reflecting important linkages between geological and biological evolution. Reconstruction of Rodinia's pre-breakup paleogeography has been controversial because sea-floor spreading data are lacking before the Jurassic, and continental paleomagnetic data become increasingly fragmentary and are subject to overprinting with older age.

Laurentia has a central position in nearly all Rodinia reconstructions because it is surrounded by >14,000 km of late Precambrian rifted margin. What conjugate piece rifted from Laurentia's western (present coordinates) margin has long been debated, and proposed links include Australia, Siberia, South China, and Tasmania (*4*). One of the most prominent models, the southwest United States–East Antarctica (SWEAT) hypothesis, postulates a connection between the southwestern U.S. (Laurentia) and East Antarctica based on correlation of Neoproterozoic rift-margin stratigraphy, continuation of Paleoproterozoic provinces (Yavapai-Mazatzal), and extrapolation of Mesoproterozoic foldbelts (Grenville Orogen) (*1*–*3*). SWEAT fell into disfavor because (i) Grenville-age basement is globally widespread, making Grenville Orogen correlations nonunique; (ii) basement provinces are discontinuous (*4*); and (iii) paleomagnetic data are lacking for many Rodinia fragments, including East Antarctica, during the time period of assembly and breakup. Reconstructions using paleomagnetic data, for example, place East Antarctica either next to, or distant from, the southwestern margin of Laurentia (*5*). Although some paleomagnetic data allow a fit between Laurentia and a composite Austral-East Antarctica during the period from ~1050 to 720 million years ago (Ma) (*6*–*9*), other data appear contradictory (*10*) and additional paleopoles of appropriate age and geologic setting are needed to resolve existing disparities. Thus, although it is generally agreed that Rodinia was assembled between about 1.3 and 0.9 Ga, uncertainty remains about its paleogeography in general, and specifically about its western Laurentian conjugate margin.

As a test of the SWEAT model, we provide geological, geochronological, and isotopic data from Antarctica that address a key piece in the Rodinia puzzle. One of the most distinctive elements of Laurentian crust is a belt of ~1.4-billion-year-old rapakivi granites that extends from the Fennoscandian shield in Baltica across Laurentia to the southwestern U.S. If the SWEAT model for Rodinia is correct, traces of this belt, as well as Archean and Paleoproterozoic crust that hosts it, should be evident in East Antarctica. Indeed, isotopic data from the Transantarctic Mountains (TAM) suggest that crustal provinces in Antarctica are similar in age and character to those in western Laurentia (*11*). Here we present detrital-zircon Hf isotope compositions and Nd isotope data from rift-margin strata, and age and isotopic data from a rapakivi granite boulder in Pleistocene moraine, which demonstrate that these distinctive Laurentian basement belts extend into Antarctica.

Upper Neoproterozoic to Lower Cambrian (~670 to 520 Ma) siliciclastic rift- and passive-margin strata in the central TAM (Fig. 1A) contain up to 22% detrital zircon ~1.4 billion years old (*12*). The detrital zircons have an age profile similar to that of A-type Mesoproterozoic granites in Laurentia (Fig. 1B) (*13*, *14*). However, this detritus cannot have a direct source in Laurentia, because by the time of Gondwanaland amalgamation in the Early Cambrian, any fragment of Rodinia juxtaposed with present-day western Laurentia must have drifted away (*6*), and because the sediment was transported outboard from the interior of East Antarctica (*15*). Therefore, the persistent signature of this distinctive ~1.4-billion-year-old detritus in autochthonous units of Antarctica indicates that the central East Antarctic shield must contain an igneous geologic province of this age.

To provide additional constraints, we analyzed the Hf isotope compositions of the ~1.4-billion-year-old Antarctic detrital zircons. Their initial epsilon-Hf (ε_{Hf}, the deviation from chondrite uniform reservoir evolution) values of –2 to +7 (table S1) closely match those from Laurentian A-type granites (Fig. 1C) (*14*). The Hf isotope compositions correspond to depleted-mantle model (or crustal extraction) ages of ~2.0 to 1.6 Ga. Crust of this type is not known from present rock exposures in Antarctica, but is well represented in the Proterozoic orogenic provinces of southwestern Laurentia (Penokean, Yavapai, and Mazatzal). Together, these detrital-zircon age and isotopic data indicate that rift-margin sediment deposited on the paleo-Pacific margin of East Antarctica had a crustal provenance of similar age and isotopic character as that known from Laurentia. Because the isotopic compositions of both the Laurentian granites and the Antarctic detrital zircons cluster tightly, it is difficult to determine which type, if any, of the Laurentian provinces best represents the source of Antarctic detrital zircons; however, many of the detrital zircons have Hf isotope compositions that overlap with those from granites intruding the Yavapai province. Thus, an extension of the Proterozoic belts in Laurentia, including 1.4-billion-year-old igneous rocks and their host terrains, probably exists as a crustal province in central East Antarctica. This interpretation is also consistent with the co-occurrence of ~1.8- to 1.6-billion-year-old zircons in the detrital populations.

We also compared the Nd isotope compositions of the Antarctic rift-margin strata with coeval successions in western Laurentia. Nd isotopes in Proterozoic (~1.1 to 0.6 Ga) strata of western Laurentia show that provenance varies substantially with age and location (*16*). Not surprisingly, sediments deposited in close proximity to the Wyoming craton contain large proportions of material derived from Archean crust. In the southwestern United States and northern Mexico, in contrast, such deposits were derived predomi-

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nantly from Paleoproterozoic crust, with variable proportions of material derived from Mojave versus Yavapai-Mazatzal provinces. Overall, sediment transport across this part of western Laurentia was dominantly east to west (present coordinates). Thus, the sedimentary provenance was almost exclusively within ~2.0- to 1.6-billion-year-old crust, presumably reflecting the fact that Paleoproterozoic rocks were the dominant exposed source material for ≥2000 km inboard from the western continental margin. Neoproterozoic (≥0.6 Ga) rift-margin siliciclastic rocks in the central TAM have $\varepsilon_{Nd} = -16$ to -20 (Fig. 1D and table S2), overlapping with sedimentary rocks of similar age and depositional setting along the southwestern margin of Laurentia. Therefore, although the sources for these autochthonous Antarctic and Laurentian rift-margin successions lie within their respective cratons, they were derived from isotopically equivalent Paleoproterozoic crust.

We also collected large clasts and matrix material from heterolithic Pleistocene moraines in the central TAM. One 24-cm granitoid clast (sample TNQ) is a coarse-grained, red, porphyritic, rapakivi-type muscovite-biotite granite with a weak foliation (Fig. 2A). This small boulder

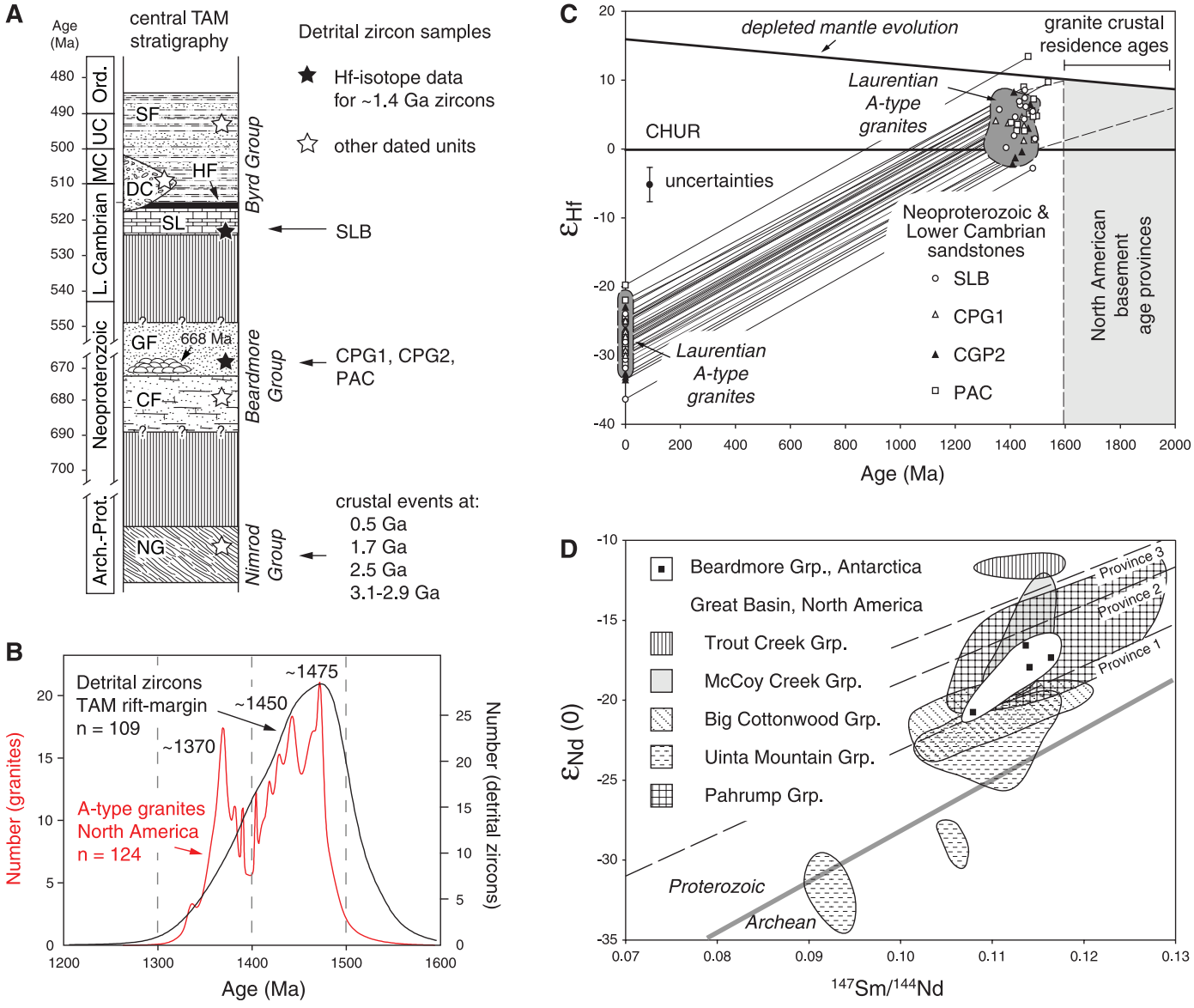

Fig. 1. Key features of Neoproterozoic and Lower Cambrian siliciclastic rocks from the central TAM margin of Antarctica. (**A**) Simplified stratigraphy of pre-Devonian sedimentary rocks overlying cratonic basement (Nimrod Group) in the Nimrod Glacier area (*15*). Stars indicate strata for which detrital-zircon age populations are known (*12*) and for which Hf isotope compositions were determined in ~1.4-billion-year-old detrital zircons (black stars). Formations are as follows: CF, Cobham; DC, Douglas Conglomerate; GF, Goldie; HF, Holyoake; NG, Nimrod Group (undivided); SF, Starshot; SL, Shackleton Limestone. SLB, CPG, and PAC are sample numbers. (**B**) Relative probability distribution of zircon U-Pb ages from Laurentian A-type granites [red (*14*)] and total detrital-zircon populations aged between ~1300 and 1600 Ma in Neoproterozoic to Lower Cambrian rift-margin sedimentary rocks in the central TAM [black (*12*)]. Curves are scaled to same height for comparison only; the smoothness of the detrital-zircon profile results from greater individual age uncertainty. (**C**) Age versus ε_{Hf} for detrital zircons from Antarctic rift-margin sedimentary rocks (table S1) and Laurentian granites [gray field (*14*)]. Uncertainties associated with individual detrital-zircon Hf isotope compositions are shown by an error bar (about ±2 ε_{Hf} units of total uncertainty for the laser-ablation inductively coupled plasma mass spectrometer method). CHUR, chondrite uniform reservoir. (**D**) Whole-rock ε_{Nd} versus $^{147}Sm/^{144}Nd$ isochron plot for Antarctic rift-margin siliciclastic rocks (table S2) compared to Neoproterozoic rift-margin successions in the Great Basin area of western North America (*16*). The gray line divides Archean from Proterozoic crustal sources; thin black lines are isochrons corresponding to different Laurentian basement provinces.

was glacially transported from the direction of the present-day polar ice cap and was collected near the upper Nimrod Glacier. This rock has high values of Zr (440 ppm), Y (90 ppm), Nb (23 ppm), Ce (180 ppm), and $K_2O + Na_2O$ [8.85 weight % (wt %)] (table S3), similar to A-type within-plate granites (*17*), including the Mesoproterozoic Laurentian suite (*13*, *18*). TNQ is strongly peraluminous (molar $Al_2O_3/(CaO + Na_2O + K_2O) = 1.01$), has a high Fe/(Fe + Mg) ratio of 0.83, and shows incompatible trace-element compositions overlapping those of 1.4-billion-year-old two-mica granites

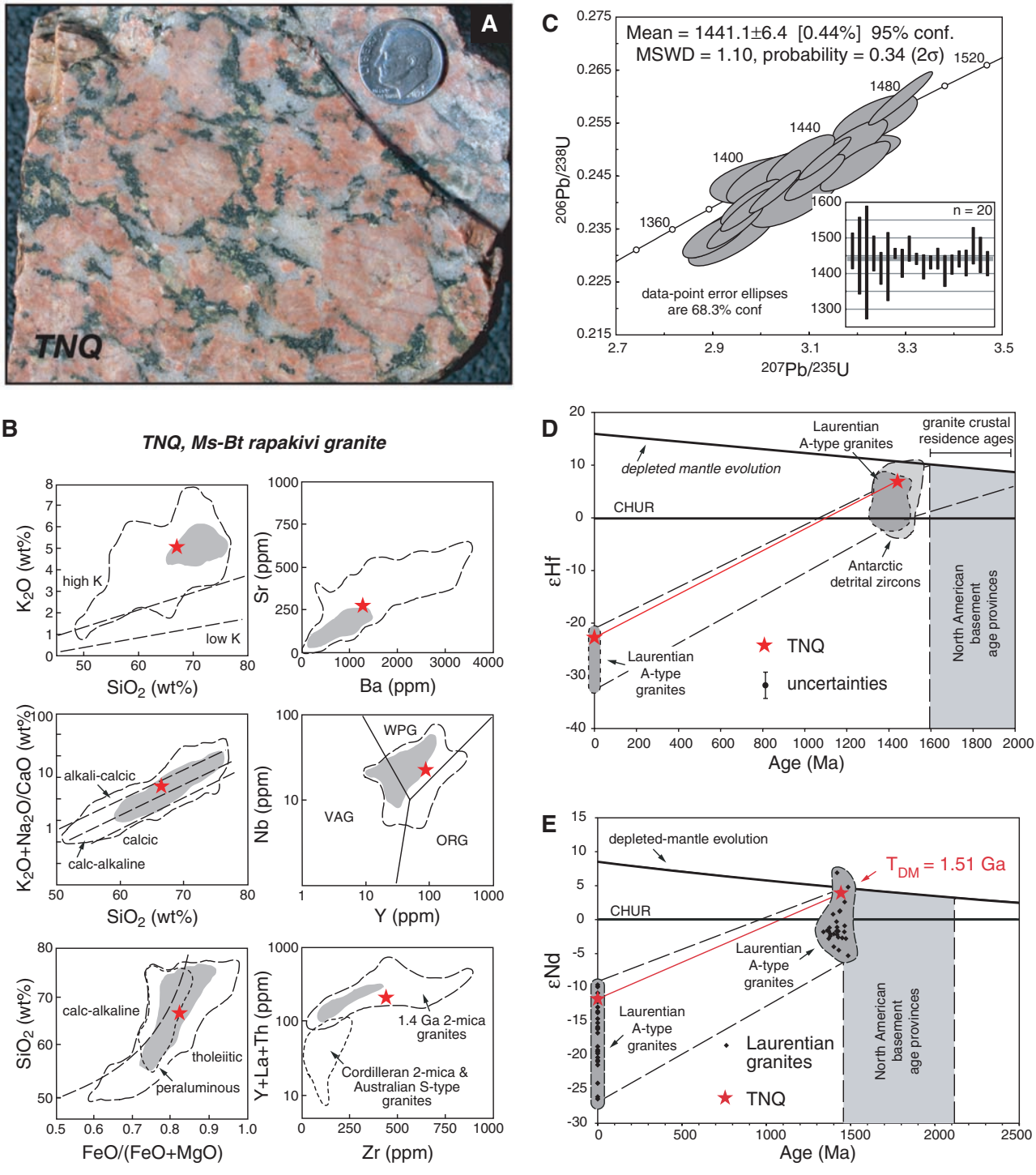

Fig. 2. Characteristics of granite boulder TNQ from a glacial moraine in the upper Nimrod Glacier area, Antarctica. (**A**) Photograph of cut slab, showing porphyritic rapakivi texture. (**B**) Geochemical characteristics of sample TNQ [red star (table S3)] compared to compositions of 1.4-billion-year-old granites in Laurentia (*18*); a dashed line bounds all analyses, and gray shading shows the densest data distribution. Ms-Bt, muscovite-biotite; ppm, parts per million. Fields for within-plate (WPG), volcanic-arc (VAG), and ocean-ridge (ORG) granites are shown. (**C**) Zircon U-Pb concordia diagram for sample TNQ (table S4). The inset shows individual SHRIMP ages included in the weighted-mean age calculation (all 20 analyses are included). MSWD, mean square of weighted deviates. (**D**) ε_{Hf} versus age for zircons in TNQ (determined by multicollector inductively coupled plasma mass spectrometry, table S5) compared to Laurentian 1.4-billion-year-old granites (dark gray field) and detrital zircons from Antarctic-margin rift sediments (light gray field; Fig. 1C). (**E**) Whole-rock ε_{Nd} versus age for TNQ (determined by thermal ionization mass spectrometry, table S6) compared to Laurentian 1.4-billion-year-old granites (*19*–*22*).

in southwestern Laurentia (Fig. 2B). Zircons from this granite are prismatic and clear, with broad growth bands observable in cathodoluminescence and no visible inherited cores, which is consistent with an origin from high-temperature Fe-rich melts (*13*). Its sensitive high-resolution ion microprobe (SHRIMP) U-Pb zircon age is 1441 ± 6 Ma (Fig. 2C and table S4). Zircons from TNQ have an average ε_{Hf} initial [$\varepsilon_{Hf(i)}$] value of +7.1 (Fig. 2D and table S5), which matches those of Laurentian A-type granites, particularly those of the central Yavapai province (*14*). TNQ has an $\varepsilon_{Nd(i)}$ value of +3.9 (Fig. 2E and table S6), yielding a depleted-mantle model age of 1.51 Ga, which corresponds to values reported for ~1.4-billion-year-old Laurentian granites (*19*–*22*). Sample TNQ thus demonstrates that the ice-covered East Antarctic shield near its paleo-

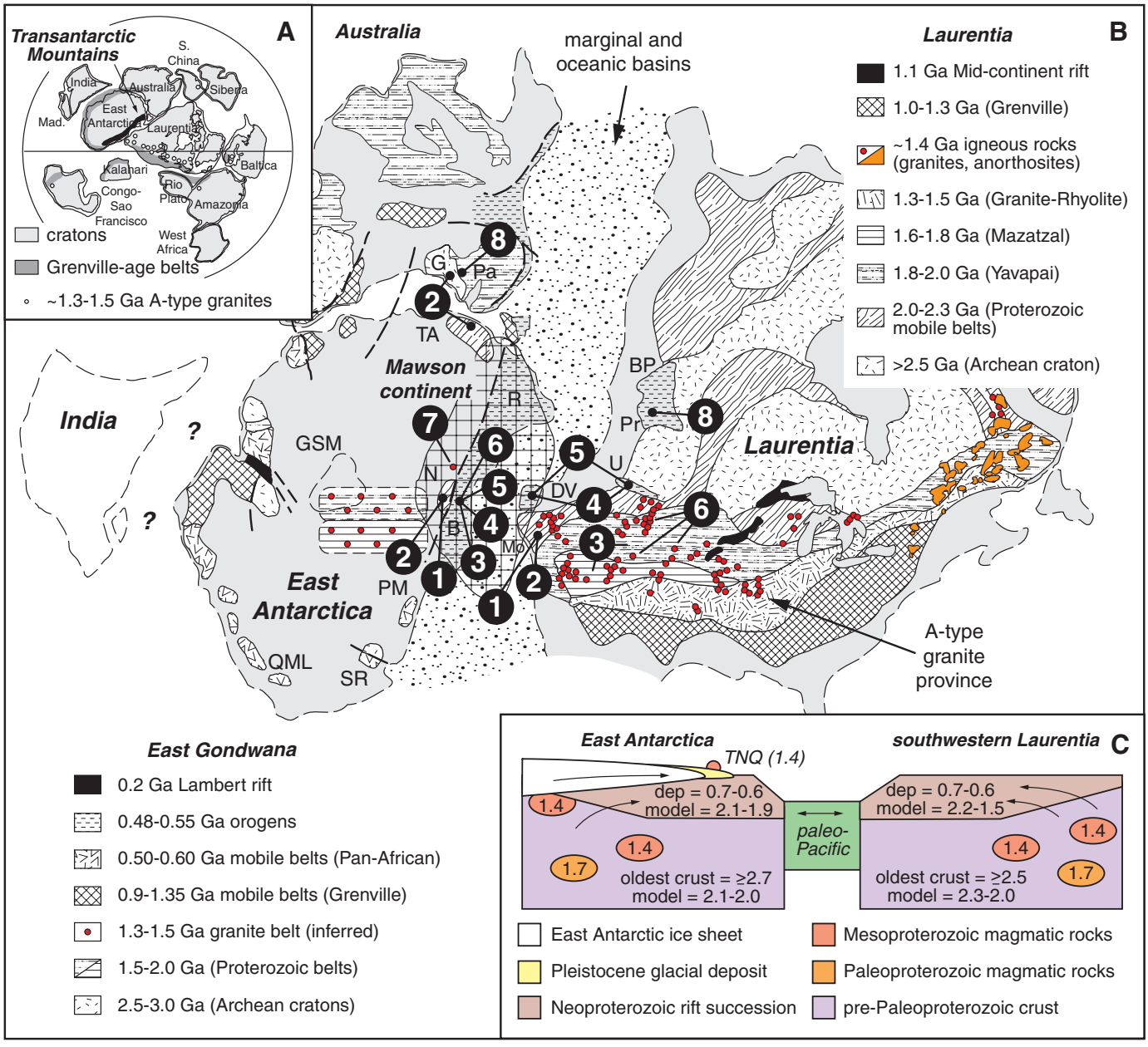

Fig. 3. Proposed paleogeographic relations of Laurentia and East Antarctica. (**A**) General Rodinia reconstruction at ~800 to 750 Ma consistent with SWEAT (*1*–*3*, *40*). Mesoproterozoic orogenic belts (dark gray) are thought to be associated with Rodinia assembly. The TAM margin of East Antarctica is shown in black. (**B**) Paleogeographic reconstruction of central Rodinia, emphasizing the postulated fit of East Antarctica–Australia and western Laurentia before rift separation, based on geologic, age, and isotopic correlations discussed in the text: 1, Nd isotope basement model ages; 2, ~1.7-Ga orogenesis and magmatism; 3, 1.8- to 1.4-Ga detrital-zircon provenance; 4, Nd isotope signature in rift-margin sediment; 5, ~0.7-Ga rift-margin sedimentation; 6, ~1.4-Ga Laurentian Hf isotope signature; 7, glacial clast of ~1.4-billion-year-old rapakivi A-type granite; 8, sediment with Gawler age and Hf signature. Also shown are Neoproterozoic and Lower Cambrian marginal-basin deposits (stippled) and the postulated extension of Proterozoic Laurentian crustal provinces into the East Antarctic shield (shown as patterned rectangles with round corners), including the ~1.4-Ga trans-Laurentian igneous province (small red circles). Crustal elements in present-day India, southern Africa, and Queen Maud Land that were assembled during Pan-African growth of Gondwana are of uncertain position in Rodinia (*40*, *41*). B, Beardmore Group; BP, Belt-Purcell basin; DV, Death Valley; G, Gawler Range; GSM, Gamburtsev Subglacial Mountains; Mo, Mojave Province; N, Nimrod Group; Pa, Pandurra Formation; PM, Pensacola Mountains; Pr, Prichard Formation; R, Ross Orogen; SR, Shackleton Range; TA, Terre Adélie; U, Uinta Mountains. (**C**) Schematic diagram showing common crustal features of the Antarctic and Laurentian margins. Numbers refer to crustal or magmatic ages (in billions of years).

Pacific margin contains Mesoproterozoic igneous crust, which further explains the large populations of detrital zircons of this age in Neoproterozoic craton-margin strata. Its age, geochemical composition, and isotopic signature indicate not only that it is the same age as the A-type granite province in Laurentia, but that it has a crustal source identical to that of the Proterozoic provinces of Laurentia. This clast thus represents a piece of rock in Antarctica with demonstrable ties to Laurentia.

Although there are as yet no known and exposed ~1.4-billion-year-old igneous bodies in East Antarctica, the high proportions of detrital zircons of this age, coupled with the discovery of a distinctive glacial clast that matches all known characteristics of the Laurentian A-type magmatic suite, suggest that a Mesoproterozoic igneous belt is present in Antarctica beneath the East Antarctic ice sheet. We fit this East Antarctic igneous source to the end of the Laurentian magmatic belt (Fig. 3).

In addition to results presented here, additional lines of evidence support a Laurentia–East Antarctic fit (Fig. 3B). Crustal-age provinces defined by Nd isotopes in East Antarctica (*11*, *23*) are similar to those in southwestern Laurentia (*20*, *24*). Proterozoic Nd-model ages from granites in the TAM are like those of known crustal provinces of southwestern Laurentia at 2.1 to 2.0, 1.9 to 1.6, and 1.2 to 1.1 Ga. Model ages of 1.45 to 1.38 Ga in the central and southern TAM (*11*) indicate the presence of Mesoproterozoic crustal sources in basement that is hidden beneath the East Antarctic ice cap. These signatures, and exposed crustal rocks having U-Pb and Nd-model ages of 3.1 to 2.7 Ga (*23*, *25*, *26*), resemble the pattern in Laurentia of Archean crust (Wyoming province) juxtaposed with Proterozoic accretionary belts (*27*). Second, granulite-facies gneiss, Caledonian-type eclogites, and intermediate-composition granitoids in the central TAM record lower-crustal metamorphism and magmatism between 1730 and 1720 Ma (*26*). Paleoproterozoic orogenesis is contemporaneous with magmatic and metamorphic events elsewhere in present-day Antarctica (*28*) and Australia (*29*) that straddle the future rift margin. The ~1.7-billion-year-old activity in East Antarctica and southern Australia reflects a phase of continental assembly that is coeval with prolific crustal growth, accretion, and reactivation in Laurentia (*30*). Both East Antarctica and southwestern Laurentia contain older parent rocks (3.0 to 2.0 Ga) that were modified substantially by events ~1.7 Ga. Third, the Antarctic-margin siliciclastic rocks noted above contain large populations of ~1.8- to 1.6-billion-year-old detrital zircons, in addition to lesser Mesoproterozoic (1.2 to 0.9 Ga) and Archean contributions (*12*). These detrital-mineral age signatures indicate that Paleoproterozoic igneous and/or metamorphic rocks are a substantial crustal component within the composite East Antarctic shield, similar to southwestern Laurentia. Notably, the ~1.8- to 1.6-billion-year-old detrital populations overlap the age of magmatic crustal growth in the Yavapai and Mazatzal provinces of Laurentia (*30*). Last, siliciclastic rocks in the central TAM contain 668-million-year-old intercalated pillow basalts and gabbros (Fig. 1A), constraining the time of rift-margin sedimentation before Early Cambrian platform development (*31*). In western Laurentia, thermal subsidence analysis indicates that the transition from rift to drift sedimentation occurred at a similar time (*32*), reflecting passive margin growth by ~625 Ma. Earlier on this margin, 689- to 667-million-year-old volcanic rocks within widespread Neoproterozoic successions date the time of rift-margin extension, sedimentation, and glacial activity (*33*, *34*). Thus, the continental rift margins in East Antarctica and western Laurentia developed synchronously after ~700 Ma. It follows that the principal breakup of Rodinia did not take place between 800 and 750 Ma, but rather was initiated between ~720 and 660 Ma (*32*, *35*, *36*).

Our reconstruction of central Rodinia (Fig. 3B) connects southwest Laurentia and central East Antarctica as in the SWEAT configuration. It is based on similarities in crustal isotopic signatures, ages of basement igneous and metamorphic events, the age and isotopic character of rift-margin sedimentary successions, sedimentary provenance tracers, and the matching of modern glacial clasts to distinctive igneous sources. Specifically, we suggest that the hallmark Proterozoic provinces of southwestern Laurentia (Mojave, Yavapai, Mazatzal, and the ~1.4-billion-year-old granite belt they host) extend into central East Antarctica. A criticism of the SWEAT model, and our modification to it, is that alleged allochthonous crustal blocks in the TAM preclude geological correlation from the East Antarctic margin (*4*). However, these units, once thought to be Neoproterozoic accreted terranes (*23*), are now recognized as autochthonous Cambro-Ordovician molasse deposits that postdate Rodinia breakup (*12*, *15*, *31*). Thus, the rift-margin deposits we have discussed do not contradict the SWEAT model; rather, their provenance characteristics reinforce it.

Together, these independent lines of geologic, age, and isotopic evidence provide a positive test of the Rodinian connection between East Antarctica and southwest Laurentia (Fig. 3C). These two areas share similar crustal, rift-margin, and sedimentary histories, including Archean crustal growth, Paleoproterozoic magmatic and metamorphic events, Mesoproterozoic magmatism, and late Neoproterozoic craton-margin sedimentation. They also share distinctive isotopic signatures, including crustal model ages, sediment model ages, and isotopic values of ~1.4-billion-year-old igneous detritus, indicating that part of the East Antarctic shield is composed of a Paleoproterozoic terrain punctuated by geochemically and temporally distinctive ~1.4-billion-year-old A-type rapakivi granites. Although Rodinia reconstructions are difficult to prove, any robust model of global Neoproterozoic paleogeography must explain the close geological, age, and isotopic ties between East Antarctica and western Laurentia. Various alternative paleogeographic configurations have been suggested, but the SWEAT model most successfully identifies the former conjugate margin to western Laurentia.

Our configuration also explains relations between Laurentia and Australia. For example, the Mesoproterozoic Belt–Purcell basin of Laurentia contains detrital zircons with ages of 1610 to 1490 Ma, including a ~1590-million-year-old population that is unknown from Laurentia and inferred to have a western source (*37*, *38*). As shown in Fig. 3B, these rocks are now adjoined to redbeds of the Pandurra Formation in South Australia, which have detrital zircons with ages of 1595 to 1585 Ma as well as overlapping Hf isotope signatures (*39*). It appears that the Pandurra and Belt successions represent proximal and distal deposits, respectively, that share a common western source in a Mesoproterozoic igneous terrain of present-day South Australia.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/321/5886/235/DC1
Tables S1 to S6
References

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Anticrack Nucleation as Triggering Mechanism for Snow Slab Avalanches

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Snow slab avalanches are believed to begin by the gravity-driven shear failure of weak layers in stratified snow. The critical crack length for shear crack propagation along such layers should increase without bound as the slope decreases. However, recent experiments show that the critical length of artificially introduced cracks remains constant or, if anything, slightly decreases with decreasing slope. This surprising observation can be understood in terms of volumetric collapse of the weak layer during failure, resulting in the formation and propagation of mixed-mode anticracks, which are driven simultaneously by slope-parallel and slope-normal components of gravity. Such fractures may propagate even if crack-face friction impedes downhill sliding of the snowpack, indicating a scenario in which two separate conditions have to be met for slab avalanche release.

Dry snow avalanches occur in two fundamentally different modes. Loose snow avalanches fan out from one point, entraining granules of snow of low cohesion by collisions. Slab avalanches originate from the extended failure of a weak subsurface layer in cohesive snow and release a large volume of the overlying snowpack (the slab) at once (Fig. 1). An apparently different type of snow instability is a sudden settling, which may occur even on horizontal terrain and may spread over large areas, often producing an audible "whumpf" sound (*1*–*3*). In practice, such whumpfs are considered clear warning signs of high avalanche hazard (*4*).

Snow is one of the most brittle materials (*5*), and the established view is that slab avalanches release by propagation of shear cracks along the weak layer (*6*, *7*). According to this viewpoint, the crack driving force is provided by the slope-parallel component of gravity. Hence, the critical crack size r_c should increase with decreasing slope angle, and fracture propagation should be impossible on flat or weakly inclined terrain. This prediction is in sharp contrast with recent experimental findings in which weak layers were artificially notched (*8*, *9*). In these experiments, critical lengths for fracture propagation, typically of a few decimeters, were found to remain constant or slightly increase with slope angle between 0° and 40° (*9*).

This discrepancy can be resolved by considering the propagation of mixed-mode anticracks that are driven by both shear and nonshear (compressive) components of the gravitational force. A mode I anticrack is a fracture mode in which the displacement field is equal in magnitude but opposite in sign to that of a classical mode I crack (*10*). In standard materials, this is not physically possible because of material interpenetration. Therefore, anticracks require loss of cohesion to be accompanied by a reduction in specific volume, clearing the space for inward displacement of the crack faces. The concept was originally introduced to characterize localized dissolution of limestone on closed, subplanar, cracklike structures (*10*), superheated ice (*11*), and in the context of earthquakes occurring under high pressure in deep subduction zones (*12*). The concept has natural applications in the analysis of failure processes in loosely packed cohesive granular materials, such as the self-sustaining progression of porosity collapse in granular sandstone under compressive tectonic loading (*13*). In snow, weak layers often have strongly anisotropic microstructures, the collapse of which may lead to a substantial reduction in specific volume (Fig. 2). It is therefore natural to apply the anticrack concept to weak-layer failure in slab avalanche release. Because the loading on a slope is not purely compressive but has important shear components, we speak of mixed-mode (mode I/II) anticracks.

We envisage a three-layer configuration consisting of a rigid collapsible layer embedded be-

Fig. 1. Crown face of a slab avalanche of exceptional size observed at Glacier du Vallonnet, France, 4 April 2007. The avalanche triggered spontaneously during precipitation. The thickness of the released slab exceeded 2 m. [Reprinted with permission of www.data-avalanche.org and A. Duclos (photo)]

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tween a cohesive slab and a rigid basal substrate, as shown in Fig. 3, A to C. The slab of thickness h is homogeneous with density ρ, plane-strain Young's modulus E, shear modulus G, and Poisson's ratio ν. The load acting on the undisturbed weak layer is composed of a compressive (negative) stress $\sigma = -\rho hg\cos\theta$ and a shear stress $\tau = \rho hg\sin\theta$, where g is the acceleration of gravity and θ the slope angle. We assume that the weak layer has failed over the crack length $2r$ as shown in Fig. 3, A to C, and that failure is accompanied by instant densification, which reduces the thickness of the weak layer by h_f (where f is fracture). As a consequence, the slab experiences both inward and lateral (slope-parallel) displacements. As the crack expands, contact between the opposing faces is established at a distance r_1 from the crack edge where the slope-perpendicular displacement of the upper crack face first reaches the value h_f. Deformation is then constrained by contact forces, which we model as Coulomb friction with coefficient μ in the slope-parallel direction and as rigid confinement in the slope-perpendicular direction.

We partition the crack formation energy $V(r)$ into $V(r) = V_f(r) + V_m(r)$, where $V_f(r)$ is the fracture energy that must be expended to destroy cohesion along the crack faces. For a crack of length $2r$, $V_f(r) = 2\,w_f r$, where w_f is the specific fracture energy per unit of crack surface, and for a notch of length r, $V_f(r) = w_f r$. The mechanical energy $V_m(r)$ comprises changes in strain energy and gravitational potential energy.

For anticrack nuclei of length significantly smaller than h, the problem is analogous to the opening of an interface crack in an infinite medium subjected to mixed-mode loading as considered by Hutchinson and Suo (*14*). The only difference is that the tensile stress is replaced by the compressive stress exerted by the weight of the overlying snow. Mathematically, the problem is treated as if the original crack faces were penetrating each other, whereas physically, there is no interpenetration but there is volume reduction of the weak-layer material. We find for the mechanical energy of a mixed-mode anticrack nucleus

$$V_{m,0}(r) = -\frac{\pi\gamma r^2}{4E}(\tau^2 + \sigma^2),\ r \ll h \qquad (1)$$

where γ is a constant of about 1 depending on Dundurs' elastic mismatch parameter (*14*).

For longer cracks, the problem must be treated differently. We approach this situation by noting that the unsupported part of the slab can be considered with reasonable accuracy as a Timoshenko beam. With the crack faces out of contact, the mechanical energy is given by

$$V_{m,1}(r) = -\frac{r^3}{3Eh}[\tau^2 H_\tau(r) + \sigma^2 H_\sigma(r)],\ r \geq h \qquad (2)$$

where $H_\tau(r) = 1 + \eta^2/[\frac{4}{9}(r/h)^2 + \frac{1}{3}\eta^2]$, $H_\sigma(r) = 3\eta^2 + \frac{4}{5}(r/h)^2(r/h + \frac{9}{4}\eta)/(r/h + \eta)$, $\eta = [E/(3kG)]^{1/2}$, and $k = 5/6$ is the Timoshenko correction factor for a rectangular beam section. Equations 1 and 2 give the mechanical energy of the crack in the respective limits $r \ll h$ and $r \geq h$. An expression for V_m that we find remarkably accurate over the entire range of $r < r_1$ is obtained by simply adding these expressions. This allows us to write the crack energy as

$$V(r) \cong V_f(r) + V_{m,0}(r) + V_{m,1}(r),\ r \leq r_1 \qquad (3)$$

For $r > r_1$, finally, the crack energy can be approximated as the energy of a shear crack with a fracture energy that is reduced by the energy recovered from gravitational collapse

$$V(r) \cong V_1 + 2(w_f + \sigma h_f)(r - r_1) - \frac{\tau_f^2(r^3 - r_1^3)}{3Eh},\quad r > r_1 \qquad (4)$$

where $V_1 = V(r_1)$ is evaluated according to Eq. 3, and τ_f is the shear stress reduced by friction; $\tau_f = \tau + \mu\sigma$ if $\tau_f \geq 0$ and is 0 if not. In the limit of $h_f \to 0$, r_1 becomes 0, Eq. 4 is valid for all r, and we recover the shear crack model (*6*). Equations 1 to 4 give the energy for crack lengths from centimeters to virtually infinity and hence cover the entire evolution from the incipient anticrack nucleus to the avalanche. The derivation of Eqs. 1 to 4, their comparison with finite element calculations, and the calculation of r_1 are presented in the supporting online material.

The size of a critical crack follows from the Griffith-Irwin criterion $\partial_r V(r) = 0$. Three cases

Fig. 2. Example of a collapsible structure in a weak interface layer of seasonal snow. The weak layer composed of hoar crystals is collapsed on the left and intact on the right. The thickness of the uncollapsed layer is 2 cm. [Reprinted from the Journal of Glaciology with permission of the International Glaciological Society (*18*)]

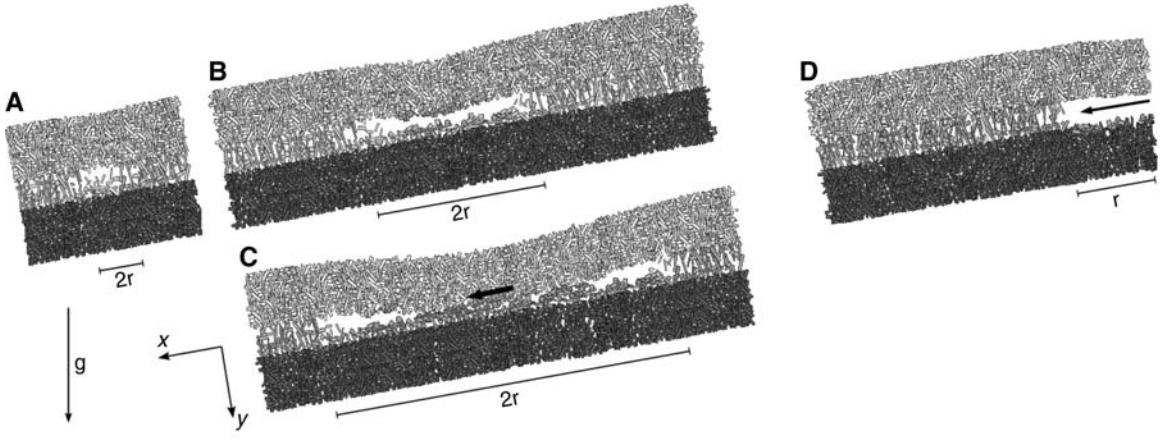

Fig. 3. Schematic of a mixed-mode anticrack cutting through a weak subsurface layer of snow (not to scale). **(A)** Anticrack nucleus. **(B)** Unconstrained anticrack. **(C)** Constrained anticrack: The opposing faces make contact. **(D)** Schematic of field experiment with an artificial notch cut into the weak layer on the uphill side. The crack or cut becomes critical for $r > r_c$ and debonds the slab as it propagates.

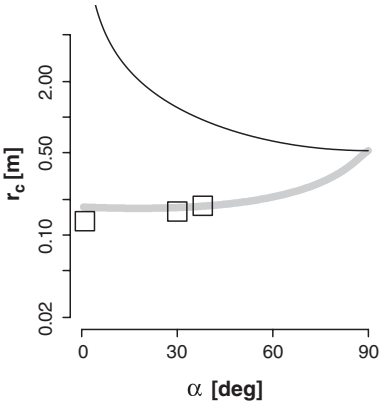

Fig. 4. Comparison of model calculations and experimental results (*9*). Squares, experiment; gray line, anticrack model of Eq. 5; black line, shear crack model (*6*). Material properties are as in Table 1.

Table 1. Data from notch experiments. The first entry refers to the 27 January 2006 field series in (*8*) and the last three entries to the 24 January 2006 field series in (*9*); for details, see (*19*).

	ρ (kgm^{-3})	*E* (MPa)	*h* (cm)	θ (deg)	r_c (cm)	w_f (J/m^2)	Cut direction
Sigrist *et al.* (*8*)	187	7.5 ± 2.5	26	30	23 ± 2	0.07 ± 0.02	Down
Gauthier *et al.* (*9*)	134	1.5 ± 0.8	11	0	13	0.03	Up
				30	19		Up
				38	22		Up

can be distinguished: (i) For $\mu \leq \tan\theta$, the crack energy function $V(r)$ has a unique maximum at r_c. The necessary condition $\partial_r V(r) < 0$ for crack propagation is met for $r > r_c$. This implies release of a slab avalanche, because the residual crack-face friction cannot support the slab. (ii) For $\mu > \tan\theta$ and $w_f + \sigma h_f < 0$, there exists again a unique maximum r_c. The supercritical crack propagates but both slope-perpendicular and slope-parallel displacements cease as the crack faces make contact. This is the case of a whumpf. (iii) For $\mu > \tan\theta$ and $w_f + \sigma h_f > 0$, the crack energy increases monotonically and the crack cannot become critical.

The theory can be tested in field notch experiments (*8*, *9*). The experimental setup is shown in Fig. 3D. For a notch, the structure of Eq. 2 is modified by the different boundary conditions and the mechanical energy is given by

$$V_{m,1}(r) = -\frac{r^3}{6Eh}[\tau^2 I_\tau(r) + \sigma\tau I_{\sigma\tau}(r) + \sigma^2 I_\sigma(r)] \quad (5)$$

where $I_\tau(r) = 1 + \frac{9}{4}\eta(r/h)^{-1} + 9\eta^2(r/h)^{-2}$, $I_{\sigma\tau}(r) = \frac{9}{2}\eta + 9\eta^2(r/h)^{-1}$, and $I_\sigma(r) = 3\eta^2 + \frac{9}{4}\eta(r/h) + \frac{9}{5}(r/h)^2$. A negative value of τ corresponds to a notch from the downhill side of the specimen, and a positive value corresponds to a notch from the uphill side. The slope dependence of the critical notch length is shown in Fig. 4 and compared with experimental results of Gauthier and Jamieson (*9*) listed in Table 1. We determine the Young's modulus from the slab density given by Gauthier and Jamieson by using a standard compilation of snow measurements (*15*) and assume a value of 0.03 J/m^2 for w_f (*5*, *8*, *16*). The average values and general trend of the measured critical lengths r_c are very well reproduced by our model.

The theory can also be tested on data of Sigrist (*8*), which comprise 21 field notch experiments yielding a mean critical length of r_c = 23 ± 2 cm on a slope of 30°. Using parameters given by Sigrist and listed in Table 1, our model predicts a critical crack length of 29 ± 5 cm. The simple shear model (*6*) yields r_c = 200 ± 30 cm for the same parameters.

The present theory predicts significantly shorter critical crack lengths than the simple shear model. For short anticrack nuclei, Eq. 1 gives

$$r_c = \frac{4}{\pi\gamma}\frac{w_f E}{(\rho g h)^2} \quad (6)$$

irrespective of the loading mode. For typical parameters of seasonal snow, this yields critical crack lengths of a few centimeter for slab thicknesses between 1 and 2 m. Because these lengths are comparable to the size of heterogeneities in weak layers (Fig. 2), the present theory plausibly accounts for the natural instability of heavily loaded slopes, for example, by wind deposition (Fig. 1).

The present model envisages slab avalanche release as a two-stage process. In the first stage, a critical mixed-mode anticrack nucleus is formed; for example, by overloading of a preexisting flaw due to an increase of σ and τ during precipitation, by deterioration of w_f, or by action of an external trigger. Once the criterion $r > r_c$ for sustained crack propagation is satisfied, this nucleus expands under the action of both slope-perpendicular and slope-normal forces. In a second stage, contact forces between the crack faces modify the boundary conditions. Crack-face friction does not lead to crack arrest but determines the nature of the instability initiated by crack propagation: If friction is small, the slab slides downhill and an avalanche is released. On slopes inclined less than the friction angle of the weak layer, on the other hand, the sliding motion of the slab is stopped, leading to a whumpf-type instability or a failed avalanche as reported in (*17*).

This two-stage scenario provides a natural explanation for several observations: (i) Weak-layer fractures may be triggered without necessarily releasing an avalanche (*17*). (ii) Slab avalanches may be remotely triggered by skiers moving on flat snowfields or on adjacent slopes. The first observation is consistent with anticrack propagation without avalanche release, whereas the second observation indicates that avalanche release may occur once such an anticrack propagates into sufficiently inclined terrain. Simple shear models (*6*) cannot explain either of these observations, because in these models fracture cannot propagate if the shear forces do not overcome the residual friction between the crack faces (*6*).

Even though Fig. 3 may suggest that our model has been formulated specifically for buried surface hoar, our mathematical description is not particular to any specific microstructural model. The key elements—volumetric collapse and residual friction in a granular assembly—apply to any weak layer composed of grains that can rearrange in a more tightly packed order. In snow, this includes layers composed of faceted crystals or depth hoar. Indeed, the present model produces accurate results for field experiments on weak layers of these types, as done by Sigrist (*8*).

The present model indicates that there is no threshold in slope angle for the disposition of a snow slope to nucleate and propagate cracks. This has important consequences for skiers moving in avalanche-prone terrain. Valid signals of instability can be detected on low-angle slopes where no avalanche is released. At the same time, skiers moving over flat ground or across small slopes cannot consider themselves entirely safe because long-distance fracture propagation may trigger avalanches on overlying slopes. These implications of the model are in line with empirical knowledge about avalanche triggering that has accumulated during many decades of snow research and presence on snow terrain. From a theoretical point of view, the antickrack model accounts for various aspects that have been cast aside by previous theories, such as remote triggering, fracture propagation on flat terrain, and the spontaneous formation of incipient cracks. In view of the characterization of snow stability, the results indicate that weak layers may fracture more easily than previously thought, and that crack-face friction may be a key factor that inhibits avalanche release on moderately inclined slopes. Hence, the granular properties that govern residual friction in weak layers may be an important factor in the assessment of avalanche hazard.

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Supporting Online Material
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SOM Text
Figs. S1 to S5
Table S1
References

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Micelles Protect Membrane Complexes from Solution to Vacuum

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The ability to maintain interactions between soluble protein subunits in the gas phase of a mass spectrometer gives critical insight into the stoichiometry and interaction networks of protein complexes. Conversely, for membrane protein complexes in micelles, the transition into the gas phase usually leads to the disruption of interactions, particularly between cytoplasmic and membrane subunits, and a mass spectrum dominated by large aggregates of detergent molecules. We show that by applying nanoelectrospray to a micellar solution of a membrane protein complex, the heteromeric adenosine 5′-triphosphate (ATP)–binding cassette transporter $BtuC_2D_2$, we can maintain the complex intact in the gas phase of a mass spectrometer. Dissociation of either transmembrane (BtuC) or cytoplasmic (BtuD) subunits uncovers modifications to the transmembrane subunits and cooperative binding of ATP. By protecting a membrane protein complex within a *n*-dodecyl-β-D-maltoside micelle, we demonstrated a powerful strategy that will enable the subunit stoichiometry and ligand-binding properties of membrane complexes to be determined directly, by precise determination of the masses of intact complexes and dissociated subunits.

Mass spectrometry of noncovalent protein complexes has made substantial contributions to structure elucidation, revealing the overall stoichiometry of protein subunits (*1*–*3*) as well as their topology (*4*) and interaction networks (*5*). Despite its undoubted power, mass spectrometry has not yet been applied in earnest to intact membrane protein complexes. This is due in part to their inherent insolubility in buffers that are compatible with electrospray (*6*), as well as the ready dissociation of subunit interactions, either between transmembrane subunits (*7*) or between transmembrane and cytoplasmic subunits (*8*). The vast excess of detergent aggregates that are present in the electrospray droplet (*7*, *9*–*12*) can also lead to the suppression of ionization (*13*). As a result of these difficulties, progress has been limited, with relatively few examples of intact membrane protein complexes having been reported. These include electrospray mass spectrometry of an intact homotrimer (*14*) and of the homomeric transmembrane complex of c-ring subunits of an adenosine 5′-triphosphate (ATP) synthase after separation from the soluble subunits and analysis with a novel ionization method (*15*). To date, however, there have been no reports of nanoelectrospray mass spectrometry of intact heteromeric membrane protein complexes containing both cytoplasmic and transmembrane domains, primarily because most previous protocols have involved the extensive removal of detergent molecules (*7*, *8*). Here, however, we show that by maintaining detergent micelles in solutions, well above the critical micelle concentration (CMC), it is possible to protect interactions between cytoplasmic and transmembrane subunits and, through gas-phase activation, release intact membrane complexes.

We selected five membrane protein complexes for our study, including some with well-defined structures and others with unknown subunit stoichiometry. We obtained mass spectra of all five complexes and chose to illustrate our approach with the well-characterized heteromeric transmembrane complex $BtuC_2D_2$, a vitamin B_{12} importer from *Escherichia coli* and a member of the ATP-binding cassette (ABC) transporter superfamily. These ABC transporters are ubiquitous membrane proteins, with representatives in organisms ranging from prokaryotes to humans, and they couple ATP hydrolysis to the transport of a diverse range of substrates across membranes (*16*). The overall structure of the $BtuC_2D_2$ assembly has been established from x-ray analysis of the complex in its ATP-free form (*17*). Two transmembrane subunits (BtuC), with a total of 20 transmembrane helices per subunit, form the translocation channel and bind to two cytoplasmic nucleotide-binding subunits (BtuD). Each subunit contacts its two immediate neighbors but has no interface with the remaining diagonally positioned subunit. A prominent cytoplasmic loop of BtuC forms two short helices that contact BtuD. The interactions between BtuC and BtuD transmit the conformational changes thought to occur upon nucleotide binding, opening the transmembrane channel for the import of vitamin B_{12}. Because this tetrameric complex consists of two different subunits and three types of intersubunit contacts, we can take advantage of its modular composition to explore interactions between subunits and different nucleotides, as well as compare gas-phase dissociation pathways and solution-phase unfolding reactions.

We used *n*-dodecyl-β-D-maltoside (DDM) to maintain the solubility of $BtuC_2D_2$, because this detergent is often preferred for retaining the native state of membrane proteins in solution (*18*) and stabilizes $BtuC_2D_2$ in an active state. Moreover, high concentrations of nonionic detergents can be tolerated more readily in electrospray than can ionic detergents (*6*), and DDM has previously been used with nanoelectrospray (*7*) and other ionization techniques (*15*, *19*). A 2-μl aliquot of a solution containing DDM above the CMC (fig. S1) (*20*) and ~5 μM membrane protein complex (*21*) was introduced from a gold-coated nanoflow capillary, and a spectrum was recorded on a mass spectrometer optimized for maintaining and focusing large macromolecular ions (*22*). Subjecting the protein-detergent assembly to maximum acceleration voltages, both within the electrospray source and across the collision cell of the mass spectrometer, gave rise to a spectrum in which the dominant peaks at low mass/charge ratios (*m*/*z*) were assigned to DDM clusters (fig. S2). At higher *m*/*z*, a broad distribution of species was observed, consistent with our previous ob-

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servations for micelles transported into the gas phase (*12*). We assigned this diffuse peak to a heterogeneous assembly containing ≥100 DDM molecules that remain associated with the complex (compare this with the solution-phase aggregation number of 130 in these buffer conditions, estimated from gel filtration). Within the same spectrum, the dissociated subunit BtuD was observed (Fig. 1A). Under these mass spectrometry conditions, all soluble protein complexes that we studied underwent substantial gas-phase dissociation (*23*), implying that this complex is protected by the detergent assembly. As the pressure inside the collision cell was increased, DDM molecules dissociated from the assembly, revealing a distinct series of peaks assigned to the intact protein tetramer.

Increasing the number of collisions and maintaining the high acceleration conditions gave rise to charge states above and below the peaks assigned to the intact tetramer. In common with mechanisms proposed for soluble complexes (*24*–*26*), these peaks were assigned to the unfolding and stripping of monomeric subunits, a phenomenon governed by the surface accessibility of the subunits as well as their size and ease of unfolding (*27*, *28*). The ejection of unfolded BtuD from the complex coincided with observations of large numbers of DDM molecules adhering to the complex. In contrast, when the complex was devoid of DDM in the gas phase, unfolded BtuC emerged as the dominant dissociation product (fig. S3). If we compare gas-phase dissociation pathways with solution-phase experiments, in which we used chemical denaturants to probe the unfolding of the soluble and transmembrane subunits, BtuD loses adenosine triphosphatase activity, consistent with some unfolding in 3 M urea (*29*). In contrast, circular dichroism studies of BtuC subunits in aqueous DDM micelles, at the same urea concentration, have shown little change in the secondary structure, implying that BtuD unfolds more readily in solution than BtuC. This is analogous to our gas-phase experiments, in which unfolding and dissociation of BtuD occurred predominantly in the presence of the gas-phase micelle. Unfolding and dissociation of BtuC, however, was observed only in the gas phase once the stabilizing effects of the detergent assembly had been disrupted.

Further support for the protective effects of the detergent assembly comes from a comparison of the average charge state of the intact tetramer with the relationship deduced for the surface area and charge of globular proteins and their complexes (*26*, *30*). We found that $BtuC_2D_2$ has ~16% lower average charge than a soluble protein complex of the same accessible surface area (Fig. 1B). The most likely explanation for this significant difference is that when charging takes place, during the droplet phase of electrospray, the complex is shielded by the DDM micelle. A similar effect to that observed for $BtuC_2D_2$ is also seen for a second ABC transporter, MacB. In this case, the average charge is ~10% lower than for a soluble complex with the same surface area. If we now subtract from the total surface area the membrane-embedded regions (*29*), we achieve closer agreement with the relationship deduced

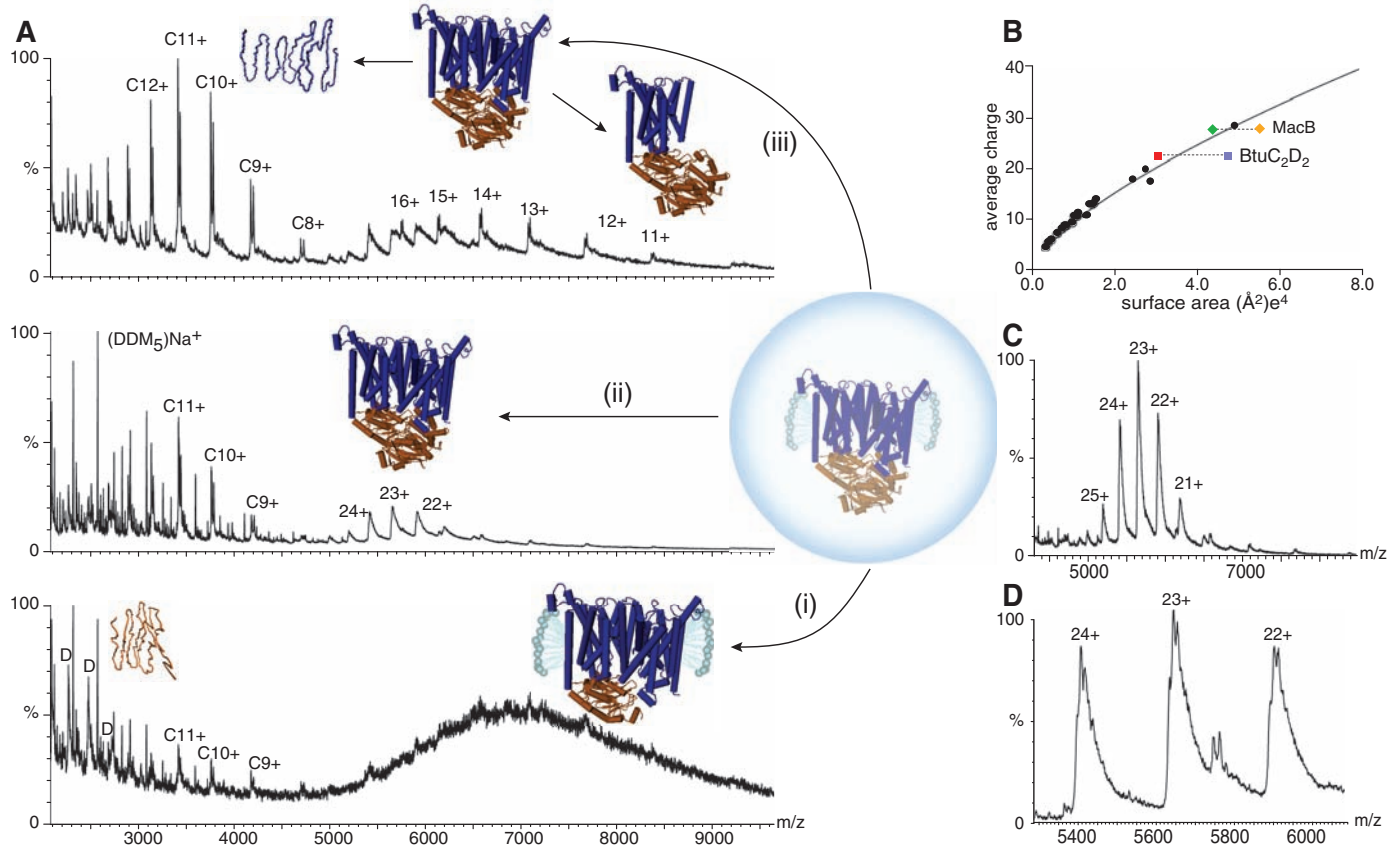

Fig. 1. Schematic representation of the emergence of the intact membrane complex from a micelle contained within an electrospray droplet and subsequent gas-phase dissociation pathways. (**A**) Populations of ions corresponding to the protein complex associated with aggregates of DDM molecules are observed above *m/z* 5000. At low *m/z*, the dominant dissociation product is the unfolded BtuD subunit [pathway (i)]. Increasing the number of collisions leads to the release of the intact tetramer [pathway (ii)]. Further increases in the number of collisions lead to the dissociation of BtuC and formation of a trimer [pathway (iii)]. (**B**) Plot of the average charge state of a series of globular and membrane protein complexes against their surface area ($Å^2 \times 10^4$) (*26*). Globular proteins are shown in black and membrane complexes are shown in blue and yellow ($BtuC_2D_2$ and MacB, respectively). The exposed surface area (that which is not surrounded by the micelle) was calculated either from x-ray structure coordinates ($BtuC_2D_2$, red) (*30*) or estimated based on secondary-structure prediction (MacB, green) (*29*, *37*). (**C** and **D**) Two different expansions across the 22+ to 24+ charge states reveal splitting assigned to a posttranslational modification. Structures were prepared from the x-ray coordinates (the Protein Data Bank file for $BtuC_2D_2$ is 1L7V). The transmembrane BtuC subunits (blue), cytoplasmic BtuD subunits (orange), and the presence of the micelle are illustrated.

for the surface area and charge (Fig. 1B). This implies that the DDM micelles are protecting the complexes, not only against the dissociation and unfolding of the transmembrane subunits during the phase transition but also from charging in the electrospray droplet.

Under the appropriate activation conditions, when extensive interactions with the DDM aggregates are disrupted, it is possible to observe the intact tetrameric protein complex in the gas phase with minimal dissociation (Fig. 1C). Close agreement between the measured and calculated masses (129,642 and 129,520 daltons, respectively) confirms the overall stoichiometry of the complex as being $BtuC_2D_2$, the majority of DDM molecules having dissociated under these conditions. Further expansion of the charge states revealed splitting of the peaks that were assigned to the intact tetramer, consistent with either binding to small molecules or posttranslational modification of the transmembrane or cytoplasmic subunits (Fig. 1D and table S1). To determine the origin of this splitting, we activated the tetramer to induce its gas-phase dissociation (Fig. 2A). All four of the anticipated dissociation products were formed, corresponding to unfolding and dissociation of either subunit and formation of the corresponding "stripped" trimers at higher *m/z*. For the $BtuCD_2$ trimer, split peaks were observed that were consistent with a ~1:1 ratio. Conversely, a 1:2:1 statistical distribution of modified forms was observed when interactions between the transmembrane subunits were maintained ($BtuC_2D$) (Fig. 2A, inset). This observation, together with the splitting observed for the unfolded BtuC monomer at low *m/z*, is consistent with a posttranslational modification of BtuC. Mass spectra recorded for the denatured protein subunits (fig. S4) confirm this modification to be an α-*N*-D-gluconyl-His tag, an established posttranslational modification for histidine-tagged proteins in *E. coli* (*31*).

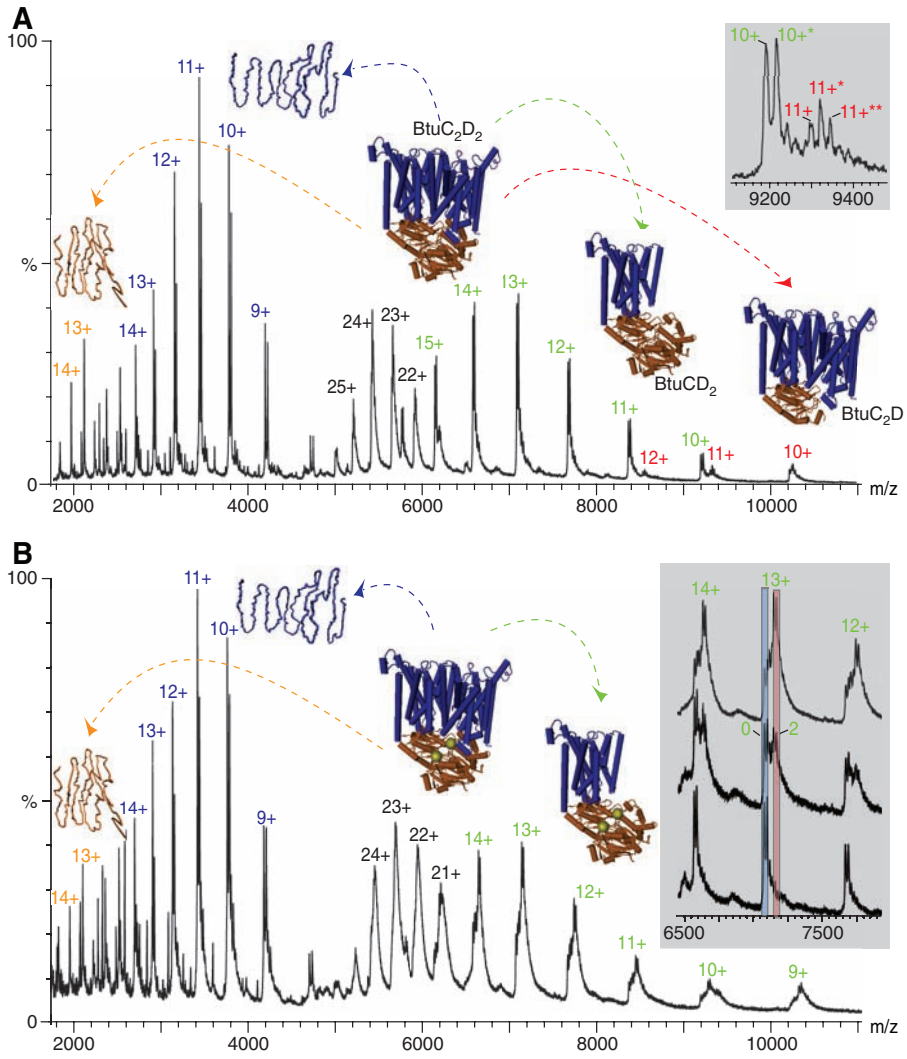

Fig. 2. Gas-phase dissociation of $BtuC_2D_2$ with and without ATP/Mg^{2+} reveals cooperative binding of ATP. (**A**) Intact tetramer, charge states 22+ to 25+ (black), undergoes asymmetric dissociation, expelling unfolded BtuD (orange) or BtuC (blue). The respective stripped trimers are observed at higher *m/z* $BtuCD_2$ (green) and $BtuC_2D$ (red). The inset shows the expansion of the 10+ and 11+ charge states of the trimeric dissociation products, showing a 1:2:1 statistical distribution of posttranslationally modified subunits in $BtuC_2D$, whereas the doublet assigned to $BtuCD_2$ corresponds to 0- and 1-modified subunits. (**B**) Dissociation of the complex in the presence of substoichiometric quantities of ATP/Mg^{2+} reveals the predominant dissociation pathway involving loss of transmembrane subunits and a distinct mass shift corresponding to binding of two molecules of ATP, represented by yellow spheres placed in the cyclotetravanadate-binding sites located crystallographically (*17*). The inset shows the expansion of the 13+ charge states of the stripped trimer $BtuCD_2$ in the absence of ATP (bottom) and in the presence of ATP 12 μM and 20 μM (middle and top, respectively). The multiplicity of peaks arises from the modified forms of BtuC as well as the 0 and 2 ATP-bound states (shaded blue and pink, respectively).

Using a similar approach to that described above, we investigated the effects of nucleotide binding by comparing the gas-phase dissociation pathways of apo $BtuC_2D_2$ with spectra recorded in the presence of ATP/Mg^{2+} (Fig. 2B). A clear increase in the average mass (1016.3 ± 9.7 daltons) is observed for the trimeric dissociation product $BtuCD_2$. This is consistent with the binding of two molecules of ATP (1022 daltons) together with Mg^{2+} adducts; however, the latter are not resolved at this high *m/z* value. The dissociation product BtuD shows no evidence of ATP binding. Moreover, it was not possible to discern a series of peaks assigned to $BtuC_2D$. This is consistent with the requirement for two BtuD subunits to form each ATP-binding site, predicted from the x-ray analysis of the cyclotetravanadate-binding sites (*17*). The low-intensity BtuD series presumably arises from dissociation of the apo form at substoichiometric ATP concentrations. Similarly, addition of adenosine 5′-diphosphate and an ATP analog, α,β-methyleneadenosine 5′-triphosphate, resulted in the binding of two nucleotides to the complex in each case. We also found that the collision energy required for gas-phase dissociation of protein subunits from all nucleotide-bound forms was higher than for the apo complex (fig. S5). Comparison with our solution-phase studies in the presence of ATP showed an increased ability of BtuD subunits to hydrolyze ATP and remain associated with BtuC when urea concentrations were raised (*29*). This shows that BtuD subunits are more resistant to unfolding when ATP is bound than when the complex is in its apo form. Together, these results imply an increase in the overall stability of the complex upon nucleotide binding, both in solution and gas phases, through increased interactions between the nucleotide-binding domains.

To investigate the cooperativity of ATP binding, proposed for all ABC transporters (*32*), we titrated increasing amounts of ATP/Mg^{2+} into solutions of $BtuC_2D_2$ (Fig. 2, inset, and fig. S6). The resulting mass spectra of the activated complex reveal that for the $BtuCD_2$-dissociation product, the population of the one ATP-bound state does not exceed either that of the apo or the two ATP-bound states, even when substoichio-

metric equivalents of ATP are added. This shows the cooperativity of ATP binding. This is evidence that ligand binding can be observed within the complex, without dissociation, even when the transmembrane subunits are stripped.

We have shown that we can maintain the seemingly unfavorable hydrophobic interactions within a heteromeric membrane protein complex by encapsulating it in a solution-phase detergent micelle and transferring this protected complex into the gas phase. Although the formation of micelles in solution has been shown to be facilitated by evaporation (*9*, *33*) and could be considered akin to the early stages of electrospray (*7*), the overall structure of gas-phase detergent assemblies is difficult to establish. Previous studies have shown preservation of micellar structure and protein encapsulation within reverse micelles (*12*). However, the fact that we have been able to employ mass spectrometry acceleration conditions far in excess of those used previously to preserve noncovalent complexes (*23*) implicates the stabilizing effects of the gas-phase detergent assembly regardless of its structure. Consequently, this enables us to release the complex intact and with ligand binding maintained. The observation that BtuD subunits are released preferentially, when large numbers of detergent molecules are present, while dissociated BtuC subunits predominate in their absence, is further evidence of the protection of the transmembrane subunits within gas-phase detergent assemblies. The low dielectric interior of lipid bilayers, which closely mimics the vacuum conditions of a mass spectrometer, may also contribute to the stability of the membrane complex once it is released from the gas-phase detergent assembly (*34*). It is established that solution-phase micelles exert pressure (*35*) sufficient to maintain the packing of the BtuC helices and subunit interfaces. We speculate that a similar effect may also be operative here, enabling us to preserve the deleterious effects of the phase transfer and to retain hydrophobic interactions between the transmembrane subunit interfaces. Irrespective of the origin of this unexpected gas-phase stability, and given the long-standing difficulties encountered in studying membrane protein complexes by mass spectrometry, as well as the ambiguities experienced in determining the stoichiometry of subunits within micelles using classical approaches, this represents a substantial advance, not only in methodology but also for assessing the effects of small-molecule drug candidates. More generally, the ability to release membrane protein complexes from detergent aggregates in the gas phase, devoid of detergent and with ligand binding intact, has great potential, not only for structural genomics but also for the many imaging techniques that are rapidly coming to the fore (*36*).

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Structural Basis of Trans-Inhibition in a Molybdate/Tungstate ABC Transporter

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Transport across cellular membranes is an essential process that is catalyzed by diverse membrane transport proteins. The turnover rates of certain transporters are inhibited by their substrates in a process termed trans-inhibition, whose structural basis is poorly understood. We present the crystal structure of a molybdate/tungstate ABC transporter (ModBC) from *Methanosarcina acetivorans* in a trans-inhibited state. The regulatory domains of the nucleotide-binding subunits are in close contact and provide two oxyanion binding pockets at the shared interface. By specifically binding to these pockets, molybdate or tungstate prevent adenosine triphosphatase activity and lock the transporter in an inward-facing conformation, with the catalytic motifs of the nucleotide-binding domains separated. This allosteric effect prevents the transporter from switching between the inward-facing and the outward-facing states, thus interfering with the alternating access and release mechanism.

Active transport proteins consume cellular energy to move substrates across biological membranes against their (electro)chemical gradients. Transport processes are regulated at various stages; this includes genetic regulation of the expression levels or control of the transporters by inhibitory cellular signals. Another mechanism is trans-inhibition, which occurs when substrates exert a concentration-dependent, inhibitory effect on the transporter after the translocation has occurred, that is, on the target side (trans side) of the membrane (*1*). This type of inhibition results in the decrease of the transport rates as the concentration of substrate increases (*2*). It is therefore a functional equivalent to product inhibition of soluble enzymes. Trans-inhibition has been reported for ion transporters (*3*) and various amino acid transporters (*4*–*8*), including glutamate transporters in astrocytes (*9*). It has also been described for binding protein–dependent ATP-binding cassette (ABC) transporters, such as those specific for

methionine [*met* system in *Escherichia coli* (*10*)], glycine/betaine [OpuA from *Lactobacillus plantarum* or *Listeria monocytogenes* (*11*, *12*)], or spermidine/putrescine [*pot* system in *E. coli* (*13*)]. Even though the functional effect of trans-inhibition has been known for a long time, its structural basis is not well understood.

We have studied *Methanosarcina acetivorans* ModBC (MaModBC), a binding protein–dependent ABC transporter specific for molybdate/tungstate. It consists of two transmembrane domains (TMDs, ModB subunits) that form a translocation pathway for the substrate, and two cytoplasmic nucleotide-binding domains (NBDs, ModC subunits) that bind and hydrolyze adenosine triphosphate (ATP) and power the transport reaction. What distinguishes MaModBC from other ABC importers such as the B_{12} transporter BtuCD (*14*, *15*) or the molybdate/tungstate transporter ModBC from *Archaeoglobus fulgidus* [AfModBC (*16*)] is the presence of a regulatory domain appended to the C terminus of the NBDs. This domain has some 120 amino acid residues, and similar extensions have been found in other ABC importers such as the methionine importer. Even though these domains had been suspected to be involved in regulation, their exact function has been somewhat of a mystery in most cases (*17*). While investigating its adenosine triphosphatase (ATPase) activity, we found that binding of molybdate or tungstate had an inhibitory effect on the ATP hydrolysis rate of the intact transporter MaModBC (Fig. 1A). The ATPase rate of MaModBC was already inhibited at low micromolar concentrations of molybdate (or tungstate), with an apparent inhibitory constant of ~5 μM. By contrast, the ATPase activity of AfModBC, which does not contain regulatory domains appended to the NBDs, was insensitive even to high concentrations of molybdate or tungstate (Table 1). Because ATP hydrolysis is a strict requirement for transport in ABC transporters, these results suggested that the regulatory domains present in MaModBC, but absent in AfModBC, mediate trans-inhibition.

We then proceeded to determine the crystal structure of the complete MaModBC transporter in a trans-inhibited conformation after cocrystallization with bound tungstate. We found that the regulatory domains form two oxyanion binding pockets that bind the substrate and lock the transporter in an inward-facing conformation, with the catalytic motifs of the NBDs separated. The structure of tungstate-bound MaModBC was determined with experimental phases from a three-wavelength MAD (multiwavelength anomalous dispersion) data set collected around the tungsten edge (*18*). The diffraction data (fig. S1 and table S1) were anisotropic and were truncated to 3.0, 3.3, and 3.5 Å resolution in the three diffraction directions (*19*). Because of the high accuracy of the MAD phases, which were further improved by solvent flattening and noncrystallographic symmetry averaging, the experimental electron density was of excellent quality (fig. S2) and allowed unambiguous building of the entire protein structure.

The TMDs and NBDs of MaModBC have folds similar to those of the previously determined AfModBC (*16*). However, the conformations of MaModBC and AfModBC are quite different. Whereas the NBDs of AfModBC have a shared interface, those of MaModBC contact each other exclusively through their regulatory domains. The catalytic (RecA-like and helical) subdomains of MaModBC have no direct contacts (fig. S3). Although separated, these domains nonetheless exhibit a "head-to-tail" arrangement, with the conserved P-loops juxtaposed to the LSGGQ motifs of the opposite subunit. Yet the distance between these conserved motifs increases from ~10 Å in AfModBC to ~23 Å in MaModBC, leaving a large gap between the NBDs in the latter. The separation is even more pronounced than that previously observed in the "open" conformation of the nucleotide-free NBD of the maltose transporter MalK (*20*).

The regulatory domains of MaModBC bind two tungstate ions at their shared interface, with both subunits contributing to both tungstate binding pockets. This is reminiscent of how ATP is bound between the NBDs of ABC transporters, where two ATP binding sites exist at the shared interface of opposite NBDs, with each domain contributing to both binding sites. Each regulatory domain contains two similar and consecutive subdomains that feature "Greek key" motifs. Similar folds have been observed for other molybdate-binding proteins, among them the C-terminal domain of the molybdate-dependent transcription regulator ModE (*21*). In the presence of molybdate, homodimeric ModE changes its conformation to form a tight, molybdate-bound interface (*22*), whereupon its N-terminal DNA binding domain acts as a repressor of the molybdate transporter and as an enhancer of molybdenum-dependent enzymes (*23*). The regulatory domains of MaModC (residues 231 to 348) and *E. coli* ModE (residues 124 to 262) are structurally similar (Fig. 2, A and B), with an overall root mean square deviation (RMSD) of 2.1 Å. However, a circular permutation places the two Greek key motifs in an opposite order, with the N and C termini of the ModE domain located at the linker between the Greek key motifs of MaModC (fig. S4). Two oxyanions are bound at the shared interface in both proteins (Fig. 2B), and the location of the binding sites, as well as the specific amino acid side chains mediating contacts (mostly hydrogen bonds) to the oxyanions, are conserved (Fig. 2C). The similarity in sequence and structure extends to proteins involved in molybdate storage and homeostasis, including the Mop protein from *Sporomusa ovata* [RMSD = 2.3 Å (*24*)] and ModG from *Azotobacter vinelandii* [RMSD = 1.3 Å (*25*)].

The architectural similarity of the regulatory domains of ModE and MaModC creates an unexpected structural link between genetic regulation and trans-inhibition in ABC transporters. In both processes, tight dimers of regulatory domains with two oxyanions (molybdate or tungstate) sandwiched at the shared interface affect the function of the fused partner domains. ModE exploits this to promote the binding of its N-terminal domains to DNA (*23*), whereas in the ABC transporter MaModBC, binding of molybdate keeps the catalytic motifs of the NBDs separated, thus disrupting the ATP hydrolysis cycle. Elevated concentrations of free cytoplasmic mo-

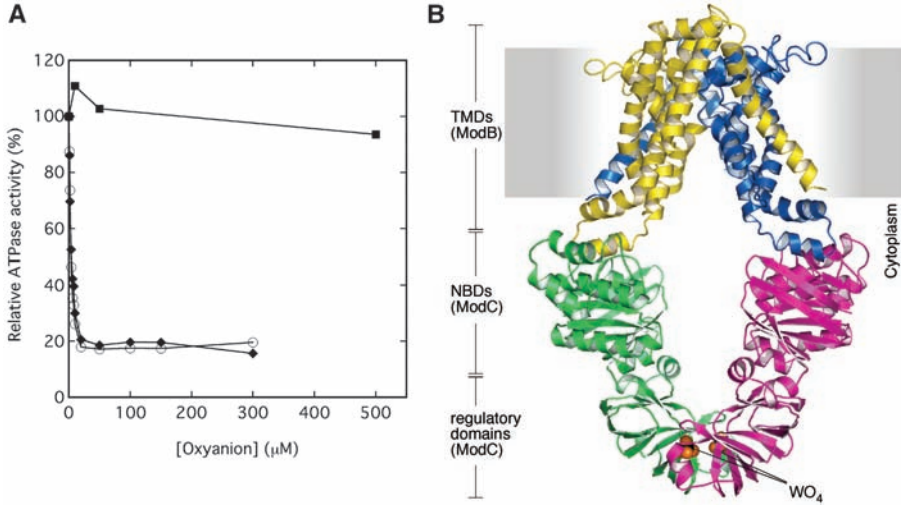

Fig. 1. ATPase activity and crystal structure of *M. acetivorans* ModBC. (**A**) Relative ATP hydrolysis rates of MaModBC in the presence of the oxyanions molybdate (open circles), tungstate (solid diamonds), and sulfate (solid squares). Only molybdate and tungstate are substrates of MaModBC. (**B**) Side view of MaModBC in ribbon representation illustrating the arrangement of the protein subunits. The gray box represents the approximate position of the lipid membrane.

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lybdate will thus lead to an inhibition of the transport activity (and hence to a decrease in molybdate uptake) through both processes. However, not all bacteria contain ModE, and in particular, there does not seem to be a homolog in the genome of *M. acetivorans*. This may indicate that trans-inhibition can substitute for the genetic regulation of the molybdate transporter. Many other organisms such as *E. coli*, *Photorhabdus luminescens*, and *Klebsiella pneumoniae* do contain genes coding for ModE and at the same time feature regulatory domains attached to their ModC subunits (fig. S5), which suggests that molybdate import in these organisms may be regulated both by trans-inhibition and by genetic control of the expression levels.

Another ABC transporter, the maltose importer MalFGK from *E. coli* (*26*), also contains a regulatory domain with a similar fold. However, maltose has not been reported to bind to MalK, nor does it seem to affect the ATPase activity of the isolated NBDs or the full maltose transporter. Instead, the regulatory domains of MalK have been reported to interact with the transcriptional activator MalT (*27*) and with the enzyme IIA component of the glucose phosphotransferase system (*28*). The arrangement of the two regulatory domains of MalK in the crystal structures is distinct from what we observe in MaModBC, and an arrangement similar to that in MaModBC is precluded by a C-terminal extension of 15 amino acid residues in MalK that would cause a steric clash (fig. S4). This difference in arrangement may explain why the activity of MalK is not trans-inhibited by elevated levels of maltose.

Table 1. Relative ATP hydrolysis rates of *A. fulgidus* ModBC free or in the presence of various oxyanions.

Substrate	AfModBC ATPase activity
—	100 ± 4.1%
1 mM Na_2MoO_4	96.3 ± 4.5%
1 mM Na_2WO_4	95.1 ± 3.7%
1 mM Na_2SO_4	101.7 ± 4.5%

Because ATPase activity is a strict requirement for transport in ABC transporters, the inhibition of the ATPase activity of MaModBC by molybdate or tungstate will result in inhibited transport activity. To demonstrate that the inhibitory effect was specific, we identified four conserved residues in MaModBC (Ser^{286}, Thr^{320}, Ser^{323}, and Lys^{340}; Fig. 2C) that serve as hydrogen bond donors in the oxyanion binding pockets. We individually mutated these residues to alanines (except for Thr^{320}, which was mutated to the isoelectronic valine) and tested the sensitivity of the ATPase activity of the resulting mutants to molybdate (Fig. 3). The mutants revealed ATPase activities similar to that of the wild type, but the sensitivity of the hydrolysis rates to molybdate was strongly reduced in two mutants (Ser^{323} → Ala and Thr^{320} → Val) and abolished in the others (Ser^{286} → Ala and Lys^{340} → Ala). A control mutation (Ser^{342} → Ala) of a non-conserved serine residue that is in the vicinity of, but does not provide a hydrogen bond to, the oxyanion, did not have any effect on the inhibitory concentration (Fig. 3). Combined, our functional data demonstrate that molybdate or tungstate binding to the oxyanion pocket at the interface of the regulatory domains allosterically inhibit the ATPase activity, and hence the reaction cycle, of MaModBC.

The transmembrane domains of AfModBC, MaModBC, and MalFGK have similar folds and TM topologies but reveal differences in conformation (Fig. 4A). Whereas MaModBC and AfModBC reveal nucleotide-free, inward-facing conformations, MalFGK is ATP-bound and outward-facing. The two ModBC structures differ in that the trans-inhibited MaModBC reveals increased angles of the two TMDs in a more pronounced inward-facing conformation, which is reflected in a larger distance of the coupling helices relative to AfModBC (Fig. 4A).

On the basis of the crystal structures of full ABC transporters, we have previously proposed a conserved coupling mechanism for ABC transporters (*16*, *29*), which is an adaptation of Jardetzky's alternating access and release mechanism (*30*) and also agrees with other mechanistic models (*31*). The basic two-state schematic suggests that the TMD-formed translocation pathways of ABC transporters adopt an inward-facing conformation when the NBDs are nucleotide-free, and an

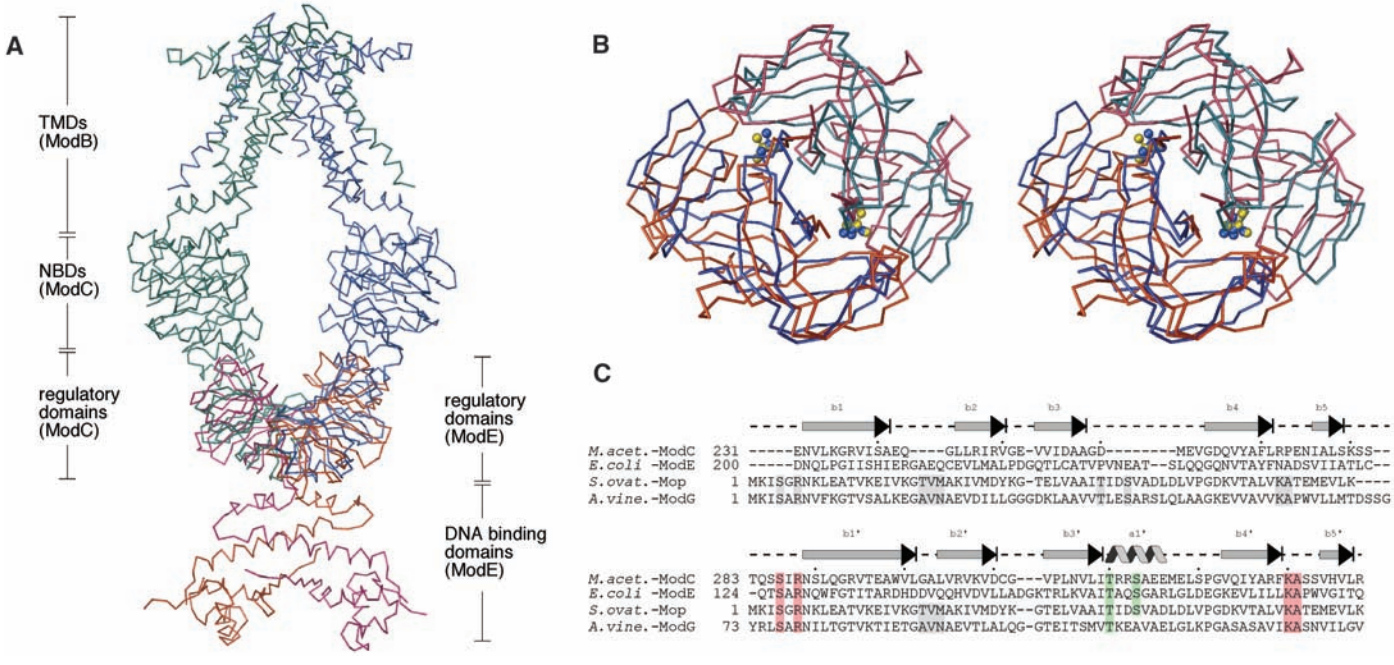

Fig. 2. Comparison of the regulatory domain of MaModC with that of the transcriptional regulator ModE. (**A**) Side view of MaModBC and *E. coli* ModE (PDB code 1o7l) after superposition of the substrate-bound regulatory domains. The MaModBC backbones are colored blue and green, whereas those of ModE are red and purple. (**B**) Stereoview of the regulatory domains of MaModC and ModE, with colors as in (A). Tungstate bound to MaModC is shown in ball-and-stick and colored blue, whereas molybdate bound to ModE is in yellow. (**C**) Structure-based sequence alignment of ModC with ModE and other molybdate-binding proteins of a similar fold. The secondary structure elements above the amino acid sequences (in single-letter code) were derived from MaModC, and residues shaded red and green contribute to the two distinct oxyanion binding pockets. Gray-shaded residues contribute to additional molybdate binding sites in Mop and ModG.

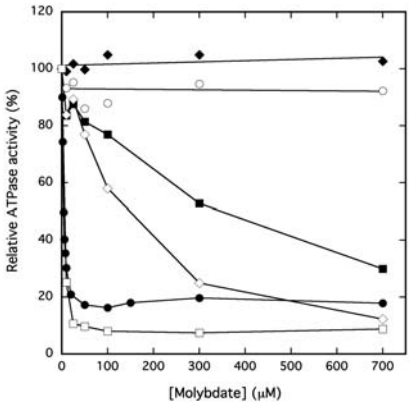

Fig. 3. Inhibition of the ATPase activity of wild-type or mutant MaModBC by molybdate. Relative hydrolysis rates are shown at various concentrations of molybdate and in percent of the uninhibited rates for wild type (solid circles), Ser^{286} → Ala (open circles), Thr^{320} → Val (solid squares), Ser^{323} → Ala (open diamonds), Lys^{340} → Ala (solid diamonds), and the control mutant Ser^{342} → Ala (open squares). All experiments were carried out twice with independent protein preparations.

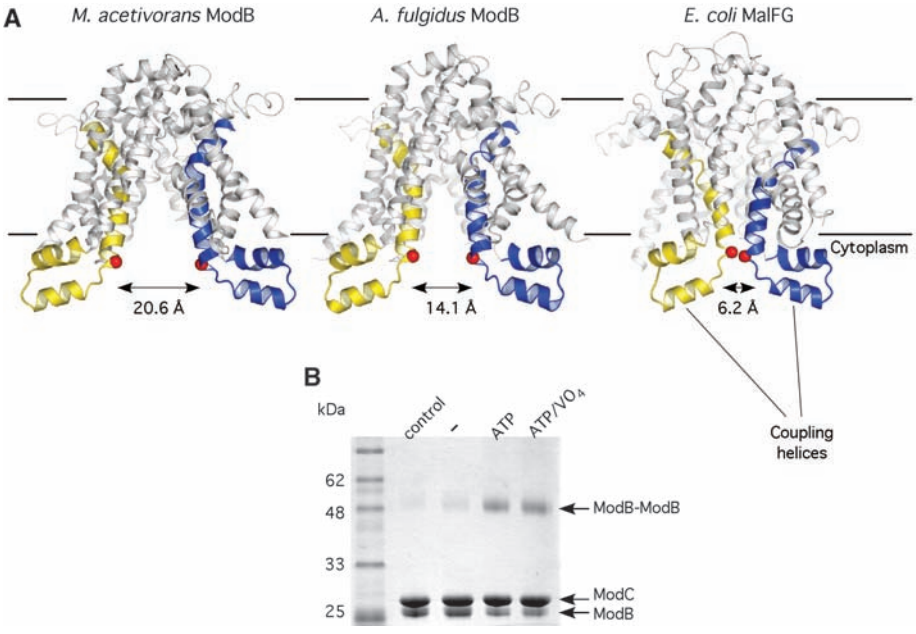

Fig. 4. TMD conformations as observed in the crystal structures of MaModBC, AfModBC, and MalFGK. (**A**) Comparison of the TMDs MaModB, AfModB, and MalFG. The key TM helices 4 and the coupling helices of each transporter are colored yellow and blue, respectively. The Cα atoms of residues MalG 183 and MalF 394 and the equivalent residues in MaModB (165) and AfModB (153) are depicted as red spheres, with the distances indicated. For clarity, only the cores of the TMDs are shown for MalFG, and the NBDs have been removed. (**B**) Chemical cross-linking of engineered cysteine residues at position 153 in AfModB. Cross-linking was performed by $CuCl_2$ in detergent solution, with or without ATP and *o*-vanadate (VO_4). No Cu was added to the control reaction. Protein markers are shown in the left lane, with molecular masses indicated.

outward-facing conformation when the NBDs form a head-to-tail sandwich with tightly bound ATP. If correct, this scheme predicts that the different conformations of the TMDs may be converted into one another, and then trapped using chemical cross-linking, depending on whether ATP is bound to the NBDs. We have thus engineered cysteines into AfModB (the TMD) at the cytoplasmic end of TM 4 at positions that are very close in the structure of MalFGK, but far apart in both ModBC structures (Fig. 4A). The engineered cysteines had no effect on the stability and ATPase activity of AfModBC, which suggests that the mutation had not induced any conformational change in the protein. The cysteines were subsequently reacted with copper, which can promote disulfide formation; this technique was introduced for membrane proteins during studies of the conformational changes in the aspartate receptor (*32*). In contrast to longer chemical cross-linkers, the use of Cu as a catalyst requires the cysteine side chains to be in the immediate vicinity and in an optimal geometrical arrangement for successful disulfide formation. One of our AfModBC mutants (Ser^{153} → Cys) revealed pronounced cross-linking that was dependent on the addition of ATP (Fig. 4B), as evidenced by the increase of a dimeric ModB band and the simultaneous decrease of the monomeric ModB band. The efficiency of the cross-linking was estimated to be above 50%, as assessed from the band intensities of several SDS gels. Such an efficiency is remarkable given the stringent requirements on geometry and the competition of the cross-linking reaction with air oxidation of the cysteines.

The results suggest that the TMDs of AfModBC can adopt a conformation similar to that of MalFGK only when ATP is bound to the NBDs. This demonstrates that binding of ATP to the NBDs is coupled to a conformational change in the TMDs, forcing the cytoplasmic TM helices together and likely converting the entire translocation pathway into an outward-facing conformation. Similar experiments with MaModBC were not successful, possibly because the relative arrangement of the introduced cysteines is not as favorable for efficient disulfide formation or because air oxidation of the cysteines was faster than the cross-linking reaction. Our results nonetheless demonstrate that in the ATP-bound state, the cytoplasmic ends of the transmembrane helices of molybdate ABC transporters approach each other and adopt a conformation similar to the outward-facing state of the maltose transporter.

Our cross-linking data lend further biochemical support to the above-mentioned two-state model of the mechanism of ABC transporters (*29*), which is in agreement with several ABC transporter structures that have been determined in recent years (*33*, *34*). The structure of MaModBC we describe here fits this two-state scenario but adds an important new element. Even though we do not know how the absence of bound molybdate or tungstate will affect the conformation of MaModBC, it will likely remain inward-facing in the absence of nucleotide and will only adopt an outward-facing conformation, similar to that observed in the maltose transporter intermediate, upon binding of ATP. The trans-inhibited MaModBC structure demonstrates that regulatory domains can keep the catalytic domains of the NBDs apart, thereby preventing efficient ATP hydrolysis. Because the NBDs are connected to the TMDs via the coupling helices, the cytoplasmic parts of the TMDs are spread apart as well, which locks the transporter in an inward-facing conformation and prevents the ATP-triggered switch to the outward-facing state. Thus, high cytoplasmic concentrations of free substrate can prevent the transporter from cycling through the alternating access and release mechanism. Even though the details of substrate binding may differ in other families of transporters, it is likely that trans-inhibition in such proteins is based on similar effects.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1156213/DC1
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The High-Affinity *E. coli* Methionine ABC Transporter: Structure and Allosteric Regulation

Neena S. Kadaba, Jens T. Kaiser, Eric Johnson, Allen Lee, Douglas C. Rees*

The crystal structure of the high-affinity *Escherichia coli* MetNI methionine uptake transporter, a member of the adenosine triphosphate (ATP)–binding cassette (ABC) family, has been solved to 3.7 angstrom resolution. The overall architecture of MetNI reveals two copies of the adenosine triphosphatase (ATPase) MetN in complex with two copies of the transmembrane domain MetI, with the transporter adopting an inward-facing conformation exhibiting widely separated nucleotide binding domains. Each MetI subunit is organized around a core of five transmembrane helices that correspond to a subset of the helices observed in the larger membrane-spanning subunits of the molybdate (ModBC) and maltose (MalFGK) ABC transporters. In addition to the conserved nucleotide binding domain of the ABC family, MetN contains a carboxyl-terminal extension with a ferredoxin-like fold previously assigned to a conserved family of regulatory ligand-binding domains. These domains separate the nucleotide binding domains and would interfere with their association required for ATP binding and hydrolysis. Methionine binds to the dimerized carboxyl-terminal domain and is shown to inhibit ATPase activity. These observations are consistent with an allosteric regulatory mechanism operating at the level of transport activity, where increased intracellular levels of the transported ligand stabilize an inward-facing, ATPase-inactive state of MetNI to inhibit further ligand translocation into the cell.

The high-affinity uptake of methionine by *Escherichia coli* is mediated by the MetD system (*1*, *2*), a member of the methionine uptake transporter family (*3*) of adenosine triphosphate (ATP)–binding cassette (ABC) transporters (*4*–*8*). ABC transporters such as MetD translocate substrates across the membrane, a process that is coupled to the hydrolysis of ATP by a cytoplasmic nucleotide binding domain (NBD). Architecturally, an ABC transporter contains four domains: two NBDs responsible for the interactions with ATP and two transmembrane domains (TMDs) that form the translocation pathway; the NBD and TMD components of the *E. coli* MetD system have been identified as MetN and MetI, respectively (*9*, *10*). Substrate translocation by ABC transporters may be described in terms of an alternating access model that involves the interconversion of outward- and inward-facing conformations driven by the binding and hydrolysis of ATP by the NBDs. Additional domains of diverse structure may be fused to the TMDs or NBDs to regulate transport activity (*11*); examples include ionic strength sensing by OpuA (*12*), the inducer exclusion phenomenon mediated by MalK (*13*), and post-translational modifications of the cystic fibrosis transmembrane conductance regulator (*14*). Structural studies of the intact ABC importers for vitamin B_{12} (BtuCD) (*15*, *16*) and the homologous HI1470/71 (*17*), molybdate (ModBC) (*18*) and maltose (MalFGK) (*19*), and the exporters Sav1866 (*20*) and MsbA (*21*) have established the basic molecular architecture of ABC transporters and provided structural models for some of the states of the transport cycle.

The MetD system was originally identified as an importer of both L- and D-methionine, either of which may be used as a source of methionine by *E. coli* (*2*, *22*). MetD is additionally capable of transporting L-selenomethionine and other methionine derivatives. Early studies by Kadner (*23*) established the phenomenon of trans-inhibition in this system, whereby uptake of external methionine is inhibited by intracellular methionine levels in a fashion consistent with the direct action of methionine on the transporter. To further investigate this system, we have determined the crystal structure at 3.7 Å resolution of the *E. coli* methionine ABC transporter MetNI solubilized in the detergent dodecylmaltoside (*24*). Together with kinetic studies, the crystallographic analyses indicate that the binding of methionine to the C-terminal domain of the ABC subunits stabilizes an inward-facing, adenosine triphosphatase (ATPase)–inactive conformation of the transporter. Furthermore, these analyses provide a structural basis for the regulatory properties discovered by Kadner.

The overall architecture of MetNI consists of two copies of the ATPase MetN in complex with two copies of the TMD MetI, with the transporter adopting an inward-facing conformation having the TMDs open to the cytoplasm and the NBDs widely separated (Fig. 1). The folds of MetN and MetI resemble those previously observed in the ModBC (*18*) and MalFGK (*19*) transporters, with three principal differences: (i) The TMD of the methionine transporter is smaller, as each MetI subunit contains only five transmembrane helices per monomer. (ii) The structures of the methionine, molybdate, and maltose transporters represent a progression of inward-facing conformations of the translocation pathway that vary from wide open to closed, respectively. (iii) The MetN ABC subunits contain a C-terminal extension that is distinct from those present in the maltose and molybdate transporters and that separates the NBDs in this inward-facing conformation.

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Each MetI subunit is organized around a core of five transmembrane helices that correspond to a subset of the helices observed in the larger membrane-spanning subunits of the molybdate and maltose ABC transporters; the membrane-spanning ModB, MalF, and MalG subunits in those transporters contain 6, 8, and 6 helices, respectively, with the common set corresponding to the five most C-terminal helices in these structures (Fig. 2A). The root mean square deviations (RMSDs) between equivalent Cα positions in MetN and these other subunits are ~2.5 Å. The region of highest sequence and structural similarity between these structures coincides with the "coupling helix" and the two transmembrane helices on either side (TM3 and TM4 in MetI). This region forms the core of the distinct conformations of the translocation pathway observed in the methionine, molybdate, and maltose transporters (Fig. 2B), with the coupling helix [including the consensus "Glu-Ala-Ala (EAA)" motif (*25*, *26*)] contributing substantially to the interface between the TMD and NBD subunits. The sequence and structural homologies extend to TM2 in these structures, including the relatively short length of this membrane-spanning helix (~14 residues) and the kinking at Pro^{67} near the periplasmic interface; these irregular features are not uncommon near the translocation pathways of various transporters (*17*). One distinctive aspect of MetI is that it contains an odd number of transmembrane helices, so that the N and C termini are on opposite sides of the membrane (the periplasm and cytoplasm, respectively); this arrangement contrasts with all other structurally characterized ABC transporters that possess an even number of transmembrane helices per TMD and have both termini in the cytoplasm.

The MetN ABC subunit consists of two domains—(i) the conserved NBD (residues 1 to 230), homologous to other ABC subunits (RMSDs to ModC and MalK ~1 Å), and (ii) the C-terminal C2 domain (residues 265 to 343)—that are connected by a linker of ~35 residues. Although the pair of NBDs are approximately related by a twofold axis, as are the pair of C2 domains, these axes differ in orientation by ~20°, which generates a pronounced asymmetry in the transporter structure (Fig. 1B). The distinct orientations of the rotation axes relating the NBD pairs and the C2 domain pairs within a transporter are accommodated through different conformations of the linker separating the NBD and C2 domains in the two MetN subunits. The C2 domain exhibits a ferredoxin-like fold with a four-stranded antiparallel β sheet (Fig. 3A) that belongs to the aspartokinase-chorismate mutase-TyrA (ACT)–domain family, proposed to represent a conserved regulatory ligand binding fold (*27*–*29*). As with other members of this family, the C2 domains from each MetN subunit in a transporter dimerize to form an eight-stranded β sheet; a similar arrangement is also observed in the structure of the isolated C2 domain determined in the course of this project (*24*).

Because ATP binds to conserved sequence motifs at the interface between two closely juxtaposed NBDs (*30*–*33*), the crystallographically observed inward-facing conformation of the MetNI transporter corresponds to an ATPase-inactive state. The extent of separation of the NBDs in this structure relative to other ABC importers (and the correlation between NBD separation and the conformation of the translocation pathway) may be assessed from the intersubunit distances between the P loops and signature motifs of different ABC subunits. Using the Cα residues of MetN residues Gly^{43} and Gly^{143} to define the positions of the P loop and signature motifs, respectively, the intersubunit distances between these two motifs are found to be between 26 and 30 Å (the range reflects the asymmetry of this complex). In comparison, the corresponding distances in the maltose and molybdate transporter structures are ~11 and ~16 Å, respectively (based on the intersubunit distances between residues Gly^{36} and Gly^{129} of ModB and Gly^{41} and Gly^{136} of MalK, respectively), and were previously reported as 14 and 16 Å in the structures of BtuCD and HI1470/71 (*17*). The short 11 Å intersubunit distance observed in the maltose transporter between the P loop and the signature motif reflects both the closed interface between the NBDs and that the translocation pathway is closed to the cytoplasm. Whereas BtuCD has a somewhat larger separation (14 Å) between NBDs than that observed in the maltose transporter, the translocation pathway is also closed to the cytoplasm. Although the intersubunit separation between NBDs is only 2 Å longer in HI1470/71 and the molybdate transporter, the translocation pathways in both structures are open to the cytoplasm, suggesting that this represents a critical parameter relating the relative positioning of the NBDs to the conformation of the translocation pathway. The ~28 Å distance observed in the methionine structure is substantially larger than the previously reported NBD separations in ABC importers and suggests that the positioning of the C2 domain dimer is crucial for stabilizing this arrangement.

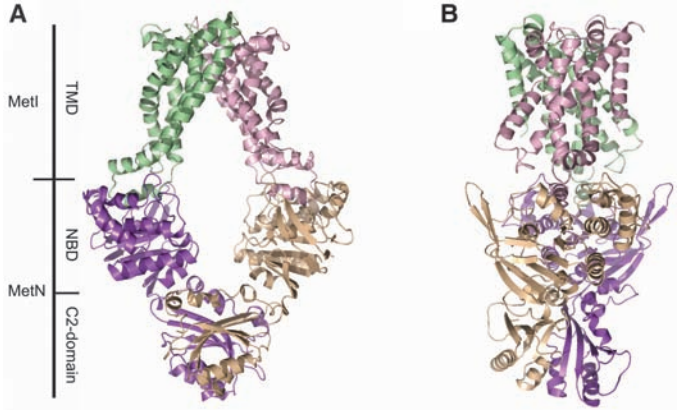

Fig. 1. (**A**) ABC transporter MetNI consists of four subunits: two membrane-spanning MetI subunits (green and pink) and two MetN ABC subunits (purple and tan). The molecular rotation axis relating the MetI subunits is vertical, with the cytoplasmic (inward)–facing surface of the transporter toward the bottom. The C2 domains forming the dimer interface between MetN subunits are at the bottom. (**B**) View of MetNI rotated ~90° about the vertical axis from that of (A), illustrating the asymmetrical orientation of the C2 domains relative to the MetI subunits and the MetN NBDs. This figure was prepared and rendered with PyMOL (*39*).

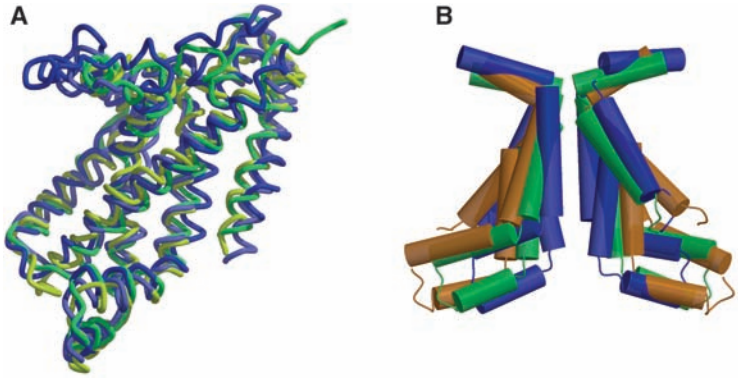

Fig. 2. (**A**) Superposition of the five membrane-spanning helices forming the common core of the TMDs of MetI (yellow), ModB (green), MalF (dark blue), and MalG (light blue). TM1 (using the MetI number) is oriented diagonally across the front, whereas remaining helices are arranged in the sequence TM2 to TM5, from left to right. The coupling helices between TM3 and TM4 are the helical elements at the bottom of the figure. (**B**) Comparison of the TM2-TM3-TM4 helices lining the translocation pathways of MetI (brown), ModB (green), and MalFG (blue), illustrating the progressive narrowing of cytoplasmic opening of the translocation pathway in the sequence from the methionine to molybdate to maltose transporters. The views in this figure are from the membrane, with the cytoplasmic surface oriented down. Figures 2 and 3 were prepared with MOLSCRIPT and RASTER3D (*40*, *41*).

To identify potential methionine binding sites, we soaked crystals of the wild-type methionine transporter in 1 mM L-selenomethionine for 2 hours and collected diffraction data to 5.2 Å resolution at the selenium edge. Anomalous difference Fourier maps revealed two binding sites for selenomethionine located near the dimer interface between C2 domains (Fig. 3A). The binding sites are located near the side chains of Met^{301} and Met^{312} (fig. S2), although the resolution was insufficient to permit a detailed modeling of the bound selenomethionine. The presence of ligand binding sites near the dimer interface parallels observations on other ACT domain containing proteins (*29*). The binding of serine to allosteric sites at the interface between ACT domains of phosphoglycerate dehydrogenase results in a sequence of conformational changes that are propagated to distinct catalytic domains (*34*). Evidence for the flexibility of the corresponding interface in MetNI is provided by a ~15° twist in the relative orientations between C2 domains present in the transporter and in free form (Fig. 3B), along with rearrangements of several loops, that could plausibly be influenced by the binding of methionine.

The juxtaposition of the methionine binding C2 domains between the NBDs in the present structure suggests they may be relevant to the transinhibition mechanism established by Kadner (*23*). To test this proposal, we characterized the effect of methionine and methionine derivatives on the ATPase activity of a detergent solubilized transporter. Although this assay does not directly monitor the influence of methionine on transport activity, it is nevertheless appropriate to assess the ability of methionine to stabilize an ATPase-inactive conformation of the transporter. As detailed in Fig. 4, L-methionine does inhibit the ATPase activity, with half-maximal inhibition occurring at ~30 μM. L-selenomethionine is an even more potent inhibitor, with half-maximal inhibition occurring at ~10 μM, whereas D-methionine has little effect. As controls, truncation of the C2 domain—although reducing ATPase activity under the assay conditions by ~60%—eliminates the inhibitory effects of methionine, whereas the $Glu^{166} \rightarrow Gln^{166}$ mutant in the NBD [corresponding to a mutation shown to prevent ATP hydrolysis in other systems (*32*)] exhibits little ATPase activity. These results are consistent with a model where an equilibrium exists between conformational states of MetNI differing in the extent of NBD displacement. Despite their separation in the crystal structure of detergent solubilized MetNI, activity assays demonstrate that these domains can associate to form an ATPase competent state. As methionine binds to the C2 domains, the equilibrium shifts toward transporter conformations with separated NBDs, and as a consequence, the rate of ATP hydrolysis decreases.

The structure of MetNI described here provides the first crystallographic analysis of a member of the methionine uptake transporter family (*3*) of the superfamily of ABC transporters. Beyond the relevance for the mechanistic understanding of this family, the MetNI structure provides two general insights into the structural organization of ABC importers: (i) a minimal core of five transmembrane helices is identified that may be elaborated by additional helices in other transporters and (ii) a mechanistic basis is proposed for the regulation of transport activity by domains fused to the core transporter. The binding of methionine by the C2 domain at the C terminus of the MetN subunit effectively stabilizes the inward-facing, ATPase-inactive conformation of the transporter. By sterically interfering with NBD association, the ATP-driven engine that powers transport will be disrupted, thereby providing a molecular mechanism for Kadner's observation that increasing levels of internal methionine inhibit transport. The ability of methionine, and particularly selenomethionine, to inhibit transport further suggests that the deletion of the C2 domain from the endogenous methionine transporter of *E. coli* strains used for selenomethionine labeling may increase the incorporation efficiency by allowing uptake to a higher intracellular concentration. Of a more general consideration, transporter activity can represent a major component of the ATP requirement of bacteria (*35*, *36*). Consequently, it is not surprising that transporters can be regulated at the level of protein function to more efficiently control the allocation of cellular energy resources. The structure of the MetNI ABC transporter provides a foundation for the characterization of regulatory mechanisms relevant to the uptake of methionine and also for how these processes are more generally integrated with cellular metabolism.

While this paper was in preparation, the report by Gerber *et al*. (*37*) was submitted, detailing the structural basis of trans-inhibition of the ModBC molybdate ABC transporter from *Methanosarcina acetivorans*; although the molybdate/tungstate binding C-terminal regulatory domain of ModC has a distinct fold from the methionine binding C2 domain of MetN, the consequences for transporter conformation are quite similar.

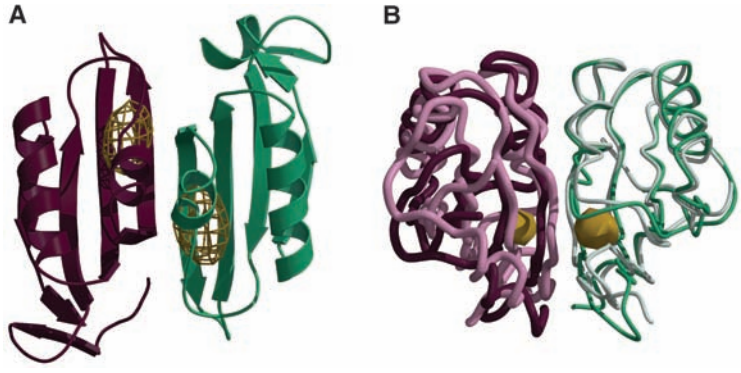

Fig. 3. (**A**) Anomalous difference Fourier map, calculated at 5.2 Å resolution, illustrating the binding of selenomethionine to the C2 domains of MetNI after a 1 mM soak of transporter crystals. The electron density is contoured at six times the SD of the map. (**B**) Comparison of the relative orientations of C2 domains observed in the MetNI and free MetN-C2 structures, after superposition of one subunit in each dimer. The maroon and teal traces correspond to the C2 domain dimer in MetNI, whereas the pink and light gray traces represent the isolated C2 domain structure. The binding site for selenomethionine is denoted by the gold surface. The view is roughly perpendicular about the horizontal axis from that in (**A**).

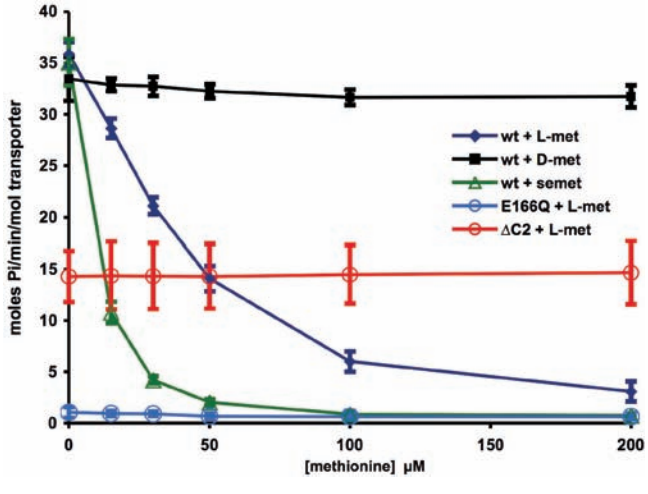

Fig. 4. Dependence of the ATPase activity of various MetNI constructs on the concentration of methionine analogs. The ATPase activity was measured from the rate of phosphate production as assayed by the method of Webb (*42*) and converted to moles of phosphate per minute per mole of transporter. An initial ATP concentration of 1 mM was used, which is about three times the apparent Michaelis constant (fig. S3). The blue diamonds, black squares, and open green triangles correspond to L-methionine, D-methionine, and L-selenomethionine, whereas the open blue and red circles represent the effect of L-methionine on the $Glu^{166} \rightarrow Gln^{166}$ (ATPase-inactive mutant) and ΔC2-MetNI truncation mutants, respectively. Error bars represent SDs calculated from eight measurements.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5886/250/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 and S2
References

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Structural Basis for Specific Substrate Recognition by the Chloroplast Signal Recognition Particle Protein cpSRP43

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Secretory and membrane proteins carry amino-terminal signal sequences that, in cotranslational targeting, are recognized by the signal recognition particle protein SRP54 without sequence specificity. The most abundant membrane proteins on Earth are the light-harvesting chlorophyll a/b binding proteins (LHCPs). They are synthesized in the cytoplasm, imported into the chloroplast, and posttranslationally targeted to the thylakoid membrane by cpSRP, a heterodimer formed by cpSRP54 and cpSRP43. We present the 1.5 angstrom crystal structure of cpSRP43 characterized by a unique arrangement of chromodomains and ankyrin repeats. The overall shape and charge distribution of cpSRP43 resembles the SRP RNA, which is absent in chloroplasts. The complex with the internal signal sequence of LHCPs reveals that cpSRP43 specifically recognizes a DPLG peptide motif. We describe how cpSPR43 adapts the universally conserved SRP system to posttranslational targeting and insertion of the LHCP family of membrane proteins.

Most membrane proteins are cotranslationally inserted into membranes by the signal recognition particle (SRP) acting as an adaptor between the ribosome and the translocation channel. Although the composition of SRP varies, the ribonucleoprotein core of SRP and its guanosine triphosphate (GTP)–dependent interaction with a membrane-bound SRP receptor is universally conserved (*1*, *2*). Helix 8 of the SRP RNA, one of the most conserved RNA structures known, was shown to play an essential, catalytic role in canonical SRPs (*3*, *4*). It accelerates the rate of SRP-receptor complex formation, it significantly stimulates the guanosine triphosphatase (GTPase) activity of the SRP-receptor complex, and the activation can be ascribed to the so-called "tetraloop" (*3*, *4*). SRP of chloroplasts (cpSRP) differs strikingly from these canonical SRP systems (*5*). cpSRP lacks the SRP RNA (*6*) and targets both chloroplast-encoded and nucleus-encoded substrates to the thylakoid membrane. Chloroplast-encoded substrates are cotranslationally targeted to the thylakoid membrane by the interaction of chloroplast ribosomes and cpSRP54 (*7*), whereas the nucleus-encoded LHCP substrates are imported into the chloroplast stroma and are posttranslationally targeted to the thylakoid membrane. LHCPs serve as antenna systems in photosynthesis (*8*, *9*). They contain three trans-membrane helices (TM1 to 3) to which chlorophyll and carotenoid cofactors bind during insertion into the thylakoid membrane (*10*). In the stroma, LHCPs and cpSRP form the transit complex, which allows the transport of the hydrophobic LHCPs to the thylakoids in an insertion-competent conformation (*11*). In this posttranslational targeting system, cpSRP forms a heterodimer consisting of cpSPR54, the chloroplast homolog of the SRP core protein, and cpSRP43, a protein specifically dedicated to this targeting complex (*12*, *13*).

cpSRP43 is a multidomain protein of 43 kD with a unique arrangement of chromodomains (CD1 to 3) and ankyrin repeats (Ank1 to 4) (Fig. 1A) that provide a robust scaffold for protein interactions (*14*–*17*). Previously, only structures determined by nuclear magnetic resonance (NMR) spectroscopy of isolated chromodomains of cpSRP43 were available (*18*). The crystal structure of cpSRP43 from *Arabidopsis thaliana* (residues 85 to 267) was determined at 1.5 Å resolution (*19*) (table S1). The structure contains the N-terminal chromodomain CD1 (residues 85 to 129) and four ankyrin repeats (Ank1 to 4, residues 130 to 267), which together form an elongated horseshoe common to ankyrin-repeat proteins (Fig. 1, B and C) (*16*, *20*). Although Ank2 and Ank3 have the typical fold of ankyrin

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repeats with a helix-turn-helix motif, the flanking Ank1 and Ank4 (Cap-repeats) contain unusual elongated helices. CD1 displays the classical chromodomain fold known from Polycomb (*21*) and other CD-containing proteins (*15*), which is built from a central three-stranded antiparallel β sheet supported by a perpendicular α helix on the concave surface of the β sheet. CD1 in the crystal structure differs significantly from the NMR structure (*18*) and forms a tight interface with the ankyrin repeats of about 240 Å^2. CD1 and Ank1 are connected by the fusion of the CD helix with the N-terminal ankyrin helix (α1a/b), making it difficult to determine the domain boundaries. The most striking deviation from the canonical ankyrin-repeat structure is, however, observed in Ank4. Here, both α helices (α7 and α8) are elongated by an extension of 16 residues that protrude from the convex side of the horseshoe. The high conservation of this extension suggests a role as a protein-protein interaction site.

Because the SRP RNA is absent in chloroplasts, cpSRP43 (isoelectric point 4.4) might be a structural and functional substitute for SRP RNA. Molecular mimicry between RNA and protein has been observed before with the complex formed by transfer RNA and the *Thermus aquaticus* elongation factor Tu (tRNA/EF-Tu) and EF-G of the translation apparatus representing the most prominent example (*22*). The analysis of the cpSRP43 surface shows high sequence conservation (fig. S1). One side exposes two predominantly hydrophobic grooves (grooves 1 and 2), which are separated by a positive ridge (Fig. 1D). The grooves may be involved in the interaction with LHCPs and/or cpSRP54. The opposite surface of cpSRP43 is highly negatively charged (Fig. 1E), with an intriguing regular spacing of negative charges of about 5.5 Å and 18 Å, reminiscent of the backbone phosphate groups of an RNA double helix. This pattern extends over the convex side of the ankyrin horseshoe. Overall, the shape and surface charge of cpSRP43 resembles helix 8 of the SRP RNA (fig. S2) (*23*), which binds to SRP54 in canonical SRPs and plays a catalytic role (*3*, *4*). cpSRP43 also interacts with cpSRP54 (*11*, *12*), but the molecular details of this interaction are not known. It induces only a moderate stimulation of the GTPase activity of the cpSRP54-receptor complex (*24*). Compared with cytosolic SRPs, a higher intrinsic rate of complex formation between cpSRP54 and cpFtsY (the chloropast receptor) was observed, which allows the requirement for the SRP RNA to be bypassed (*25*). Preliminary data indicate that cpSRP43 does not further accelerate complex formation (*25*). Therefore, cpSRP43 may only partially substitute for SRP RNA function. In cotranslational targeting with a translating ribosome present, cpSPR43 and the SRP RNA seem dispensable, whereas in posttranslational targeting, cpSRP43 is crucial for the formation of the transit complex.

In the transit complex, cpSRP43 binds to the L18 region, a highly conserved region in the stromal loop connecting TM2 and TM3 [residues 152 to 169 of Lhcb1 (Fig. 2A)] (*12*, *26*), whereas cpSRP54 interacts with the TM regions of LHCP with a possible preference for TM3 (*12*, *27*). The TM regions of LHCPs were shown to contribute to, but not to be sufficient for, pro-

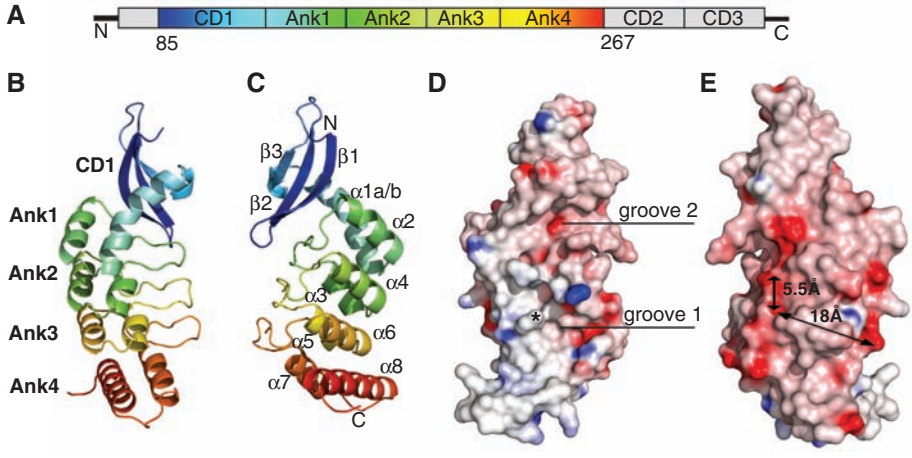

Fig. 1. Structure of cpSRP43. (**A**) Scheme of the domain structure of cpSRP43 with chromodomains (CD1 to 3) and ankyrin repeats (Ank1 to 4). Domains present in the crystal structure are given by residue numbers and are indicated in rainbow colors. (**B**) Side view of cpSRP43 in ribbon representation. The domains are labeled. (**C**) Side view of cpSRP43 [90° rotation with respect to (B)]. Secondary structure elements are numbered. The N- and C-termini are labeled. (**D**) Front view of cpSRP43. The surface representation shows two hydrophobic grooves separated by a positive ridge. The molecular surface is colored blue and red according to positive and negative electrostatic potential, respectively. The asterisk highlights Tyr^{204} in Ank3. (**E**) Back view of cpSRP43 [same view as in (C)] showing the highly negatively charged surface with a spacing of negative charges reminiscent of RNA (see fig. S2).

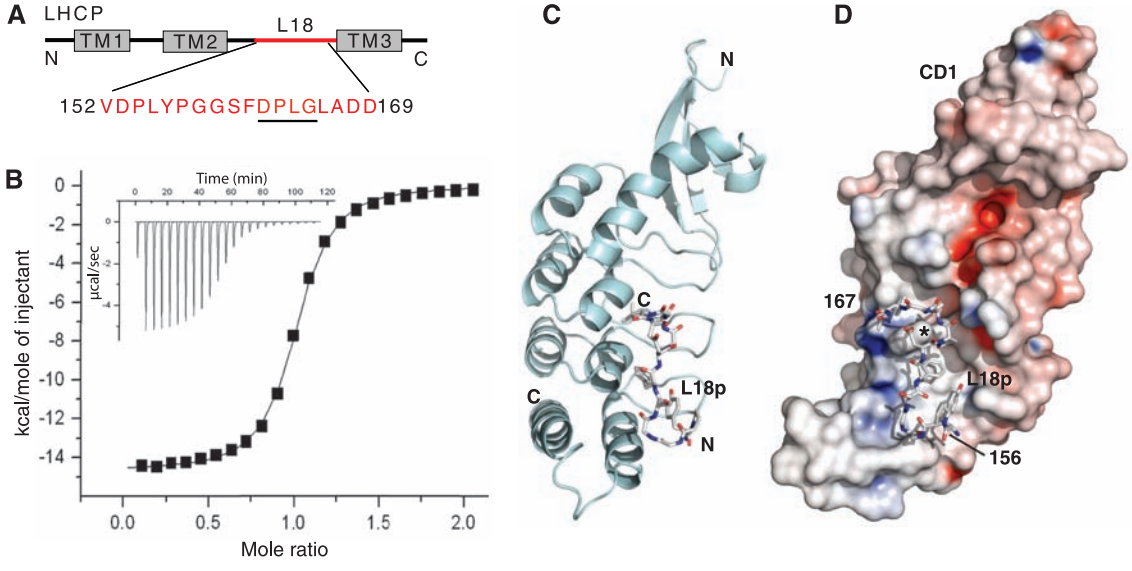

Fig. 2. Structure of the cpSRP43/L18p complex. (**A**) LHCP topology with three transmembrane helices (TM1 to 3). The sequence of the L18 region is given for the major LHCP, Lhcb1 from *Pisum sativum*, which was used in this study (red, the DPLG motif is underlined). TM3 starts immediately after the L18 region. (**B**) Typical isothermal titration calorimetry (ITC) experiment of the cpSRP43 interaction with L18p. (**C**) Ribbon representation of cpSRP43 (blue) with bound L18p (as a ball-and-stick model, gray). The N- and C-termini are indicated. (**D**) Surface representation of the cpSRP43/L18p complex. The peptide (labeled by residue numbers) binds in the hydrophobic groove 1. Four residues at the N terminus and two residues at the C terminus are not resolved.

moting transit complex formation. However, the L18 region is strictly required (*26*, *28*). In order to characterize this complex in more detail, we performed binding studies of cpSRP43 variants with a synthetic L18 peptide (L18p) using isothermal titration calorimetry (ITC). A high-affinity interaction with a 1:1 stoichiometry was observed (Fig. 2B and fig. S3). Because a truncated cpSRP43 construct comprising only CD1/Ank1 to 4 is sufficient for binding L18p without a significant drop in affinity (fig. S3), it was used for cocrystallization. The crystal structure of cpSRP43 in complex with L18p (Fig. 2C) is very similar to the free cpSRP43 (table S1). L18p binds to groove 1 across the inner (concave) surface of Ank2 to 4 (Fig. 2, B and C). It interacts with the ankyrin repeats at a similar location, as recently described for an ankyrin-repeat protein involved in transcriptional control (fig. S3) (*29*). L18p adopts an extended conformation except for a DPLG signature motif (Asp-Pro-Leu-Gly) typical for type I turns (residues 162 to 165) (Fig. 3A and fig. S4). The structure of the DPLG motif bound to cpSPR43 is basically identical to the one observed in the LHCP crystal structures (*8*, *9*) and in other proteins containing a DPLG motif (*30*, *31*), which emphasizes the stability of this structural element (fig. S5). We characterized the importance of the DPLG motif for the cpSRP43/L18p interaction by ITC with truncated and mutated L18 peptides (Fig. 3B and fig. S3). The motif is essential for binding to cpSRP43, as no interaction was observed in its absence. Replacement of Leu^{164} by Lys also results in a loss of binding to cpSRP43 (Fig. 3B). Although the replacement of the leucine in the DPLG motif does not alter the conformation of the type I turn (fig. S5), the long and charged side chain of a lysine cannot be accommodated in the hydrophobic binding groove. To test the functional relevance of this observation, membrane insertion of LHCP was analyzed by in vitro thylakoid import assays (Fig. 3C) (*32*). Wild-type LHCP is efficiently inserted as judged by the presence of a characteristic protease-resistant fragment [asterisk (Fig. 3C)], but the Leu^{164}Lys mutant of LHCP does not insert into thylakoids at all. Therefore, the recognition of the DPLG motif by cpSPR43 is essential for the formation of a productive transit complex.

The structural requirements for DPLG recognition are reflected by the specific properties of groove 1 with a pronounced bend, marked by Tyr^{204} of Ank3. The DPLG motif wraps around Tyr^{204} (Fig. 3A), and the replacement of Tyr^{204} by alanine abolishes the interaction with L18p (Fig. 3B). Adjacent positively charged residues seem to stabilize the interaction with L18p. Replacement of the arginines Arg^{161}, Arg^{192}, and also Arg^{226} by alanines (fig. S4) results in increased dissociation constants (fig. S3). Arg^{161} is in direct contact with L18p and, therefore, shows the strongest effect. Sequence analysis of FCPs (fucoxanthine chlorophyll a/c–binding proteins) of diatoms shows that, in this family of antenna complexes, the DPLG motif is present at the N terminus (fig. S6) (*33*). FCPs are also targeted by cpSRP, which further supports the role of the DPLG motif as a specificity determinant for transit complex formation. In LHCPs, the DPLG motif is part of a conserved interaction site for carotenoids (*9*), which are crucial for LHCP folding (*10*). When the cpSRP43/L18p and LHCP structures are superimposed (fig. 5A), Tyr^{204} superimposes with the carotenoid head group. Therefore, the interactions in the transit complex might be a prerequisite for the attachment of cofactors during membrane insertion.

cpSRP43 forms a stable heterodimer with cpSRP54 also in the absence of LHCP (*12*). Whereas the third chromodomain (CD3) is dispensable for the interaction with cpSRP54, CD2 is strictly required (*34*). Although CD2 is not present in the crystal structure, its location with respect to CD1 and the ankyrin repeats can be inferred from crystal symmetry (Fig. 4A and fig. S7). In different crystals obtained for cpSRP43 (*19*) CD1 always packs against the extension of Ank4 (helix α8) protruding from a symmetry-related molecule. A contact of about 270 $Å^2$ is formed by this interaction. Two conserved, surface-exposed hydrophobic residues (Phe^{249} and Ile^{253}) within helix α8 form the core of this interaction. Phe^{249} is accommodated in an aromatic cage (fig. S7), which is reminiscent of the interaction between chromodomains and methylated lysines in chromatin remodeling (*21*). We therefore propose that the conserved extension of Ank4 serves as binding site for CD2 of cpSRP43 (Fig. 4B). CD2 was recently shown to interact with an RRKR peptide from the C terminus of cpSRP54 (*34*). Together, these data prompt us to propose a model for the interaction between cpSRP54 and cpSRP43 in which the signal sequence–binding site located in the M domain of cpSRP54 would be positioned in the immediate vicinity of the L18 binding site in cpSRP43.

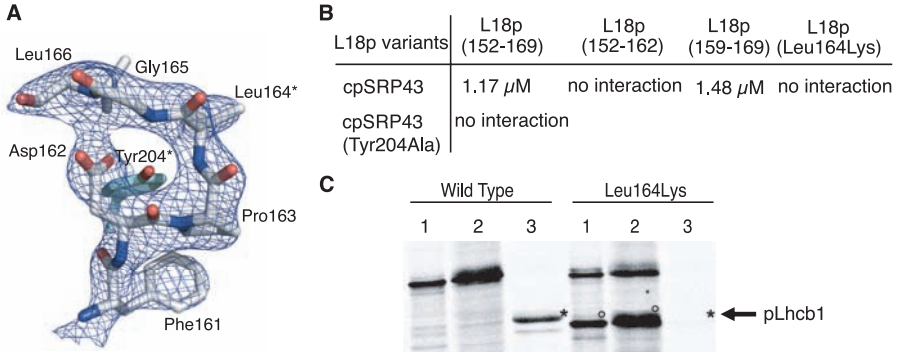

Fig. 3. Specific recognition of the DPLG motif by cpSRP43. (**A**) Close-up view of the DPLG motif wrapped around the Tyr^{204} hook. The peptide is shown together with a $2mF_{obs} - DF_{calc}$ electron density contoured at 2 σ. Residues essential for binding as tested by mutagenesis [see (B)] are marked with an asterisk. (**B**) Summary of ITC data. The DPLG motif within L18p is essential for cpSRP43 binding. The Tyr^{204}Ala mutation in cpSRP43, as well as the Leu^{164}Lys mutation in L18p, totally abolish the interaction (see fig. S3). (**C**) Thylakoid import assay. Wild-type pre-Lhcb1 (pLhcb1) and the Leu^{164}Lys mutant were synthesized by in vitro translation (lanes 1), incubated with pea thylakoids (lanes 2) and treated with trypsin (lanes 3). The asterisks (*) mark the protease-protected fragment of membrane-inserted p-Lhcb1, which is absent in the Leu^{164}Lys mutant. Note: The Leu^{164}Lys mutant was in vitro translated in the presence of a suppressor Lys-$tRNA^{amber}$, which always yields a mix of full-length protein (pLhcb1) and truncated protein (marked by ○) (*19*).

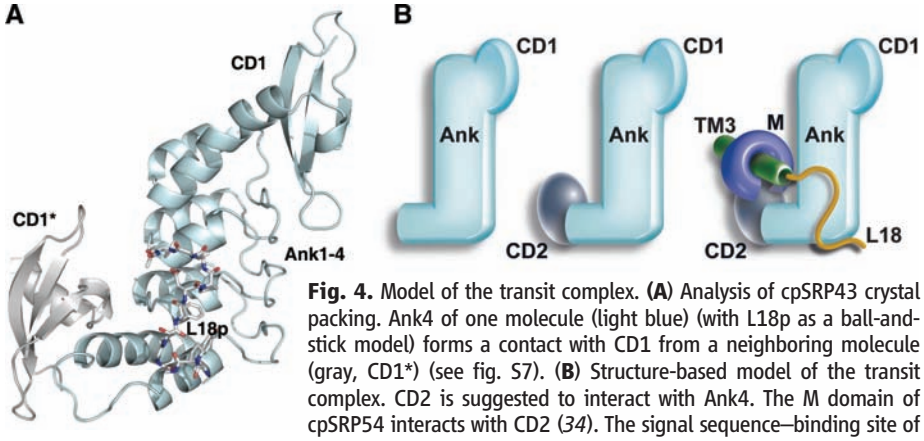

Fig. 4. Model of the transit complex. (**A**) Analysis of cpSRP43 crystal packing. Ank4 of one molecule (light blue) (with L18p as a ball-and-stick model) forms a contact with CD1 from a neighboring molecule (gray, CD1*) (see fig. S7). (**B**) Structure-based model of the transit complex. CD2 is suggested to interact with Ank4. The M domain of cpSRP54 interacts with CD2 (*34*). The signal sequence–binding site of the M domain might preferentially bind TM3 of LHCPs (*12*, *27*) and should therefore be in close proximity to L18 in groove 1 of cpSRP43.

Thereby, TM3 and the L18 region of LHCPs could be recognized simultaneously as suggested before (*12*). The location of the DPLG motif within LHCPs might be critical to position TM3 in a way that it can interact with cpSPR54. Although it cannot be predicted how TM1 and TM2 of LHCP are bound in the transit complex or where the GTPase domain of cpSRP54 would be located, the model presented here provides the basis for further experiments to characterize these interactions.

LHCPs are multispanning membrane proteins and present in high amounts in the thylakoids. The specific requirements for efficient, posttranslational LHCP targeting and insertion have been met by recruiting cpSRP54 to the transit complex. The subsequent interaction between cpSRP54 and cpFtsY allows the use of the conserved downstream components of SRP-mediated membrane targeting. Although signal sequences are usually recognized without sequence specificity in both co- and posttranslational targeting (*35*), cpSPR43 adds specificity and provides additional interaction sites. The formation of the transit complex may compensate for the absence of the ribosome with cpSRP acting as a chaperone for the hydrophobic regions of the LHCPs before membrane insertion. Taken together, we show how posttranslational membrane insertion is accomplished by the adaptation of a universally conserved machinery.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5886/253/DC1
Materials and Methods
Figs. S1 to S7
Table S1
References

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Genetic Determinants of Self Identity and Social Recognition in Bacteria

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The bacterium *Proteus mirabilis* is capable of movement on solid surfaces by a type of motility called swarming. Boundaries form between swarming colonies of different *P. mirabilis* strains but not between colonies of a single strain. A fundamental requirement for boundary formation is the ability to discriminate between self and nonself. We have isolated mutants that form boundaries with their parent. The mutations map within a six-gene locus that we term *ids* for identification of self. Five of the genes in the *ids* locus are required for recognition of the parent strain as self. Three of the *ids* genes are interchangeable between strains, and two encode specific molecular identifiers.

About 60 years ago, different clinical isolates of the swarming bacterial species *Proteus mirabilis* were shown to form visually apparent boundaries between colonies growing on agar (*1*). By contrast, swarms of a single strain merge with each other (*2*) (Fig. 1A). This phenomenon is still used in diagnostic laboratories to type clinical isolates of *P. mirabilis* (*3*). Many clinical isolates of *P. mirabilis* secrete proteins called proticines that kill sensitive strains. An individual strain of *P. mirabilis* can be identified by a combination of the proticine it produces and the proticines to which it is sensitive (*4*, *5*). Boundaries form between swarms of strains differing in proticine production and sensitivity. However, some strains do not produce any proticines but still form boundaries, even with other non–proticine-producing strains. Thus, proticine production and sensitivity do not explain boundary formation. We sought to identify self versus nonself discrimination factors required for boundary formation by screening for and isolating mutants that recognize their parent as different from self.

We chose *P. mirabilis* strain BB2000 as a model because it is genetically tractable (*6*). We used an agar plate assay to screen 3600 BB2000 mutants, generated by random transposon mutagenesis, in a format where each mutant swarm had two, three, or four adjacent neighbors (*2*). We found a single mutant that formed a boundary with every adjacent mutant, and we named the mutant phenotype "identification of self" (Ids) because mutant and parent swarms did not merge with each other. To show that the transposon insertion was responsible for the phenotype, we crossed the insertion in the Ids transposon mutant into the BB2000 parent by homologous recombination and isolated four recombinants, all of which formed boundaries with the parent but not with each other (fig. S1).

Boundaries between strain BB2000 and the independent isolate HI4320 contained individual cells of both strains at a low density as well as round bodies and debris. Cells of BB2000 and HI4320 made contact with each other within the boundary, but we did not observe cells that penetrated the opposite swarm (Fig. 1B). In boundaries between swarms of the Ids transposon mutant and the BB2000 parent, we also observed a low density of cells, but round bodies and debris were not evident. Cells from the BB2000 parent swarm appeared to traverse the boundary and penetrate the Ids transposon mutant swarm (fig. S2). During the merger of two swarms of the parent strain BB2000, cells from each swarm penetrated the opposite swarm without apparent hindrance (Fig. 1C).

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By using the sequenced genome of strain HI4320 (*7*), we found that the *ids* mutation mapped to codon 1030 of a 1033-codon open reading frame occurring between base pairs 3,282,912 to 3,286,013 and residing in a cluster of six genes (Fig. 2A). A homologous cluster was found by sequencing the parent BB2000 strain (*2*). We refer to the six-gene cluster as *idsABCDEF* for identification of self. We constructed an *idsA-F* deletion mutant of strain BB2000 and found that boundaries formed between swarms of the *idsA-F* deletion mutant and the BB2000 parent but not between the deletion mutant and the Ids transposon mutant (Fig. 1D). Complementation of the *idsA-F* deletion mutation with an *idsA-F* expression vector (which included the 800-bp region directly upstream of *idsA*) resulted in a transformant that merged with the BB2000 parent but formed boundaries with the deletion mutant (Fig. 1E). The complementation analysis confirmed that the *idsA-F* locus encodes self-recognition factors.

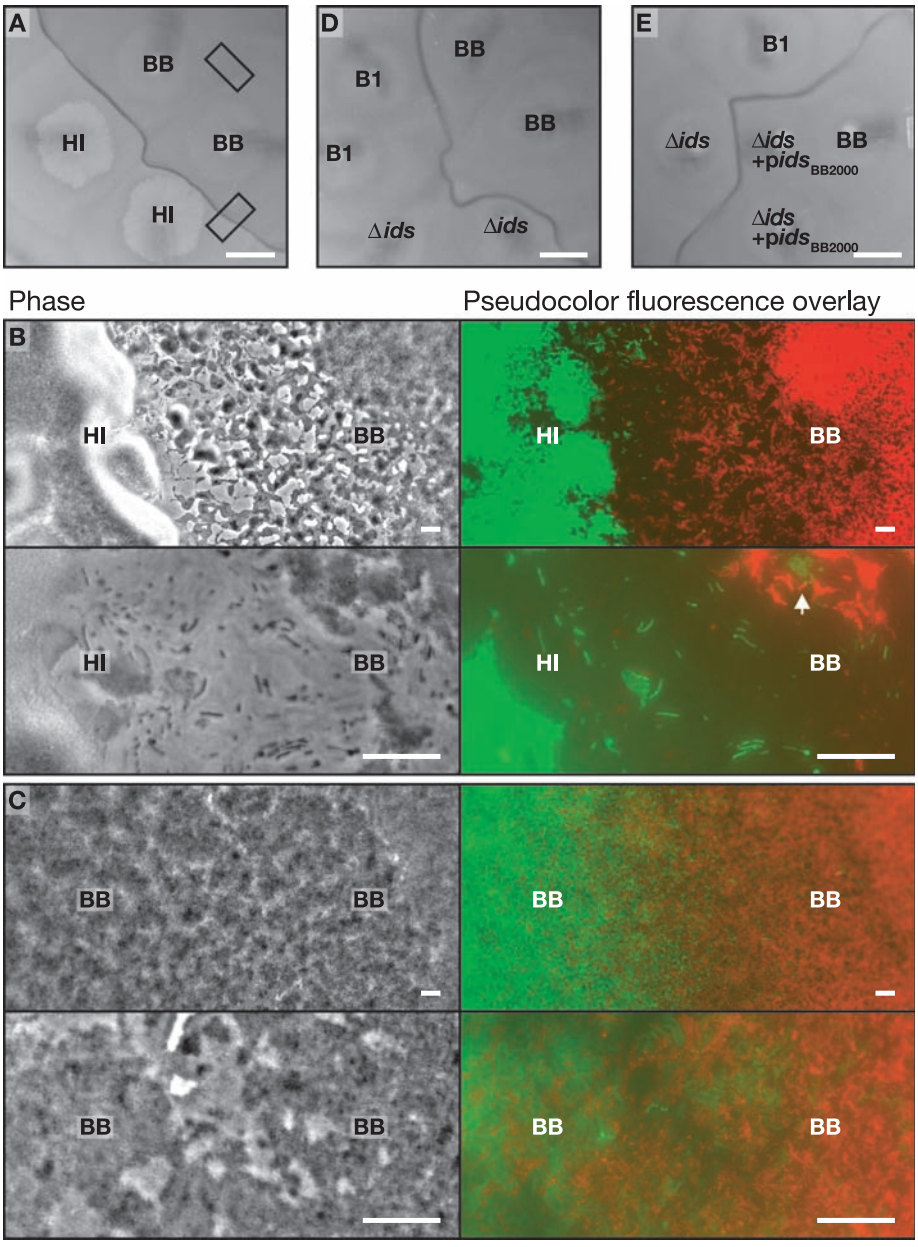

Fig. 1. Images of swarm boundaries between different *P. mirabilis* strains. (**A**) Section of an agar plate with swarms of *P. mirabilis* strains HI4320 (HI) and BB2000 (BB). The boxes indicate intersections visualized in (B) and (C). (**B**) Microscopy showing (top) the boundary between a green fluorescent protein (GFP)–labeled HI swarm (green) and a red fluorescent protein (DsRed)–labeled BB swarm (red) and (bottom) a higher magnification of the boundary. The arrow indicates HI cells among BB cells in the boundary. (**C**) Microscopy showing (top) the merger of two BB swarms and (bottom) a higher magnification of the merger. The left BB swarm (green) expressed GFP, and the right BB swarm (red) expressed DsRed (*2*). (**D**) Section of an agar plate with swarms of BB, the Ids transposon mutant (B1), and the *idsA-F* deletion mutant (Δ*ids*). (**E**) Section of an agar plate with swarms of B1, Δ*ids*, BB, and two swarms of the *idsA-F* deletion mutant carrying an *idsA-F* expression vector (Δ*ids*+ p*ids*$_{BB2000}$). The scale bars indicate 1 cm [for (A), (D), and (E)] and 10 μm [for (B) and (C)].

To assess individual *ids* gene functions, we introduced plasmids containing *idsA-F* gene clusters in which individual genes were disrupted into the *idsA-F* deletion mutant (*2*). We then tested all of the *ids* plasmid–carrying strains to determine whether they merged with each other or formed boundaries on swarm plates (Fig. 2B). We classified the constructs into recognition groups that were composed of strains whose swarms merged with each other but not with swarms of strains in different recognition groups (Fig. 2C). An *idsA*-deficient strain merged with swarms of wild-type BB2000 but formed boundaries with the *idsA-F* deletion mutant (Fig. 2, B and C). In contrast, *idsB*-, *idsC*-, *idsD*-, or *idsE*-deficient strains merged with the *idsA-F* deletion mutant but formed boundaries with wild-type BB2000. The *idsF*-deficient mutant likewise formed boundaries with wild-type BB2000 but had the additional property of forming boundaries with the *idsA-F* deletion mutant, and, in fact, swarms of the *idsF*-deficient mutant formed boundaries with swarms of any construct but itself. We conclude that *idsA* is not required for recognition of the BB2000 parent as self, but *idsB*, *idsC*, *idsD*, *idsE*, and *idsF* are required for self-recognition. The *idsF* gene appears to encode a recognition factor distinct in function from *idsB*-, *idsC*-, *idsD*-, or *idsE*-encoded factors, as indicated by the fact that *idsF* mutants merged only with themselves.

To further investigate the function of the *ids* genes in self-recognition, we introduced DNA containing either the complete BB2000 *idsA-F* gene cluster or a combination of disruptions in the BB2000 *idsA-F* gene cluster into wild-type HI4320 by conjugation to create transgenic diploids (*2*). The diploid HI4320 strains partitioned into those that merged with wild-type HI4320 and those that formed boundaries with wild-type HI4320 (Fig. 2D and fig. S3). A diploid HI4320 strain carrying the complete BB2000 *idsA-F* gene cluster formed boundaries with wild-type HI4320 but merged with swarms of diploid HI4320 strains carrying the BB2000 *idsA-F* gene cluster with disruptions of *idsA*, *idsB*, *idsC*, or *idsF* (Fig. 2D). In contrast, swarms of diploid HI4320 strains carrying the BB2000 *idsA-F* gene cluster with disruptions in either *idsD* or *idsE* merged with wild-type HI4320 (Fig. 2D). Therefore, *idsB*, *idsC*, and *idsF* encode essential self-recognition functions, and the *idsB*, *idsC*, and *idsF* alleles can be complemented by alleles from a different strain. However, *idsD* and *idsE* are essential for self-recognition and appear to encode identity determinants.

To confirm that *idsD* and *idsE* encode identity determinants, we conjugated DNA containing the HI4320 *idsA-F* gene cluster with gene disruptions in *idsD* and separately in *idsEF* into wild-type BB2000 (*2*). Swarms of both the *idsD*-deficient and *idsEF*-deficient diploid BB2000 strains merged with wild-type BB2000. However, a diploid BB2000

strain carrying the complete HI4320 *idsA-F* gene cluster formed boundaries with both *idsD*-deficient and *idsEF*-deficient diploid BB2000 strains (Fig. 2D). Thus, *idsD* and *idsE* encode identity determinants, which we refer to as molecular identifiers.

We note that a diploid BB2000 strain carrying the complete HI4320 *idsA-F* gene cluster formed boundaries with all other strains, including the diploid HI4320 strain carrying the complete BB2000 *idsA-F* gene cluster (Fig. 2D). Therefore, the *idsA-F* gene cluster is probably not the sole determinant of boundary formation between different strains. Consistent with the presence of additional unidentified determinants, boundaries formed even in situations where one of the swarming strains did not carry any of the *idsA-F* genes (i.e., the *idsA-F* deletion mutant).

The *idsA* and *idsB* genes encode polypeptides with substantial sequence similarity to the conserved bacterial proteins Hcp and VgrG, respectively. Recently, *hcp* and *vgrG* were shown to form the first two genes in the type VI protein secretion system of *Vibrio cholerae* (*8*), and both *hcp* and *vgrG* homologs occur in multiple copies in many bacterial species, including *P. mirabilis* (*7*–*9*). We have included *idsA* as part of the *ids* cluster even though it is not required for self-recognition because it is linked to *idsB* homologs in other bacteria and because it is possible that another *hcp* homolog may be recruited to replace *idsA* in *idsA*-deficient *P. mirabilis* strains. The *idsC*, *idsD*, and *idsE* gene products do not show significant similarity to other known polypeptides. The *idsF* gene encodes a conserved hypothetical bacterial protein.

We sequenced the *ids* loci from five additional isolates of *P. mirabilis*: CW677, CW977, G151, I5/5, and S4/3 (*2*). Swarms of the five strains formed boundaries with BB2000, HI4320, and each other. All strains had the six-gene *ids* locus except strain CW677, which had a seven-gene *ids* locus that contained an additional gene with similarity to *idsE* (fig. S4). In all strains, *idsA*, *idsB*, and *idsC* were identical in length, and each polypeptide encoded by *idsA*, *idsB*, *idsC*, and *idsF* had over 96% identity with its homologs from the other strains (Fig. 3A). Both IdsD and IdsE could be separated into two distinct subfamilies with 30% pairwise identity. Within a single IdsD or IdsE subfamily, there was 97 to 99% pairwise identity across the majority of the sequence. However, within each subfamily, there was a C-terminal region in IdsD with only 72 to 84% pairwise identity and a similar region of only 32 to 80% pairwise identity in IdsE (between amino acids 80 and 169). The variable regions of *idsD* and *idsE* are reminiscent of alleles encoding antigenic variation in some bacterial pathogens (*10*).

The DNA immediately downstream of the *idsA-F* locus in strain BB2000 contains a gene coding for a polypeptide with sequence similarity to IdsF and two genes coding for polypeptides with similarity to IdsE (fig. S4). We do not know

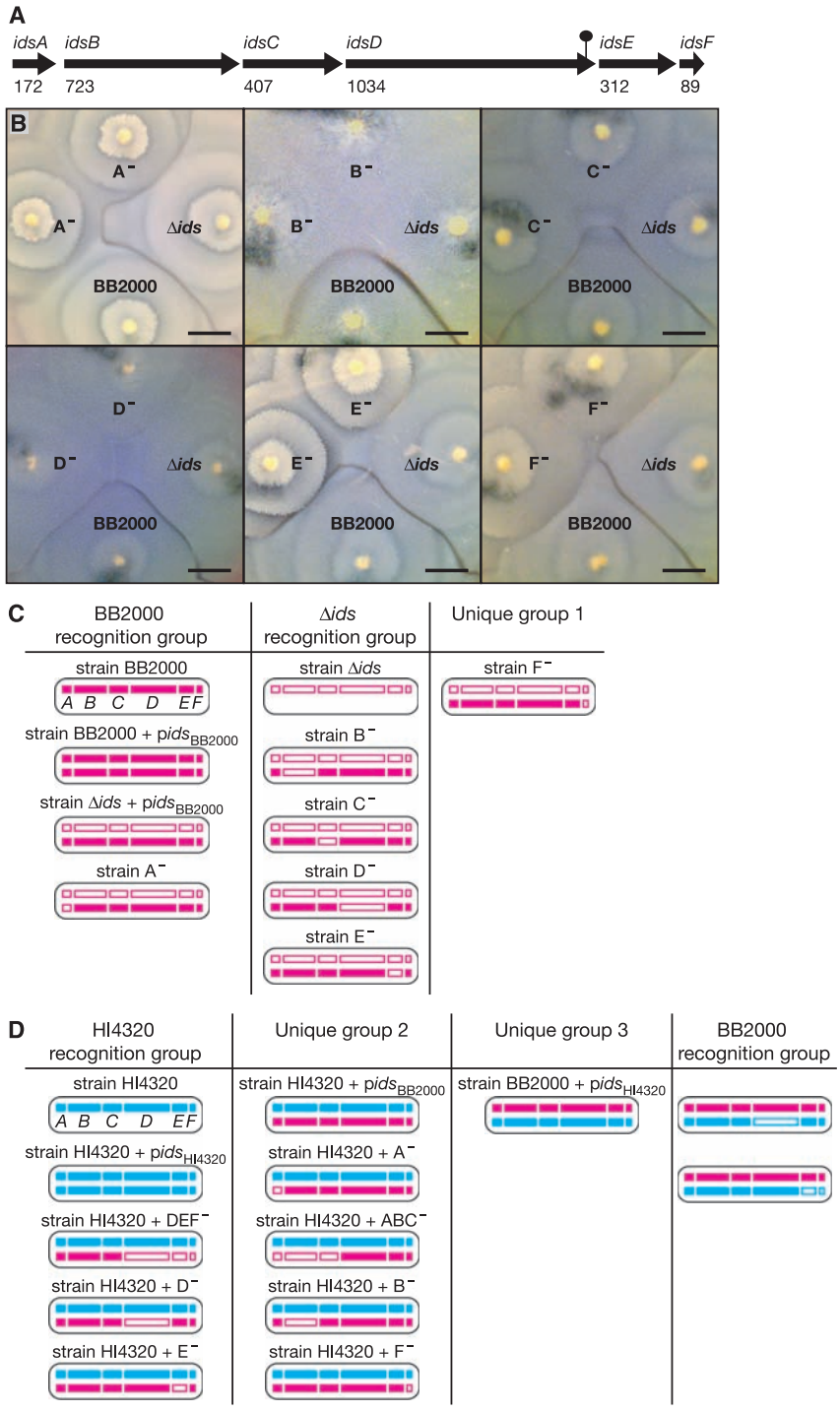

Fig. 2. Genetic analysis of the *ids* gene cluster. (**A**) The length of the encoded polypeptides in the six-gene *ids* cluster of strain BB2000 is shown underneath each gene. The lollipop marks the site of the transposon insertion in the Ids transposon mutant. (**B**) Sections of agar plates with the BB2000 parent, the *idsA-F* deletion mutant (Δ*ids*), and the *idsA-F* deletion mutant carrying a plasmid-borne BB2000 *idsA-F* gene cluster with single-gene disruptions in *idsA* (A^-), *idsB* (B^-), *idsC* (C^-), *idsD* (D^-), *idsE* (E^-), or *idsF* (F^-). Scale bars, 1 cm. (**C**) Recognition groups of strains constructed in the BB2000 and Δ*ids* backgrounds. A subset of the strains is shown in (B). (**D**) Recognition groups of transgenic diploid derivatives of wild-type HI4320 and wild-type BB2000. Agar plates with swarms of representative strains are shown in fig. S3. For (C) and (D), a cell of each strain is represented by an oval in which the chromosomal and plasmid-borne *ids* gene clusters are on the top and bottom, respectively. A white box denotes a gene disruption. The BB2000 and HI4320 *ids* gene clusters are in red and blue, respectively. Each strain was tested against every other strain to determine the recognition groups (*2*). Each recognition group was composed of strains whose swarms merged; boundaries formed between swarms of strains in different recognition groups.

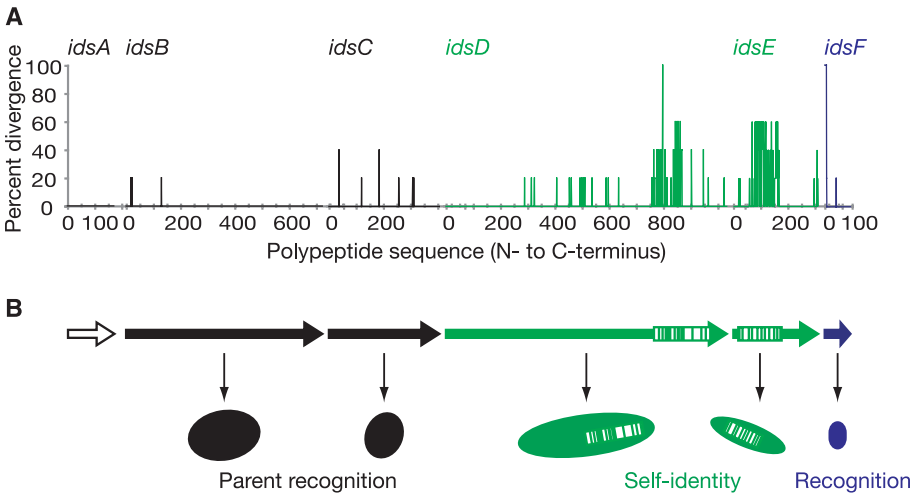

Fig. 3. Organization and model of the *ids* gene cluster. (**A**) A plot showing the percent divergence of the encoded polypeptides in the *idsABCDEF* gene cluster among *P. mirabilis* strains BB2000, HI4320, CW977, G151, and S4/3. (**B**) A model for self-recognition. The *idsB*, *idsC*, *idsD*, *idsE*, and *idsF* genes are required for recognition of the BB2000 parent as self. IdsA is not required for self-recognition. IdsF has a function in self-nonself recognition that is distinct from that of IdsB and IdsC. The *idsD* and *idsE* genes encode specific molecular identifiers of self.

whether there are additional IdsE or IdsF family members coded in the BB2000 genome, but the sequenced HI4320 genome contains a six-gene repeat between base pairs 84,801 and 91,381 that codes for polypeptides with similarity to IdsE (*7*) (fig. S5). It is possible that the putative IdsE homologs could act as additional molecular identifiers.

We have not yet succeeded in detecting the products of any of the *ids* genes in *P. mirabilis* cells, and so we do not know their cellular locations or how they might function to allow swarms to discriminate themselves from other encroaching swarms. It is unlikely that this is a toxin-antitoxin system because we do not see evidence of dead cells in the boundaries between the Ids transposon mutant and its parent (fig. S2) and because the *idsA-F* deletion mutant and the BB2000 parent grew equally well in mixed cultures. When inoculated at a 1:1 ratio, the ratio of the parent to the *idsA-F* deletion mutant in stationary phase remained 1:1. Instead, our data are consistent with a model for self-recognition in which *idsD* and *idsE* encode specific molecular identifiers of self. The *idsB*, *idsC*, and *idsF* products are devices necessary for self-nonself recognition, and the *idsF* product has a function distinct from those of *idsB* and *idsC* (Fig. 3B).

Self-recognition may play a role in maintaining clonal *Proteus* infections (*11*). It also seems likely that other species of bacteria have genes encoding self-recognition. In fact, there is a report of swarm boundary formation between strains of the opportunistic pathogen *Pseudomonas aeruginosa* (*12*). The *P. mirabilis* genetic model of swarm identity provides a simplified system to further examine the molecular mechanisms of self-nonself recognition.

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13. We thank B. Senior for generous sharing of many *P. mirabilis* strains and helpful comments on the swarm assay; R. Belas for providing strain BB2000; H. Mobley for providing strain HI4320; M. Visalli for providing strain G151; S. Chugani, B. Duerkop, and A. Schaefer for thoughtful scientific discussions; and the W. M. Keck Foundation for support. K.A.G. was supported by training grant AI55396 from NIH. The sequences of the *ids* loci and flanking regions from strains BB2000, CW677, CW977, G151, I5/5, and S4/3 were deposited at GenBank, and the accession numbers are EU635876, EU635877, EU635878, EU635879, EU635880, and EU635881, respectively.

Supporting Online Material

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Materials and Methods
Figs. S1 to S5
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Modulation of Gene Expression via Disruption of NF-κB Signaling by a Bacterial Small Molecule

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The control of innate immune responses through activation of the nuclear transcription factor NF-κB is essential for the elimination of invading microbial pathogens. We showed that the bacterial *N*-(3-oxo-dodecanoyl) homoserine lactone (C12) selectively impairs the regulation of NF-κB functions in activated mammalian cells. The consequence is specific repression of stimulus-mediated induction of NF-κB–responsive genes encoding inflammatory cytokines and other immune regulators. These findings uncover a strategy by which C12-producing opportunistic pathogens, such as *Pseudomonas aeruginosa*, attenuate the innate immune system to establish and maintain local persistent infection in humans, for example, in cystic fibrosis patients.

The innate immune system is activated in response to invading microbial pathogens through evolutionary conserved receptor-dependent mechanisms (*1*). For example, in mammals, the Toll-like receptor 4 (TLR4) recognizes lipopolysaccharide (LPS) as a generic signal for an infection by Gram-negative bacteria (*2*). This in turn leads to the rapid activation of the nuclear transcription factor NF-κB and the expression of genes encoding proinflammatory cytokines, including tumor necrosis factor–α (TNF-α). Upon cellular stimulation with TNF through its receptors (TNFR), a positive signaling feedback loop in the NF-κB pathway prolongs LPS-induced gene expression (*3*, *4*). NF-κB–dependent processes, in concert with other signaling cascades such as the p38 protein kinase pathway (*5*, *6*), result in coordinated physiological responses that are critical for pathogen elimination (*7*–*10*). Despite the activation of the innate immune system, highly virulent bacteria are able to cause acute severe disease resulting from extensive bacteremia, and

some opportunistic pathogens have evolved mechanisms to establish persistent infections.

We hypothesized that the distinct pathogenesis of highly virulent and opportunistic pathogens is the result of differences in their ability to affect signaling in macrophages induced through TLR-responsive pathways. The Gram-negative bacterium S*almonella typhimurium* and the Gram-positive bacterium *Staphylococcus aureus* were chosen as representative pathogens that activate cells mainly through TLR4 and TLR2, respectively. As a classic example of an opportunistic pathogen, the Gram-negative bacterium *Pseudomonas aeruginosa*, which causes persistent infections in humans, especially in patients with cystic fibrosis (CF), was selected (*11*, *12*). To initiate our investigation, we exposed bone marrow–derived macrophages (BMDMs) to either *S. typhimurium*, *S. aureus*, *or P. aeruginosa* (*13*), and the macrophage responsiveness was compared by Western blot analysis for the inhibitor of NF-κB (IκB) alpha (IκBα) protein degradation and resynthesis, a distinct feature of NF-κB signaling (*14*), and the phosphorylation of p38 (p-p38), an additional marker of TLR pathways. The stimulation with these bacteria resulted in similar p-p38 amounts. Also, the macrophage activation in response to *S. typhimurium* and *S. aureus* was evident in comparable temporal patterns of IκBα protein degradation and resynthesis; however, we observed a substantial delay in IκBα resynthesis when the cells were treated with *P. aeruginosa* (Fig. 1A).

This difference in NF-κB pathway regulation might be linked to the presence of the bacterial *N*-(3-oxo-dodecanoyl) homoserine lactone (C12), a product of *P. aeruginosa* (*15*), because it was measured in significant concentrations in the samples of *P. aeruginosa* culture (C12 = 4.7 μM, $P < 0.001$, $n = 5$ independent experiments) but not in *S. typhimurium* or *S. aureus* samples. We found that the BMDM responses to *P. aeruginosa* deficient in *lasI*, the gene responsible for C12 synthesis, showed the normal profile in IκBα degradation and resynthesis, providing evidence that C12, secreted by wild-type *P. aeruginosa*, caused the observed effect on the NF-κB pathway in macrophages (Fig. 1, A and B, and fig. S1).

C12 is a small bacterial signaling molecule (Fig. 1C) that, in contrast to LPS, activates mammalian cells through TLR4-independent mechanisms (*16*). In our *P. aeruginosa* experiments, the macrophages were exposed to a complex mixture of bacterial products containing not only C12 but also LPS. To distinguish whether the observed abnormal NF-κB signaling was mediated by C12 itself or whether it altered LPS response, we investigated the biochemical effects of LPS, C12, and their combination. The temporal profiles of IκBα expression and phosphorylation in response to LPS were substantially impaired in the presence of C12, whereas prolonged p38 phosphorylation was observed (Fig. 1D). The natural stereochemistry of C12 and its structural integrity are required for disruption of LPS-mediated NF-κB signaling (fig. S2). These effects of C12 on LPS-induced signaling were not limited to BMDM, but were also observed in alveolar macrophages (Fig. 1E) and human cells from the THP1 cell line (fig. S3).

The highly coordinated degradation and resynthesis of IκB proteins are critical for stimulus-induced NF-κB activation, whose transcriptional activity is additionally regulated through phosphorylation of its subunit RelA (*17*, *18*). Therefore, we next examined the ability of C12 to modulate LPS-induced phosphorylation of RelA. Because IκBβ plays an important role in LPS-responsive NF-κB regulation (*19*), especially in the absence of IκBα (*20*), IκBβ protein levels were also monitored. We found that temporal profiles of RelA phosphorylation, as well as IκBβ degradation in response to LPS, were substantially disrupted in the presence of C12, whereas IκBβ protein levels remained relatively high in cells stimulated with LPS plus C12 (Fig. 2A). Metabolic labeling experiments independently confirmed the effects of C12 on LPS-induced RelA phosphorylation and demonstrated that the phosphorylated form of IκBβ remains bound to RelA in the absence of IκBα (Fig. 2B).

The IκB kinase complex (IKK) phosphorylates IκBα and RelA (*21*); thus, we examined the IKK activity in macrophages stimulated with LPS, C12, or LPS and C12 together. The results of in vitro kinase assays revealed that although C12 did not induce IKK activity (fig. S4) and modestly reduced the early phase of IKK activation by LPS (fig. S5), the LPS-responsive kinase activity was down-regulated faster in the presence of C12 at late time points, especially after 5 min (Fig. 2C, top). Correlations between the profiles of IKK activity and the corresponding patterns of IκBα and RelA phosphorylation prompted the investigation of whether C12 is able to affect IKK activity in the late stage of LPS stimulation, when newly synthesized IκBα becomes abundant (Fig. 2C and fig. S6). IKK activity was evident by the presence of p-IκBα and p-RelA in cell extracts from macrophages pretreated with LPS for 2 hours, whereas the addition of C12 to these cells resulted in rapid reduction of p-IκBα and p-RelA (Fig. 2D). In contrast, the phosphorylation of p38 and the transcription factor adenosine 3′,5′-monophosphate (cAMP) response element–binding protein (CREB), a target of the p38 pathway (*22*), was induced in response to C12 (Fig. 2D). These observations support our interpretation that C12 selectively causes abnormal regulation of LPS-induced IKK activity. Although further investigations are required to identify the mechanisms of this regulation, it is unlikely that C12 is a direct inhibitor of IKK activity, because both the purified IKK complex and a constitutive active form of its

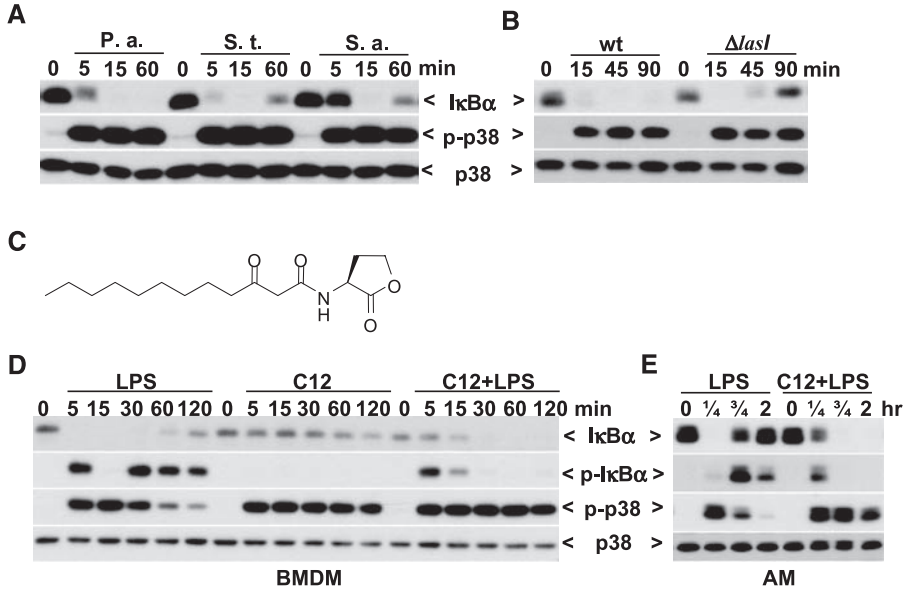

Fig. 1. C12-producing *P. aeruginosa* alters inducible NF-κB signaling. (**A**) Comparison of the macrophage responsiveness to *P. aeruginosa* (P. a.), *S. typhimurium* (S. t.), and *S. aureus* (S. a.). Western blot analysis of IκBα, p38, and its phosphorylated form (p-p38) in BMDM extracts after treatment with bacteria are shown. (**B**) BMDM were stimulated with *P. aeruginosa* wild type (wt) or *lasI* mutant (Δ*lasI*), and total protein extracts were prepared and analyzed as in (A). (**C**) Chemical structure of C12. (**D** and **E**) Western blot analysis of IκBα, p38, and their phosphorylated forms in BMDM extracts (D) or alveolar macrophages (E) after treatment with LPS, C12, or a combination of both.

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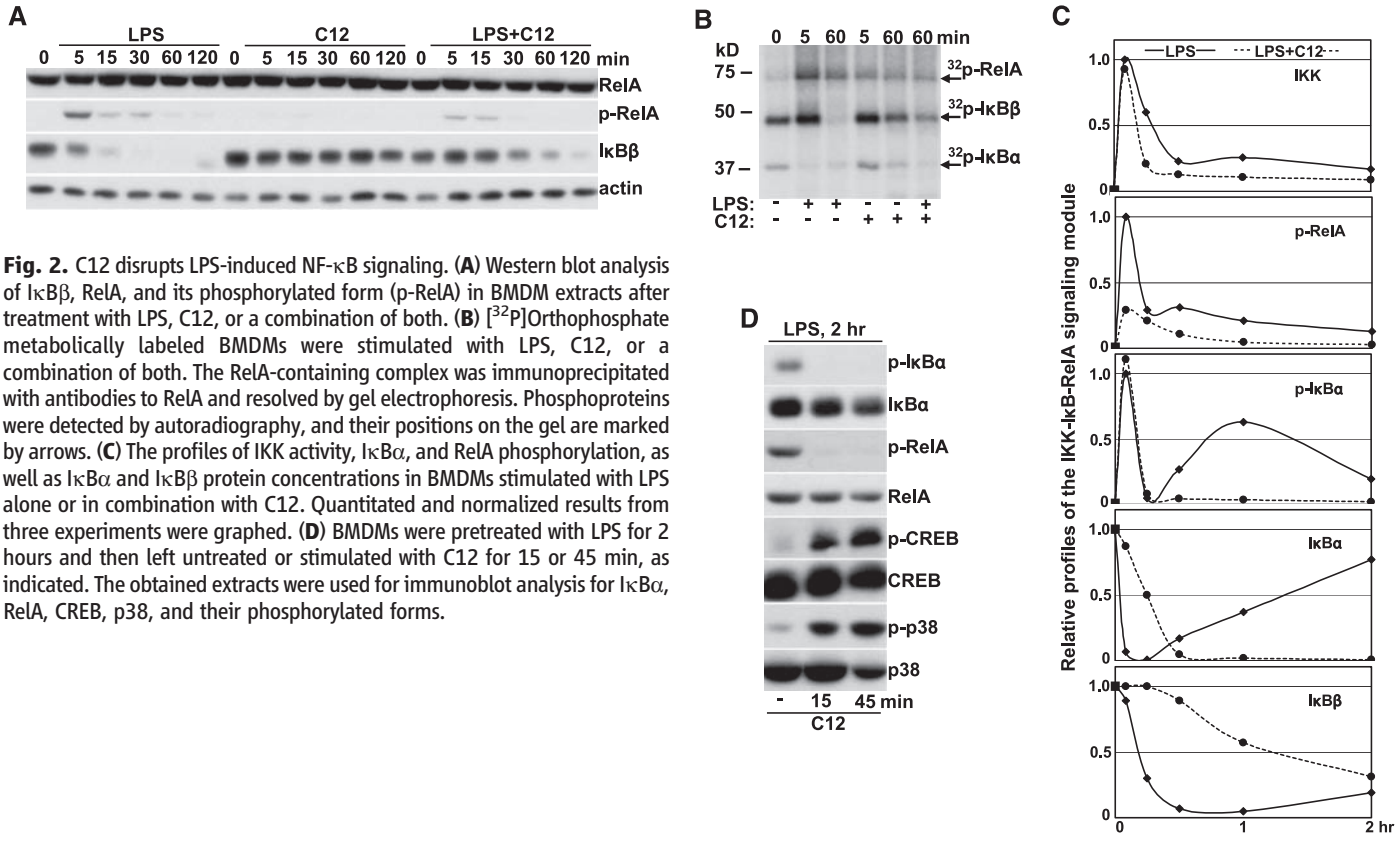

Fig. 2. C12 disrupts LPS-induced NF-κB signaling. (**A**) Western blot analysis of IκBβ, RelA, and its phosphorylated form (p-RelA) in BMDM extracts after treatment with LPS, C12, or a combination of both. (**B**) [^{32}P]Orthophosphate metabolically labeled BMDMs were stimulated with LPS, C12, or a combination of both. The RelA-containing complex was immunoprecipitated with antibodies to RelA and resolved by gel electrophoresis. Phosphoproteins were detected by autoradiography, and their positions on the gel are marked by arrows. (**C**) The profiles of IKK activity, IκBα, and RelA phosphorylation, as well as IκBα and IκBβ protein concentrations in BMDMs stimulated with LPS alone or in combination with C12. Quantitated and normalized results from three experiments were graphed. (**D**) BMDMs were pretreated with LPS for 2 hours and then left untreated or stimulated with C12 for 15 or 45 min, as indicated. The obtained extracts were used for immunoblot analysis for IκBα, RelA, CREB, p38, and their phosphorylated forms.

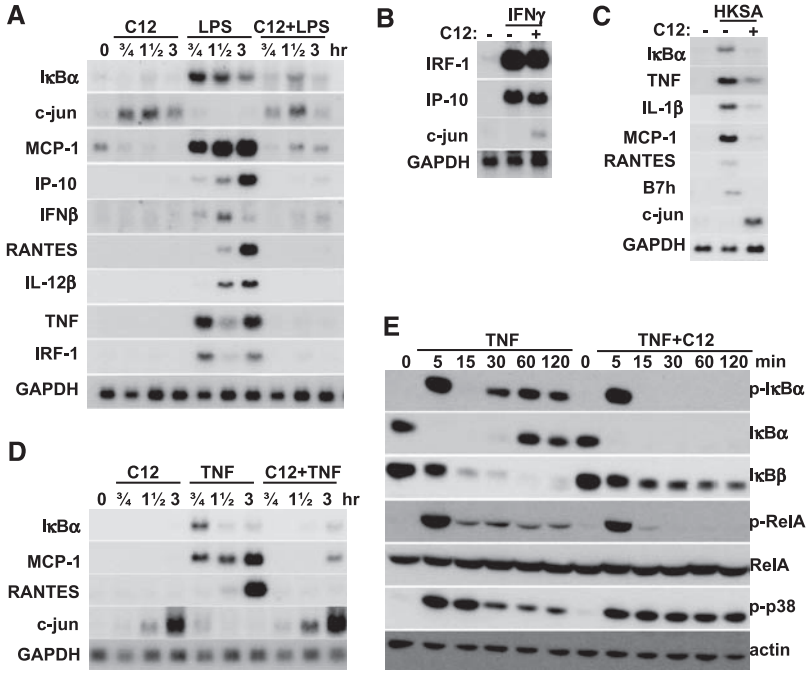

Fig. 3. C12 specifically impairs inducible NF-κB–regulated gene expression. (**A** to **C**) Northern blot analysis of total RNA prepared from BMDMs monitors the effect of C12 on the mRNA expression levels of genes indicated on the right and induced by LPS (A), IFN-γ (B), or HKSA (C). (**D**) Mouse embryo fibroblasts (MEFs) were stimulated with C12, TNF or a combination of both, and total RNA was prepared. Northern blots showing the mRNA levels of genes are indicated on the right. (**E**) Western blot analysis of IκBα, IκBβ, RelA, p38, and their phosphorylated forms in extracts prepared from MEFs after treatment with TNF, C12, or a combination of both.

subunit IKKβ displayed normal kinase activity in the presence of even high doses of C12 (fig. S7).

To define functional consequences of the abnormal NF-κB signaling, we investigated how C12 affects the induction of known NF-κB–responsive genes (table S1) in response to pro-inflammatory stimuli. To control for specificity, we monitored the mRNA levels of *IκBα, IRF-1*, and *IP-10*. The transcription of *IκBα* is predominantly regulated through the classical NF-κB pathway, whereas the induction of *IP-10* and *IRF-1* can also be induced independently of NF-κB, such as in response to interferon-γ (IFN-γ) through the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (*23*). Northern blot analysis revealed that LPS induction of *IκBα* and other NF-κB–regulated genes in BMDMs was substantially impaired in the presence of C12 (Fig. 3A). Similar results were obtained in lung macrophages and embryonic fibroblasts (fig. S8). This C12 effect was dose-dependent and occurred in cells that remained stimulus-responsive, as evident by the C12-mediated induction of c-*jun* mRNA (fig. S9). To confirm the specificity of C12-mediated modulation of NF-κB signaling, we measured its effect on IFN-γ–dependent induction of *IP-10* and *IRF-1* mRNAs. The expression of these mRNAs in response to IFN-γ was unaffected by the presence of C12 (Fig. 3B).

Next, we examined the effect of C12 on the macrophage responsiveness to heat-killed *S. aureus* (HKSA) containing Gram-positive bacteria-derived TLR ligands. Northern blot analysis demonstrated

that C12 substantially impaired HKSA-mediated induction of *IκBα* and other NF-κB–dependent genes (Fig. 3C). Thus, C12 modulates the ability of distinct TLR ligands to activate NF-κB–dependent gene expression.

To clarify whether C12 targets molecular components of the TLR signaling cascades or the NF-κB pathway itself, we examined the effect of C12 on the induction of NF-κB–regulated genes in response to TNF. Northern blot analysis demonstrated that C12 substantially altered the TNF-mediated induction of *IκBα* and other NF-κB–dependent genes (Fig. 3D), providing evidence that C12 acts as a general modulator of the NF-κB pathway. Moreover, the patterns of signal processing within the IκB-RelA modules in cells stimulated with TNF or TNF and C12 (Fig. 3E and fig. S10) matched those that were previously observed for C12-mediated effects on the cellular responses to LPS.

To extend our studies, we used transgenic mice harboring a luciferase reporter driven by an NF-κB–responsive promoter of the *IκBα* or *TNF* gene. The luminescence of both IκB- and TNF-reporter animals was monitored after injection of vehicle, LPS, C12, or a combination of both. These experiments in mice confirmed our in vitro data that LPS-induced responses were substantially suppressed by C12 (Fig. 4, A and B).

In TNFR-mediated cellular responses, proper function of the NF-κB pathway is required for protection from TNF cytotoxicity (*24*); for example, RelA-deficient fibroblasts and macrophages are sensitive to TNF-induced apoptosis (*25*). The treatment of lung fibroblasts and bronchial epithelial cells with TNF plus C12 resulted in enhanced poly (adenosine 5′-diphosphate–ribose) polymerase (PARP) cleavage (Fig. 4C), which is a biochemical marker indicative of apoptosis (*26*). As expected, this proapoptotic response was accompanied by the loss of IκBα protein and its phosphorylated form, confirming the abnormal regulation of TNF-mediated NF-κB activation by C12. Macrophages are major producers of TNF that functions in septic shock pathology through proinflammatory autocrine and paracrine mechanisms (*27*). Therefore, rapid inhibition of TNF expression in LPS-stimulated leukocytes by C12 may indeed be beneficial for the survival of both C12-producing bacterial pathogens and the surrounding cells (Fig. 4, D to F, and fig. S11). This effect might occur in pathological conditions, such as chronic *P. aeruginosa* colonization of the airways of CF patients or biofilm formation in patients with catheters and on respirators. Further study will determine if these condtions lead to host-cell exposure to local micromolar concentrations of C12 and subsequent effects on host immunity (*28*).

These findings suggest that, in the case of *P. aeruginosa*, C12-mediated disruption of NF-κB signaling attenuates TLR4-dependent innate immune responses, thereby potentially promoting persistent infection. The abrogation of LPS-induced NF-κB activity in a variety of mouse strains bearing defects in the TLR4 pathway correlates with the prolongation of some Gram-negative bacterial infections [fig. S12 and (*29*)]. Therefore, the identification of a mammalian C12 receptor will provide additional insights into how to regulate the interactions between pathogen and host.

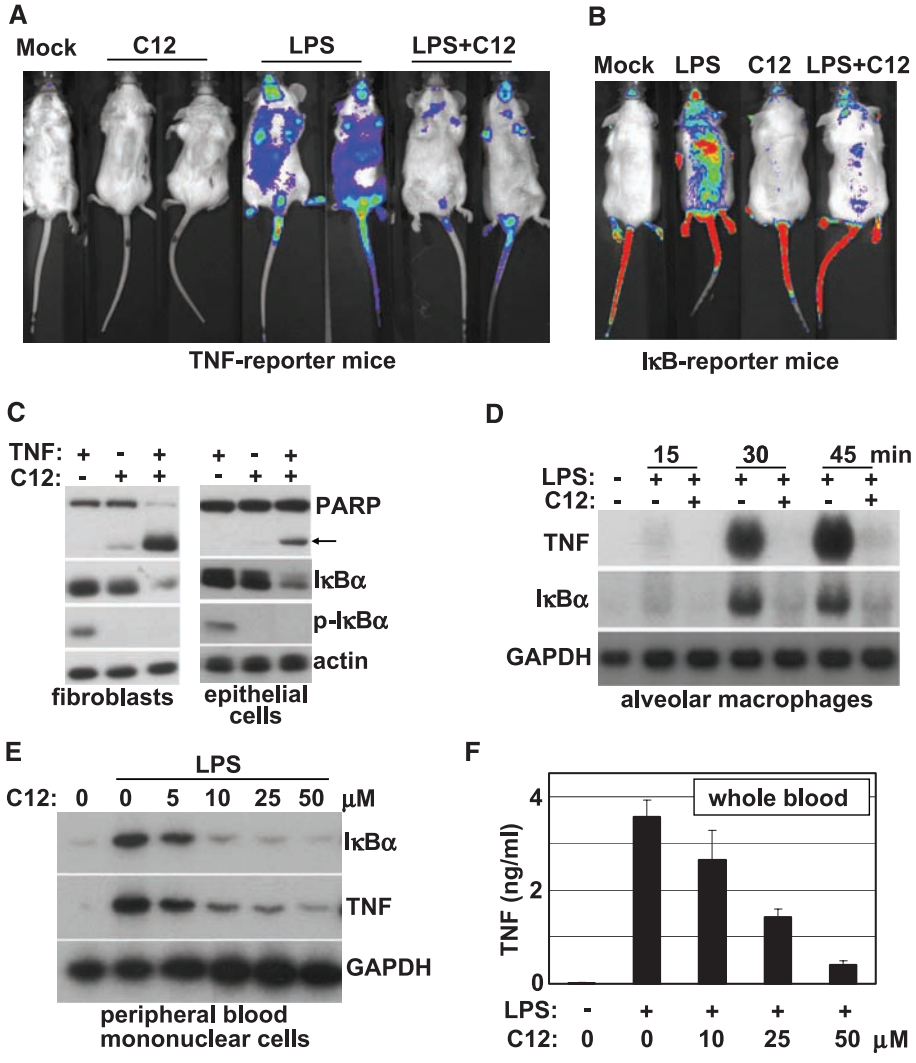

Fig. 4. C12-mediated inhibition of TNF expression in activated leukocytes could be beneficial for surrounding cells. (**A** and **B**) Bioluminescence emission from TNF-reporter (A) or IκB-reporter (B) mice 2 hours after injection with vehicle, LPS, C12, or a combination of both, as indicated. (**C**) Western blot analysis monitors PARP cleavage as well as the amounts of IκBα and its phosphorylated form in WI38 lung fibroblasts and bronchial epithelial cells stimulated for 2 hours, as indicated. (**D** and **E**) Northern blot analysis shows the effect of C12 on rapid LPS-mediated induction of *TNF* and *IκBα* mRNAs in primary cells. (**F**) Inhibitory effect of C12 on LPS-induced TNF production in whole blood.

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Drug Target Identification Using Side-Effect Similarity

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Targets for drugs have so far been predicted on the basis of molecular or cellular features, for example, by exploiting similarity in chemical structure or in activity across cell lines. We used phenotypic side-effect similarities to infer whether two drugs share a target. Applied to 746 marketed drugs, a network of 1018 side effect–driven drug-drug relations became apparent, 261 of which are formed by chemically dissimilar drugs from different therapeutic indications. We experimentally tested 20 of these unexpected drug-drug relations and validated 13 implied drug-target relations by in vitro binding assays, of which 11 reveal inhibition constants equal to less than 10 micromolar. Nine of these were tested and confirmed in cell assays, documenting the feasibility of using phenotypic information to infer molecular interactions and hinting at new uses of marketed drugs.

The treatment of human diseases with carefully selected drugs provides a long-lasting controlled chemical perturbation experiment in a complex organism. Its readout includes the regulated recording of side effects summarized in the package inserts (also known as patient information leaflets or drug labels). Drug side effects are complex phenomenological observations that have been attributed to a number of molecular scenarios including the interaction with the primary or additional targets (off-targets hereafter), downstream pathway perturbations, kinetic and dosage effects, drug-drug interference, insufficient metabolization, effects of active metabolites, and aggregation or irreversible target binding of the drug (*1*). Of these, direct interaction with proteins seems to be one of the most important scenarios (*2*, *3*).

Although unexpected activities derived from off-targets are usually unwanted and harmful, they can sometimes be beneficial and have led to new therapeutic indications for drugs. For instance, sildenafil (Viagra, Pfizer Incorporated, New York, New York) was developed to treat angina, but its side effect of prolonged penile erections in human volunteers led to a change in the therapeutic area of the drug (*4*).

Similar side effects of unrelated drugs can be caused by their common off-targets. For example, the two dissimilar drugs cisapride and astemizole both cause cardiac arrhythmias because they inhibit the cardiac ion channel hERG in addition to their primary targets (serotonin and histamine receptors, respectively) (*5*). In general, drugs with similar in vitro protein binding profiles tend to cause similar side effects (*6*, *7*), implying a direct correlation between target binding and side-effect similarity and hence a possibility to predict off-target binding. So far, additional targets for known drugs have been systematically identified through phenotypic assays, by exploiting chemical similarity measures, and through docking strategies [e.g., (*8*–*12*), reviewed in (*13*)]. All of these discoveries had their bases in the analysis of cell assays or binding studies and did not consider the entire human system.

Therefore, we explored side-effect information generated from the use of marketed drugs to infer molecular activities of drugs that are not implicit by their chemical similarity or the sequence similarity of their known targets. We developed a measure for side-effect similarity

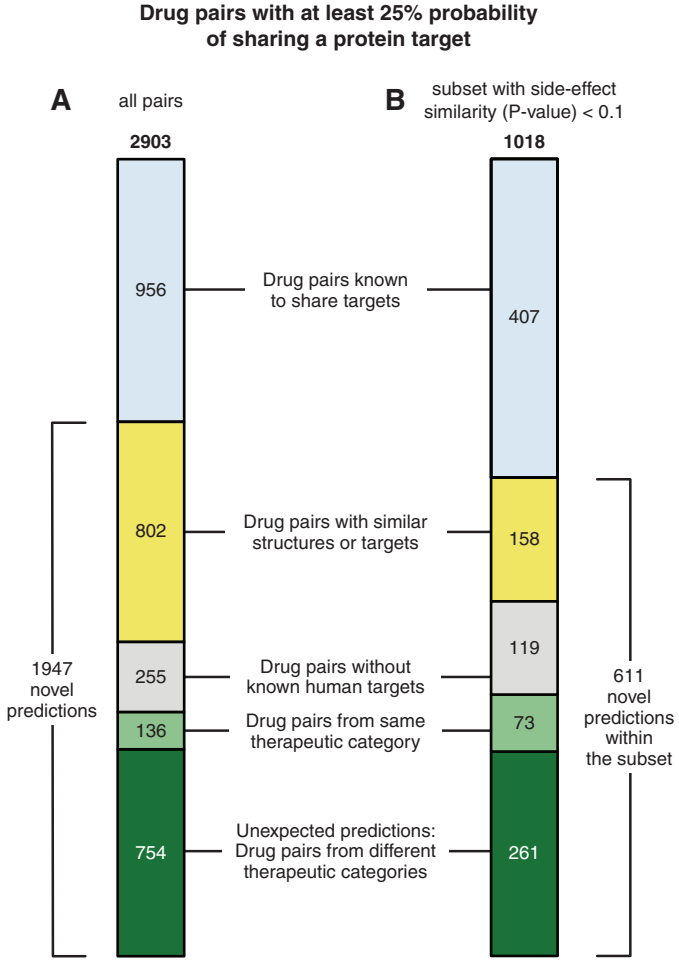

Fig. 1. Breakdown of drug pairs predicted to share a target. (**A**) We subjected the initial set of 2903 to a series of stringent filters, leaving 754 pairs that imply unexpected drug-target relations. In particular, we filtered out pairs of structurally similar drugs (Tanimoto 2D similarity > 0.6) and drug pairs with similar known targets [normalized bitscore > 0.12, which corresponds to ~28% sequence identity (fig. S6)] because both molecular features can be used independently to infer targets (*13*, *20*–*22*). Thus, the unexpected relations contain combined contributions from weak chemical similarities and a range of side-effect similarities. (**B**) The subset of drug pairs that are predominantly based on strong side-effect similarity [*P* value < 0.1 (*15*)] was used for network analysis (Fig. 2).

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(fig. S1), analyzed the likelihood of sharing protein targets for 277,885 pairs of 746 marketed drugs, and confirmed experimentally that side-effect similarity indeed indicates common protein targets of unrelated drugs. Thus, we are able to propose additional targets for many existing drugs, often implicated in different therapeutic categories.

To classify side effects, we used the Unified Medical Language System (UMLS) ontology for medical symptoms (*14*) and extracted relevant terms from drug package inserts (*15*). We used the relations between terms in the ontology to capture also similarities between drugs annotated with distinct but closely related terms. Not all side effects are independent of each other; for example, most drugs that cause nausea also cause vomiting. We corrected for this redundancy by weighting side effects in a manner analogous to the down-weighting of similar protein sequences within multiple alignments (*15*, *16*).

The recorded side effects vary greatly in abundance: Some, like megaloblastic anemia, are caused by only a few drugs, whereas others, like dizziness, occur for most. Within a reference set of 502 drugs with 4857 known human drug-target relations (*15*) from the Matador (*17*), DrugBank (*18*), and PDSP K_i (Psychoactive Drug Screening Program inhibition constant) databases (*19*), we observed an inverse correlation between side-effect frequency and the

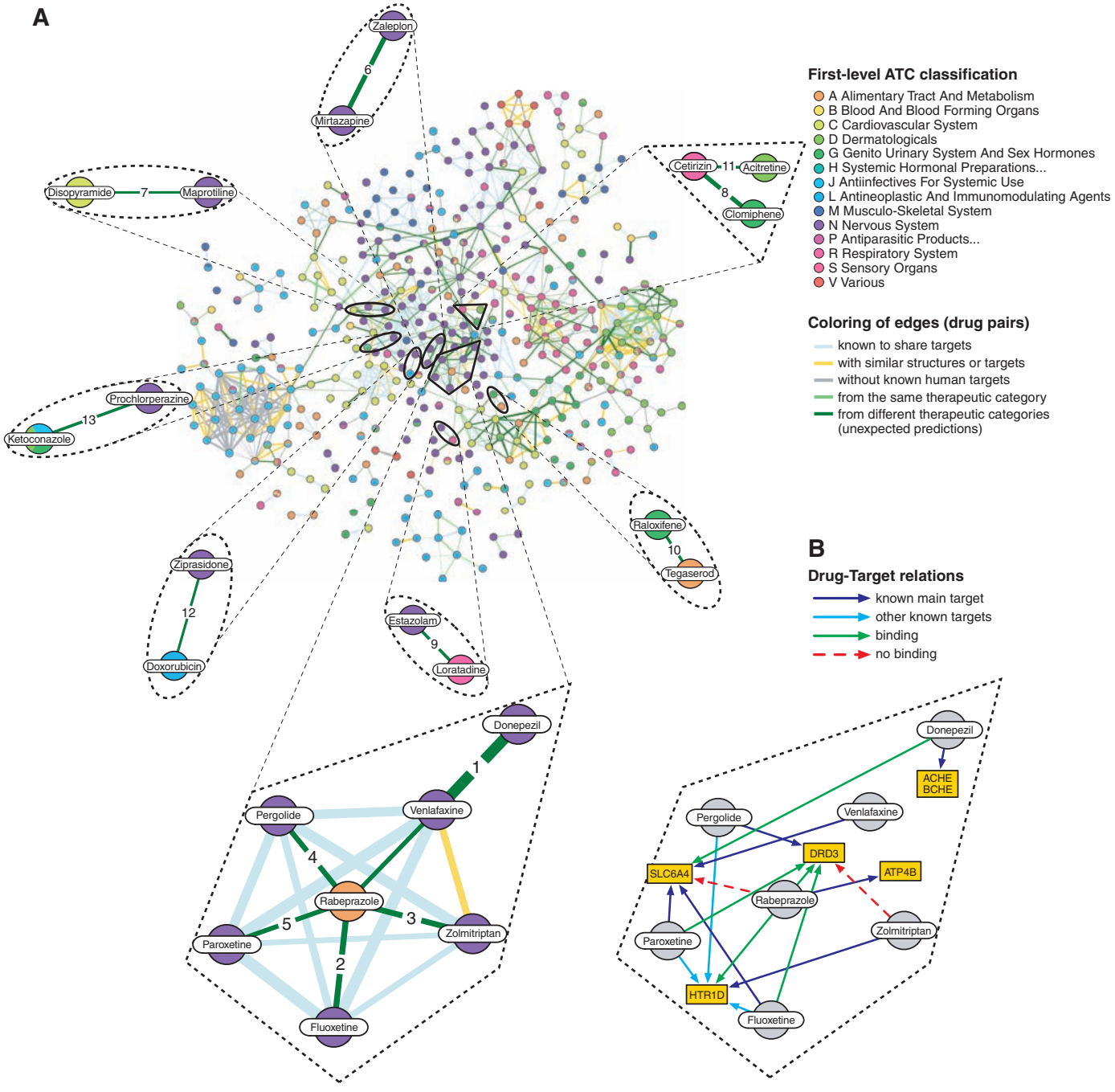


Fig. 2. Network of drugs predicted to have common protein targets. (**A**) 424 drugs (nodes) form 1018 pairs with strong side-effect similarity and above 25% probability of sharing a target (edges, width proportional to probability). Drug subnetworks around the antiulcer drug rabeprazole and other experimentally confirmed predictions are magnified. (**B**) Selected drug-target relations in the subnetwork around rabeprazole (see fig. S10 for other drug-target pairs). Predicted drug-target relations that were experimentally validated (Fig. 3) are shown with green arrows; dashed red arrows indicate that the predicted targets could not be confirmed. The confirmed relations are sufficient to prove the predicted drug-drug relations in the rabeprazole subnetwork.

likelihood of two drugs to share a protein target, and we weighted side effects accordingly (fig. S1D).

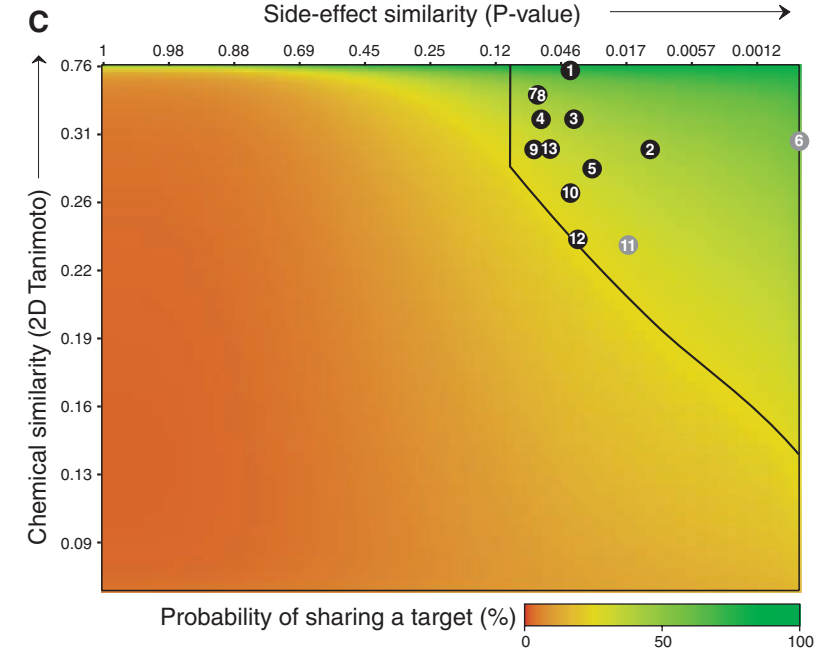
A measure for side-effect similarity was established by using these weighting schemes and by incorporating statistical significance assessments (*15*). We tested the predictive power of this side-effect similarity measure on our reference set of 502 drugs with known human targets and observed a clear correlation between side-effect similarity and the likelihood that two drugs share a protein target (fig. S1H). Side-effect similarity can thus be used to predict new targets for old drugs.

Consistent with previous studies [e.g., (*20*–*22*)], we observed in our reference set that chemically similar drugs [according to the two-dimensional (2D) Tanimoto chemical similarity score (*15*)] are likely to have the same targets (fig. S1H). The corresponding predictions showed only a small overlap with those based on side effects: In the reference set, only 35 drug pairs are in common between the 198 and 301 pairs with more than 50% probability of sharing targets according to their side-effect similarity and chemical similarity, respectively. Consequently, we combined side-effect similarity and chemical

A

Pair	Prob.	Tested Drug	Reference Drug	Target	K_i(μM)	Cellular Act. Antag./Inhib.	Ago.
1	75	donepezil	venlafaxine	5HTT	9 ❶	**100**	
2	40	fluoxetine	rabeprazole	DRD3	2 ❷	27	**60**
3	40	rabeprazole	zolmitriptan	HTR1D	7.6 ❸	*Assay not available*	
4	36	rabeprazole	pergolide	DRD3	1.6 ❹	**87**	<20
5	33	paroxetine	rabeprazole	DRD3	3.8 ❺	<20	*46*
6	57	zaleplon	mirtazapine	HRH1	26 ⑥	**74**	<20
7	44	disopyramide	maprotiline	HRH1	2.7 ❼	**106**	<20
8	43	clomiphene	cetirizine	HRH1	6.5 ❽	*44*	<20
9	30	loratodine	estazolam	BZRP	5 ❾	*Assay not available*	
10	29	raloxifene	tegaserod	HTR1D	0.3 ❿	*Assay not available*	
11	29	acitretin	cetirizine	HRH1	15 ⑪	**59**	<20
12	25	doxorubicin	ziprasidone	HRH1	10 ⑫	*Compound interference*	
*13	30	ketoconazole	prochlorperazine	Serot. Rec.	2.8 ⑬	*45*	<20

$K_i \leq 10$ 11 ● **Activity>50** 6
$10 < K_i < 30$ 2 ● *40<Activity<50* 3



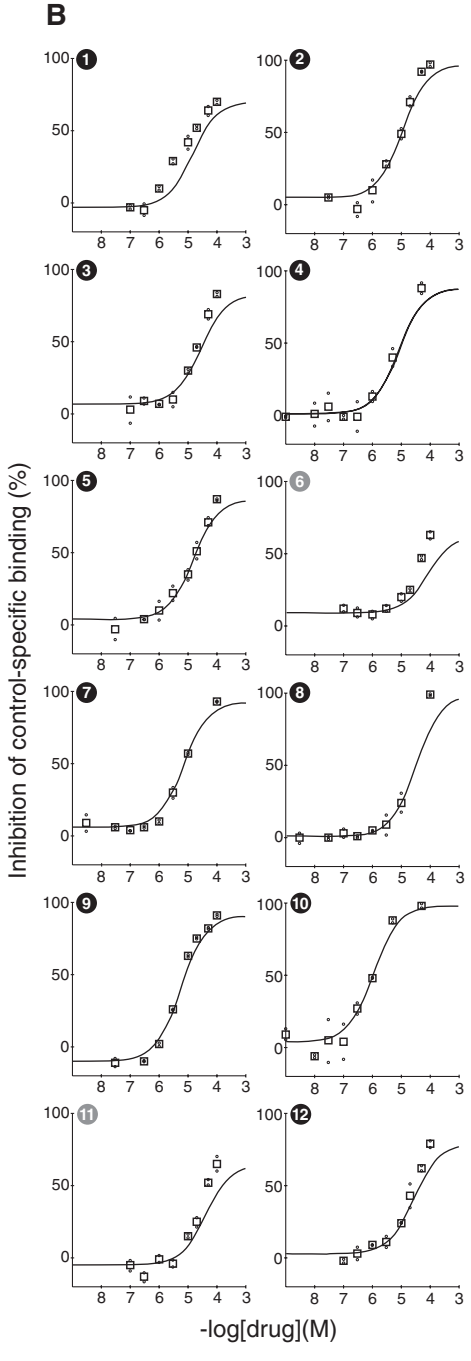


Fig. 3. Novel drug-target relations. (**A**) Values of K_i for the 13 drug-target relations were measured for those drugs that showed an in vitro binding activity higher than 40% at 50 μM. When possible, drug-target relations were validated in cell assays by measuring the activity of the compounds at 50 μM. The asterisk denotes a candidate that was partially insoluble (fig. S8B). (**B**) Concentration curves from competition assays for the novel drug-target relations. (**C**) By using our reference set of 4857 drug-target relations (*15*), we assigned probabilities on the basis of a combination of side-effect similarity and chemical similarity. The line delimits the area used to construct the network in Fig. 2 with shared target probability >25% and side-effect similarity *P* value < 0.1. Drug pairs that were experimentally confirmed to share a target are denoted by black and gray dots according to K_i value [see (A)].

similarity and benchmarked the result against our reference set to obtain the final probabilities for any two drugs to share a protein target (*15*). Both specificity and sensitivity improved considerably (fig. S3).

We next applied our target prediction method to a larger set of 746 human-marketed drugs for which side-effect information is available (table S1), including 244 drugs that have no annotated human targets in our reference set (for example, antibiotics). After exclusion of 44 drugs with less than seven side effects (too few to make specific predictions) (fig. S4), we predicted 2903 pairs of drugs to share a target with over 25% probability (Fig. 1A and fig. S5). We use this arbitrary 25% cutoff in the following because, above this value, the combined method was more sensitive than chemical similarity or side-effect similarity alone (fig. S3D). The actual chance of sharing a target is likely to be higher than our scoring scheme indicates because many binding partners for known drugs are not known yet and were thus counted as false negatives in our benchmarks. Among the 2903 predicted pairs, 956 were known to have common targets (Fig. 1A), which is fivefold higher than what would be expected by random chance (*15*). From the remaining 1947 drug pairs that imply novel predicted interactions, we removed 1193 drug pairs that could be expected to share targets because the drugs are in related indication areas, are chemically similar, or have similar targets (Fig. 1A and fig. S6) (*13*, *15*). Thus, we predicted unexpected, shared targets for 754 drug pairs.

To get an overview of the subset of predictions that are driven by side-effect similarity (1018 of the total 2903 predictions, Fig. 1B), we constructed a network of the corresponding 424 drugs with at least 25% probability of sharing a target (Fig. 2; see fig. S7 for the complete network from all predictions as depicted in Fig. 1A). Of these, 261 pairs were examined in more detail because they involved dissimilar drugs from different therapeutic indications ("unexpected relations" in Fig. 1B and table S2).

We focused on areas in the network that contain drugs from different therapeutic categories (Fig. 2). For example, there is a subnetwork of several drugs targeting the nervous system around the antiulcer drug rabeprazole, a proton pump inhibitor. Within this subnetwork, five drug pairs were predicted to share targets with a probability in the range from 30 to 75%, four of which involve rabeprazole. We validated all our predictions in this subnetwork with both in vitro and cell assays (Fig. 3). We found that rabeprazole inhibits the dopamine receptor DRD3 and binds the serotonin receptor HTR1D (Fig. 2B). The nervous system drugs pergolide, paroxetine, and fluoxetine share these targets with rabeprazole (Fig. 2B), whereas zolmitriptan seems to have only its primary target, serotonin receptor HTR1D, in common with rabeprazole (Fig. 2B). Taken together, the sharing of side effects of the proton pump inhibitor rabeprazole revealed two nervous system off-targets with affinities (Fig. 3) that have been shown to cause side effects (*23*) and should be physiologically relevant given rabeprazole's plasma concentrations (*24*). Our experimental validations also imply that all drug-drug associations in this subnetwork (Fig. 3B) are indeed caused by shared targets.

To generalize our validations, we experimentally tested predictions derived from another 15 drug pairs in addition to the five predictions around rabeprazole (Fig. 2A). All predictions involve at least one drug with a human target and are from the "unexpected" category (261 candidate pairs comprising dissimilar drugs from different indication areas in Fig. 1B). In total, for 13 of the 20 pairs tested, we confirmed binding activity to at least one of their predicted targets in vitro (Fig. 3 and figs. S8 and S9). Eleven of the observed binding affinities are strong enough to lead to side effects [median inhibitory concentrations < 50 μM (*23*)], 11 can be considered biologically active [inhibition constant (K_i) < 10 μM (*12*)], and 7 appear relevant in vivo (K_i values within one order of magnitude of the measured average drug plasma concentrations, table S3). For 9 of the 13 drug-target relations with in vitro activity, cell assays were available, and all confirmed the predicted activity (Fig. 3). Both the observed phenotypic similarity (shared side effects) that led to these predictions and the cellular activities confirmed here support the possible physiological relevance of the newly identified drug-target relations.

All verified predictions imply binding of existing drugs to proteins associated with different therapeutic categories. For example, we have found a relation between the nootropic drug donepezil and the antidepressant venlafaxine (Fig. 2B). Indeed, it has been proposed that donepezil can be used to treat depression (*25*). Although it is still unclear whether the activities we found are sufficient for direct medical applications, the respective drugs certainly can be used as leads for further optimization toward new targets (*26*–*28*).

Many aspects of the current method can be improved (*15*); for example, the inference of the shared target between drug pairs involves a manual step, and we also cannot relate the target to particular side effects. Yet, when taking into account each individual probability of sharing a drug target, the 1947 predicted drugs pairs with >25% probability (Fig. 1A) roughly translate into 860 true drug-drug relations, each implying at least one new off-target protein, more than two-thirds of them in distinct therapeutic categories. The numerous off-targets for marketed drugs suggest that many of them have a broader spectrum of targets with physiological relevance than expected.

The use of direct readouts (side effects) of a perturbed human system to reveal molecular drug-target interactions should be applicable in a number of ways. First and foremost, existing drugs could be routinely checked for additional hidden targets and potential use in different therapeutic categories. Newly uncovered off-target effects will provide insights into the molecular basis of the drug's side effects but will also increase the reference set, which, in turn, then helps improving the method. The strategy could also be used in a preclinical setting through integration of candidate drugs into the network presented here or through application to animal models.

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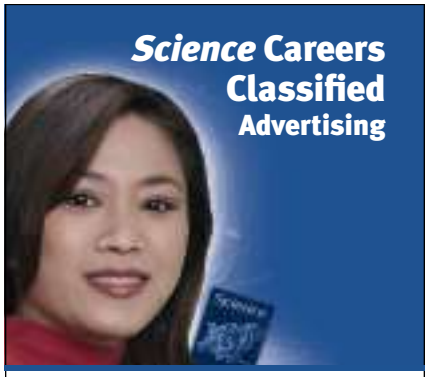

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European research institutions seek to diversify funding sources as well as their work force. **By Jill U. Adams**

In 2002, the European Union (EU) set a goal, referred to as the Lisbon strategy, that member states should be spending 3 percent of their gross domestic product on research and development by the year 2010. At present only a few countries are at that level, such as Sweden (3.9 percent) and Finland (3.5 percent). Powerhouses such as Germany (2.5 percent), France (2.1 percent), and the UK (1.7 percent) strongly support R&D, like the US (2.6 percent); Spain (1.1 percent) and Italy (1.1 percent) have some catching up to do.

Funding statistics are useful, but they cannot tell the whole story. "As a young scientist, you don't care about politics, you care about your own career," said **Ernst-Ludwig Winnacker,** secretary general of the European Research Council (ERC), which is part of the Seventh Framework Programme (FP7) to boost research, education, and innovation in the European Union. "You don't care about the European research area and these sorts of things; you go to places where your career is best served. Scientists vote with their feet."

The ERC awards grants to individual investigators of any nationality strictly on the basis of scientific excellence, says Winnacker. "The idea is to fund pioneer grants or frontier research," he says, without preference for geographical location or field of science. The only other condition is that the host institution must be in Europe (including the 27 European Union member states and eight other participating countries).

The 300 new awardees of the ERC's starting grants—for scientists who are 2-9 years from earning their Ph.D.s—were selected from more than nine thousand applications. Grants averaged €1.2 million for five years, and the winners voted with their feet for a total of 21 countries. The top vote getters, in rank order, were the UK, France, Germany, the Netherlands, Italy, and Spain. Switzerland and Israel also did extremely well.

Other countries came up empty, like Poland, Turkey, and the Baltic states. "Not because they don't like Poland," says Winnacker of the newly funded young scientists, "but because they don't think the institutions are good enough as yet for them."

Countries like the UK, France, and Germany are no surprise, as they always measure up in assessments of European science, whether by funding or citations. Spain jumps ahead of all but the Netherlands when the number of grants is expressed in relation to national expenditures for research. Italians, from a country where research funding has been flat for a decade, applied for the ERC grants in droves, with some 1,900 applications and earning nearly 12 percent of the awards, second only to Germans. continued »

Ernst-Ludwig Winnacker

Silvio Garattini

"You don't care about the European research area and these sorts of things; you go to places where your career is best served."

UPCOMING FEATURES

Careers and Graduate Programs for BS/MS Scientists—August 15

Annual Postdoc Survey—August 29

Faculty Feature: Enriching Your Academic Career—September 12

Federal Ministry of Education and Research

ZENTREN FÜR INNOVATIONSKOMPETENZ
UNTERNEHMEN REGION
Die BMBF-Innovationsinitiative Neue Länder

IN SEARCH OF EXCELLENCE!

The German Federal Ministry of Education and Research (BMBF) is supporting the establishment of fast-track research groups at eight new Centres for Innovation Competence. Scientists with excellent references and international expertise are invited to send in their applications by **5 Sept. 2008**. Women are especially invited to apply. Preference will be given to disabled applicants with equal qualifications. For further details on the application requirements please see:
www.unternehmen-region.de

B CUBE – Molecular Bioengineering Dresden
Dresden Technical University

Nature offers an enormous arsenal of functional systems and properties that could answer a wide range of unmet technological needs. The vision of the newly founded Centre for Innovation Competence Molecular Bioengineering, B CUBE, is to identify natural functional units, characterise them at the molecular level, and adapt them to specific needs and so design the materials and technologies of the future. The centre will initially comprise three complementary Junior Research Groups that will closely interact with three newly created B CUBE professorial chairs and be supported by a pool of state-of-the-art technology platforms. B CUBE, in collaboration with the German Federal Ministry of Education and Research, invites applications for the following positions:

Research Group Leader "Bionanotechnological Analysis and Manipulation" or "Biofunctional Nanostructures"
Equivalent to Junior (DE) or Assistant (USA) Professorship

The successful candidate will establish an international team of young, talented scientists to investigate biomolecules and complexes to unlock adaptive functional units. This requires the continual development of bionanotechnological methods to characterise and manipulate biomolecular structures and functions. Applicants should possess a PhD in a bionanotechnological field and demonstrate postdoctoral experience in molecular imaging and manipulation.

Or:

The successful candidate will establish an international team of young, talented scientists to study and imitate adaptive processes observed in natural systems. The goal of this research group is to establish novel synthetic pathways for innovative nanomaterials that may lead to future technological applications. Applicants should possess a PhD in biochemistry and/or polymer chemistry and demonstrate postdoctoral experience in the analysis and control of biomolecular processes and/or supramolecular chemistry.

Research Group Leader "Bioresponsive Materials"
Equivalent to Junior (DE) or Assistant (USA) Professorship

The successful candidate will establish an international team of young, talented scientists to transfer adaptive principles seen in nature into synthetic polymer materials. The aim of the group is to combine biomolecules, bioanalogous components and synthetic polymer architecture to yield reversible, self-regulating systems. Applicants should possess a PhD in chemistry and demonstrate postdoctoral experience in preparative biochemistry and/or organic or macromolecular chemistry.

The positions are initially funded for a period of five years. The salary is aligned to TV-L and commensurate with age and experience. Candidates are required to demonstrate a commitment to excellence in both research and teaching. B CUBE offers a unique research environment with excellent facilities and an internationally recognised expertise in biology, biotechnology and materials research.

Applications should be addressed to:

B CUBE
Technische Universität Dresden
Professor Carsten Werner
Tatzberg 47
01307 Dresden
Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
office@bcube-dresden.de; http://www.bcube-dresden.de

Virtuhcon
Freiberg Technical University

The Freiberg Technical University holds a leading position worldwide in the fields of metallurgy and fuel conversion, both topics ranking among the core themes of the university's research profile. Scientific top-rate performances in these fields are pushed by extending the excellent infrastructure. A further leap in quality will be achieved by the establishment of the Centre for Innovation Competence Virtuhcon (Virtualisation of High Temperature Conversion Processes). The centre will focus on the improvement of sustainability of the most resource- and energy-intensive processes in the energy conversion and material supply sector. High temperature conversion processes will be modelled, simulated and virtualised on a new scientific level by using high-performance computing.

Research Group Leader "Multiphase Systems"

The group leader sets up an international team of young scientists. The tasks of the team are the comprehensive analysis of material systems of real high temperature conversion processes and the development of consistent property data sets allowing the thermodynamic description of complex material systems and the creation of mathematical and natural-scientific models.

Requirements for engagement are:
- PhD (e. g. process engineering, metallurgy, technical/physical chemistry, technical mineralogy)
- Experience in R & D and high temperature conversion processes technology
- Scientific professional experience abroad and experience in international cooperation
- Managerial skills, capacity for teamwork, motivation and interdisciplinarity

Research Group Leader "Reactive Flow Systems"

The research group leader and his team will investigate the characteristics of reactive flows at different reaction room geometries for high temperature conversion processes. The team will analyse and develop models of flow behaviour under different conditions. The combination of those models serves as a basis for realistic numerical simulation and virtualisation of high temperature conversion processes for diverse applications. The tasks include the utilisation of CFD software and visualisation and the virtualisation of a large amount of data.

Applicants should possess:
- PhD (fluid dynamics, mechanical/process engineering, physics, numerical mathematics)
- Experience in one or various fields of reactive flows simulation, modelling and numerical simulation of highly particle loaded turbulent flows, visualisation and virtualisation as well as research, development and engineering of high temperature conversion processes
- Experience in project coordination and in leading an interdisciplinary, international team
- Team spirit, ability to work under pressure and high motivation

The initial appointments are for a period of five years and renewable upon satisfactory performance and continuation of funding for another five years.

Applications and enquiries should be addressed to:

TU Bergakademie Freiberg
Dezernat für
Personalangelegenheiten
09596 Freiberg
Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
Professor Meyer (bmeyer@iec.tu-freiberg.de); http://www.virtuhcon.de

Humoral Immune Reactions in Cardiovascular Diseases
Ernst Moritz Arndt University Greifswald

The Ernst Moritz Arndt University Greifswald is a traditional German university located in the beautiful region of West Pomerania on the Baltic Sea coastline. Life Sciences constitute one of its major research areas. The university is setting up a new interdisciplinary Centre for Innovation Competence. Its establishment is supported by the German Federal Ministry of Education and Research through the funding of two research groups. The focus of the centre is the humoral immune response in cardiovascular diseases. It will elucidate nanostructures of antigens in cardiovascular diseases and the reactions of the immune system upon contact with these complex structures.

For this purpose, two research groups will be established: "Nanostructure" (applying biochemical, biophysical and nanotechnological methods), and "Cell Response" (modern immunological techniques), with both groups collaborating closely. The research group leaders will build a team of 4–5 scientists each. Both groups will be funded initially for five years, with about 3 million euros each. This includes personnel, laboratory set-up and consumables.

Research Group Leader "Nanostructure"

We seek to recruit an outstanding scientist with experience in separation and analysis of proteins, especially in structural changes of proteins and protein aggregates. Candidates should be familiar with nanotechnological techniques such as atomic force microscopy or photon correlation spectroscopy. Knowledge of immunology is considered an advantage.

Candidates should have a PhD in biochemistry, chemistry, biology or biophysics and should have a strong publication record. They are expected to show a high degree of flexibility and be willing to work in interdisciplinary teams. Ideally, candidates should already have some experience in leading a work group and are team players with very good social skills.

Research Group Leader "Cell Response"

Candidates should possess a PhD, preferentially in biology, biochemistry or medicine (immunology, haematology) and should be experienced in advanced immunological techniques with a strong knowledge of molecular biology and biochemistry. Ideally, they should have well-established experience in B-cell immune responses. Experience with transgenic animals and molecular imaging systems is considered an asset.

Interdisciplinary thinking and a high degree of flexibility is expected. Ideally, candidates have had some experience in leading a work group and are team players with very good social skills.

Ernst Moritz Arndt University Greifswald
Institute for Immunology and
Transfusion Medicine
Professor Andreas Greinacher
Sauerbruchstrasse
17487 Greifswald, Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
greinach@uni-greifswald.de; http://www.hike-autoimmunity.de

innoFSPEC Potsdam

innoFSPEC Potsdam, the Centre for Innovation Competence for Fibre-Optical Spectroscopy and Sensing, is established as a joint initiative of the Astrophysical Institute Potsdam (AIP) and the University of Potsdam (UPPC). The centre's research focus is the unique combination of multichannel spectroscopy and fibre-optical chemical sensing.

innoFSPEC Potsdam invites applications for:

Research Group Leader "Multichannel Spectroscopy"

Research Group Leader "Innovative Fibre-Optical Sensing"

Both positions are equivalent to Junior (DE) or Assistant (USA) Professorship,tenure track option.

We are seeking outstanding candidates with strong research records in one or several of the following fields: astronomical instrumentation, physical chemistry, photonics, laser spectroscopy, fibre-optical sensing. Successful candidates should have several years of postdoctoral experience, preferably in an international context.

Both group leaders will head teams of 4–5 postdoctoral researchers/doctoral students with secured funding for an initial period of five years. They will be supported by a dedicated centre manager and by administrative and scientific staff of the two hosting institutions.

The innoFSPEC headquarters are located at AIP in Potsdam-Babelsberg. Research will take place on the thriving, attractive campuses of AIP and the University of Potsdam. In addition to the existing research infrastructure of modern laboratories, computer facilities and workshops, new buildings will provide dedicated lab space for the centre. innoFSPEC will be embedded in an active academic and student environment and will build upon the exciting research and the dynamic teams of AIP and UPPC.

Potsdam, the capital of Brandenburg, is a beautiful city with Prussian castles and attractive lakes, featuring an outstanding scientific environment with about 25 research institutions. Moreover, the vicinity of the national capital Berlin provides unique benefits in terms of science, culture, education and leisure.

Applications should be addressed to:

Universität Potsdam
Dezernat 3
Am Neuen Palais 10
14469 Potsdam
Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
Dr Martin Roth (mmroth@aip.de) or Professor Hans-Gerd Löhmannsröben; (loeh@chem.uni-potsdam.de); http://www.innofspec-potsdam.de

plasmatis – Leibniz Institute for Plasma Science and Technology (INP) Greifswald

With "plasmatis – plasma plus cell", an interdisciplinary Centre for Innovation Competence for the investigation of interactions between physical plasma and living matter is established as a cooperation between INP Greifswald and Greifswald University. The centre seizes the potential of plasma applications in medicine. plasmatis will conduct fundamental research with the goal of understanding the complex mechanisms of the impact of plasma on cellular structures in order to derive systematic therapy options, particularly for wound healing. With plasmatis, a modern and well-equipped research centre arises on a new university campus shaped by medical and natural science that offers young scientists optimal working conditions in the scenic surroundings of West Pomerania in the close vicinity of the Baltic Sea islands of Rügen and Usedom. The two junior research group leaders to be appointed have the opportunity to form their own research group and realise their own research concept within the framework of the goals of plasmatis.

Research Group Leader "Cellular Effects"

The leader, along with 4–5 staff members, is responsible for the investigation of the direct impact of growth and the vitality of cells and tissues under the influence of physical plasmas.
Applicant expectations:

- Distinguished PhD (e. g. biology, biochemistry, pharmacy, medicine)
- Core competence in the field of cell biology
- Internationally accounted publication list
- Experience with project management and management of personnel
- Work experience abroad is an advantage

Research Group Leader "Extracellular Effects"

The leader, along with 4–5 staff members, is responsible for the investigation of the indirect impact on cells and tissues of mutations of the vital environment through physical plasma.
Applicant expectations:

- Distinguished PhD (e. g. physics, engineering)
- Core competence in the field of plasma physics or biophysics
- Internationally accounted publication list
- Experience with project management and management of personnel
- Work experience abroad is an advantage

Please address your application to:

INP Greifswald
Professor Klaus-Dieter Weltmann
Felix-Hausdorff-Strasse 2
17489 Greifswald
Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
weltmann@inp-greifswald.de; http://www.plasmatis.de

Silicon and Light: from macro to nano – SiLi-nano
Martin Luther University Halle-Wittenberg

We are establishing a leading centre for light conversion processes in silicon: Silicon and Light: from macro to nano – SiLi-nano. The topic of "Optoelectronic Reciprocity" will be covered by two research groups – "Silicon-to-Light" and "Light-to-Silicon" – in close collaboration with the Institute for Physics of the Martin Luther University Halle-Wittenberg, the Max Planck Institute for Microstructure Physics, the Fraunhofer Institute for Mechanics of Materials and the Fraunhofer Centre for Silicon Photovoltaics. SiLi-nano will be largely independent and organised as an independent interinstitutional scientific entity under the roof of the Martin Luther University Halle-Wittenberg.

Research Group Leader/Assistant Professor "Silicon-to-Light"

The group leader should be an outstanding expert in the field of silicon technology, in particular silicon photonics and non-linear optics. The scientific focus of the group is a broad range of optical solid state spectroscopy as well as the preparation of nanostructured silicon.

Candidates should have a PhD (preferably in physics). Experience in leading a research group and in university teaching is desirable.

Research Group Leader/Assistant Professor "Light-to-Silicon"

The group leader is expected to be an outstanding expert in the field of phosphors and fluorescent glasses and glass ceramics. The scientific focus of the group is a broad range of solid state spectroscopy as well as the preparation of fluorescent glasses and glass ceramics. Experience in the field of photon management for solar cells is necessary.

Candidates should have a PhD (preferably in physics) and experience in leading a research group and in university teaching.

After five years of funding from the German Federal Ministry of Education and Research and a positive evaluation, a tenure track position will be provided for the two group leaders (Associate Professorship at the Institute for Physics at the Martin Luther University Halle-Wittenberg or a leading position at the Fraunhofer Institute for Mechanics of Materials).

Please send your application to:

Professor Heinrich Graener
Dekan der Fakultät für
Naturwissenschaften II
Martin-Luther-Universität
Friedemann-Bach-Platz 6
06108 Halle (Saale), Germany

and

Project Management
Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
heinrich.graener@physik.uni-halle.de; http://www.sili-nano.de

SEPTOMICS
Friedrich Schiller University Jena

The newly founded Centre for Innovation Competence SEPTOMICS in Jena will be established as an integrated research centre with the vision to improve the molecular understanding of life-threatening infections and the ensuing host response. Three complementary and interacting groups of scientists will be established. They will benefit from close collaboration within an academically well-established cluster of sepsis research in Jena allowing translational and early clinical proof of concept studies.
SEPTOMICS is seeking young, outstanding scientists as:

Research Group Leader (Associate Professor, W2)"Fungal Septomics"

The position is available for an outstanding young scientist with PhD/MD background and experience in molecular biology and functional genomics of human pathogenic fungi, preferentially candida albicans. The group leader is expected to possess a strong publication record. He/She is to establish a scientific team that utilises tools of systems biology to identify and characterise molecular patterns in the response of the fungus to the innate immune system.

Research Group Leader (Associate Professor, W2) "Host Septomics"

The position is available for an outstanding young scientist with PhD/MD background and experience in molecular biology, functional genomics, immunology and the general field of host response. He/She is expected to possess a strong publication record. He/She is expected to establish a team of scientists that utilises tools of systems biology to improve understanding of innate and adaptive immune responses to bacterial and fungal infections.

Funding of the groups will include laboratory set-up, consumables and additional personnel (postdoctoral as well as technical positions) for five years. A successful group leader will be offered a tenure track by the university.

The Friedrich Schiller University Jena aims to increase the number of women in those areas in which they are underrepresented and therefore urges them to apply. Suitably qualified disabled individuals will be preferred and are especially encouraged to apply.

Jena has been elected "Science City 2008" and is a friendly university town with excellent cultural, recreational and living facilities.

Applications should be addressed to:

RG Fungal Septomics:
Friedrich Schiller University Jena
Dean of the Biological-Pharmaceutical Faculty
Professor J. Lehmann
Fürstengraben 26
07743 Jena, Germany

or

RG Host Septomics:
Friedrich Schiller University Jena
Dean of the Medical Faculty
Professor K. Benndorf
Bachstrasse 18
07743 Jena, Germany

and

Project Management Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
axel.brakhage@hki-jena.de (RG Fungal Septomics),
konrad.reinhart@med.uni-jena.de (RG Host Septomics);
http://www.septomics.de

HALOmem
Martin Luther University Halle-Wittenberg

The Centre for Innovation Competence HALOmem at the Martin Luther University Halle-Wittenberg seeks to initiate an expertise platform for the determination of membrane protein structure. In this context, two independent research groups are to be established: "Recombinant Expression of Membrane Proteins" and "Reconstitution of Membrane Proteins". Both groups will have the opportunity to work and collaborate within the stimulating multidisciplinary environment that has established Halle as an internationally recognised centre for pure and applied protein biochemistry, biotechnology and biophysics.
HALOmem seeks highly motivated and outstanding junior scientists for the positions of:

Research Group Leader "Recombinant Expression of Membrane Proteins"

The successful candidate will establish a team to develop technologies for recombinant expression of functional membrane proteins (preferentially in prokaryotes) suitable for structural biology. The applicant will have trained in protein biochemistry and/or biophysics, with documented experience in these fields at postgraduate and postdoctoral level.

Research Group Leader "Reconstitution of Membrane Proteins"

The successful candidate will establish a team to develop technologies for the functional reconstitution of membrane proteins and the analysis of their interactions with membrane components. The applicant will have received training in physical chemistry/membrane biophysics covering a wide array of methods. Documented experience in these fields at postgraduate and postdoctoral level is expected.

Furthermore, applicants should ideally have experience in:
- Working in an international and interdisciplinary scientific environment
- Project coordination and leadership
- Obtaining third party funding

Each group will receive funding from the German Federal Ministry of Education and Research, including laboratory set-up, consumables and additional personnel of 4–5 co-workers for five years. Successful candidates are expected to participate in undergraduate teaching to a limited extent and to contribute to the "Graduate School of Molecular Life Sciences".

Both positions offer the possibility of subsequent tenure at the Martin Luther University, which may or may not be submitted for tender depending on the success of the group leader.

The Martin Luther University Halle-Wittenberg strives to promote equal opportunities in science. Female and disabled applicants, qualified according to the above criteria, will be given preference over other candidates with equivalent relevant qualifications.

Applications and enquiries should be addressed to:

Professor Milton T. Stubbs
ZIK HALOmem
c/o Institut für Biochemie und Biotechnologie
Martin-Luther-Universität Halle-Wittenberg
Kurt-Mothes-Strasse 3
06120 Halle (Saale), Germany

and

Project Management Organisation Jülich
Berlin Office
Zimmerstrasse 26–27
10969 Berlin, Germany
E-mail: k.-d.husemann@fz-juelich.de

For further information please contact:
info@halomem.de; http://www.halomem.de

"We have a better situation than five years ago. More centers, more activities, more grants."

—Jordi Cami

CREDIT: JORDI CASAÑAS/PRBB

Italy

In Italy, training abroad is encouraged. "It's important for a scientist to get another point of view of research," says **Silvio Garattini,** director and vice president of the Mario Negri Institute for Pharmacological Research, a private organization that employs 900 scientists at four locations in Italy.

When Italian students and postdocs go abroad, whether elsewhere in Europe or to the US, "The problem is trying to get them back," says Garattini. The issue of brain drain is of much concern in Italy and the funding situation in the country over the past decade has played a prominent role.

Ten years ago, the Italian government spent 1 percent of its gross domestic product on scientific research, says **Enrico Garaci,** president of the Istituto Superiore di Sanita (ISS) in Rome, which as the primary scientific arm of the Italian National Health Service employs some 1,500 scientists. "Now it is 1.1 percent," he says.

The recent elections in Italy aren't likely to make an impact anytime soon. Political parties both right and left have overseen the decade of flat funding. "Politicians are not very interested in research," says Garattini.

Another critical aspect is the low number of researchers compared to other countries, says Garaci. "For every thousand workers there are three scientists in Italy. In the US, it's nine." The European average is between five and six.

So far, Italy has remained influential on the world stage. Citation indexes show that Italy's long tradition of research is continuing. Prominent scientists like Garattini and Garaci are focusing on the strengths at their respective institutes, investing in specific research areas, establishing formal collaborations across their borders, and doing all they can to change the climate for the better.

Under Garaci, ISS has an agreement with George Mason University to apply the latest methods in proteomics to discover new cancer biomarkers and drug targets. Garaci emphasizes the benefits of focusing on a few areas in which to excel, rather than trying to cover "all of medicine." The agreement includes trading clinical samples and research trainees, as well as shared profits from any commercialization. Garattini points to joint research the Mario Negri does with the Weizmann Institute in Israel.

Mario Negri has upgraded its facilities, moving its Milan headquarters closer to the Polytechnic University of Milan to encourage collaboration, and fitting its new, larger building with modern laboratories and core equipment. "We also have a residence where we can host foreign visitors," says Garattini.

In short, the lesson of Italy is to look at the positives at the institutional level, which may well override the negatives at the national level for a scientist considering a position there.

Spain

The mood in Spain is optimistic. A country whose name is not often mentioned in the same breath with the United Kingdom or Germany when talking about scientific discovery is gaining notice in Europe and beyond. Having suffered its own brain drain, the country is now welcoming returning Spaniards home.

The Spanish government has created new programs and has substantially increased funding for science, biomedical science in particular. "We have a better situation than five years ago," says **Jordi Cami,** the general director of the Barcelona Biomedical Research Park (PRBB). "More centers, more activities, more grants."

Mariano Barbacid, who directs the new Spanish National Cancer Research Center in Madrid (CNIO), returned to Spain in 1998 after working for 23 years in the United States. Like two other national research centers in Spain, which focus on cardiovascular research and genomic regulation, the CNIO is a public institution with about 50 percent of its budget coming as hard money from the government. The other 50 percent comes from grants.

Barbacid has built the CNIO, now with more than four hundred scientific staff, to be research—and researcher—friendly. The national centers have the advantage of being autonomous in terms of strategic planning and daily operations. "That is something the other research centers cannot do; they have to ask permission for everything either to the [Spanish] research council or to the university."

One of the first things Barbacid did was to create a good startup package to attract the best people, including luring back Spaniards who have done their postdoctoral training abroad. "We give them three [support] positions, and everything they need for the first three years, within reason," he says. continued »

"Nine universities won the so-called future concept grants, which Germany hopes will boost those schools into the international ranking."
—Beate Konze-Thomas

In addition, CNIO employees are not civil servants, which requires passing a national exam. "Almost everyone in Spain is a civil servant, whether you belong to the university or the research council," says Barbacid. Skirting that requirement, he says, gives his center tremendous flexibility in hiring scientists from abroad.

"We are dying to get more foreigners here. We are starting an international postdoctoral program where we are paying more competitive salaries, comparable to EMBL, the European Molecular Biology Laboratory," says Barbacid. Currently 25 percent of the CNIO's postdocs and graduate students are foreign, as are five of the 35 group leaders.

Still there's room for improvement. Researchers are limited to only one individual grant at a time, although they may get additional funds from the central government if they are part of a large network grant. The size of grants is limited as well. "As long as you demonstrate you are productive with two different projects, why should you only get funded for one?" asks Barbacid.

Another source of funding in Spain is regional governments. "Several of the regional governments, like Catalonia, really have emerged as new and important means of support," says Cami, which was not the case 10 years ago. Private sector funding on the other hand is scarce; science-focused philanthropy is not part of the Mediterranean culture and industry funding tends to be concentrated in other countries in Europe, and in the United States.

Cami is working to improve relations with industry by holding workshops at the PRBB, simply to bring together academics and industrial scientists, sit them at the same table, and have them share ideas. "Our idea is to survey the different research groups and help scientists see their own research as an opportunity that can be useful or interesting for industry or commercial purposes."

Language can be another barrier to movement among countries. Five years ago, the PRBB switched to using English to teach the graduate level program. "Now almost 70 percent of all our Ph.D. students are non-Spaniards," says Cami. The percentage of foreigners is 20 percent for the total staff of 1,200, 30 percent for scientists. "The current language in the elevators and the restaurants and the seminars is English," he says.

Germany

Germany has a long history of scientific excellence, both in the life sciences and the physical sciences. While Germany typically ranks high in measures of funding for science and output measures like citations and Nobel prizes, scientific research in the country has been stifled somewhat by old-fashioned policies at universities, in state funding schemes, and in intellectual property law.

Change is afoot, starting with the new Excellence Initiative from the German Research Foundation (DFG), the primary federal funding agency. The DFG will spend €1.9 billion over five years on the initiative—a huge addition to the DFG's regular budget of €1.7 billion. The Excellence Initiative funds three broad programs to effect change in graduate education, to encourage research clusters, and to bring back a sense of competitiveness and prestige to German universities.

Nine universities won the so-called future concept grants, which Germany hopes will boost those schools into the international rankings, says **Beate Konze-Thomas,** head of the department for coordinated programs and research infrastructure at the German Research Foundation. The review process was comprehensive, looking at measures of international status, research performance, management, education, the degree of collaboration, and the success in attracting funding from a variety of sources.

Many people see the Excellence Initiative as a welcome challenge to the old system that considered all German universities to be equivalent. Some feel that it may even succeed in inspiring Germans to take more pride in their science. "Scientists in the UK and US have much more self-confidence," says **Enno Aufderheide,** director, research policy and external relations for the Max Planck Society.

Aufderheide says that even the general public in Germany may underestimate what German science can accomplish. "This has been changed a little bit by two things. The first is that, with this Excellence Initiative, there is this feeling that yes we do have excellent universities. The second important thing was the two Nobel prizes for physics and for chemistry, which went to Germany last year." continued »

Featured Participants

Barcelona Biomedical Research Park
www.prbb.org

European Research Council
erc.europa.eu

German Research Foundation
www.dfg.de/en

Istituto Superiore di Sanita
www.iss.it

Mario Negri Institute for Pharmacological Research
www.marionegri.it

Max Planck Society
www.mpg.de/english

Medical Research Council
www.mrc.ac.uk

Spanish National Cancer Research Center
www.cnio.es/ing/index.asp

The Wellcome Trust
www.wellcome.ac.uk

CREDIT: DFG/LICHTENSCHEIDT

Current Opportunities – and an Invitation for Expressions of Interest

UCL

The Faculty of Biomedical Sciences at University College London represents one of the largest and most prestigious aggregations of academics in biomedicine in Europe today. Active groups are working in almost all of the major themes of medical science ranging from basic research to the clinic. UCL itself is now rated as one of the top 10 universities in the world. Performance indicators (e.g. Thompson's ESI) recognise that UCL is already top in Europe for both neuroscience and clinical medicine, second in Europe in Immunology and has enormous strengths across a range of other biomedical research themes including Cancer, Cardiovascular Medicine, Women & Children's Health, Population Health and Ophthalmology.

UCL FBS delivers excellence in research and teaching across 12 prestigious Divisions and Institutes, including Child Health, Neurology, Ophthalmology and the Wolfson, working closely with our partner NHS Trusts at UCLH, Great Ormond Street, Moorfields, Queen Square, the Royal Free and the Whittington. Within this integrated structure, UCL has a powerful tradition of a liberal and individualised approach to academia which allows talented individuals extensive freedom and scope to pursue their research interests, with a minimal level of central control. This tradition is one of the great attractions of UCL and is highly valued by many academics – as well as postgraduate students - as one of their reasons for wishing to work and study at UCL.

1 We welcome Expressions of Interest from academic staff interested in coming to UCL. You should have a track record of excellence in research in any area of biomedicine. You should either already be a leader in your field, or have a CV that demonstrates the capacity to become a leader within the next 3-5 years. Similarly we invite Expressions of Interest for two UCL Research Fellowships which will shortly become available for young international candidates of the highest quality, in any field of Biomedicine.

UCL offers competitive salaries, a central-London location and an environment of unrivalled research excellence supported by excellent facilities for both basic science and clinical research.

If you are interested, please email Mrs Vanessa Havercroft (v.havercroft@ucl.ac.uk) with a copy of your CV and a statement (no more than 1000 words) on your current research activities and research plans.

2 In addition, we have a number of specific current opportunities for academic staff, postdoctoral fellows and postgraduate students, including:

- **Institute of Neurology, Wellcome Trust Centre for Neuroimaging various Academic opportunities. Contact E. Bertram at personnel@ion.ucl.ac.uk, details at http://www.ucl.ac.uk/hr/vacancies/adverts/INE35.html. Closing Date 21.7.08**
- **Division of Population Health, Chair in Health Economics, Contact: Floriana Bortolotti (f.bortolotti@ucl.ac.uk), details at http://www.ucl.ac.uk/epidemiology/jobs/index.htm. Closing Date: 15.8.08**
- **Institute of Child Health, Clinician Scientist award, Contact Professor David Goldblatt (d.goldblatt@ich.ucl.ac.uk) to discuss, k.white@ich.ucl.ac.uk to apply. Details at http://www.ich.ucl.ac.uk/ich/html/humanresources/jobs.html. Closing Date: 29.8.08**
- **Cancer Institute, Senior Statistician, contact Mandy Verdon at human.resources@wibr.ucl.ac.uk, details at http://www.ucl.ac.uk/hr/vacancies/adverts/G414.html. Closing Date: 31.7.08**
- **Division of Population Health, Senior Lecturer/Reader in Public Health, Contact: Floriana Bortolotti (f.bortolotti@ucl.ac.uk), details at: http://www.ucl.ac.uk/epidemiology/jobs/index.htm. Closing Date: 1.8.08**
- **Division of Population Health, Lecturer in Health Psychology, Contact: Floriana Bortolotti (f.bortolotti@ucl.ac.uk), details at http://www.ucl.ac.uk/epidemiology/jobs/index.htm. Closing Date: 1.8.08**
- **Division of Medicine, Oliver Bird 4 year PhD studentships, Contact: Professor David Isenberg, d.isenberg@ucl.ac.uk;**
- **Division of Medicine, Centre for Respiratory Medicine, PhD Studentship (MRC Capacity Building Postgraduate Award). Contact: Professor Jadwiga Wedzicha (j.wedzicha@ucl.ac.uk), Closing Date: 31.7.08**
- **Division of Medicine and the Department of Neuroscience, Physiology and Pharmacology, PhD Studentship - project aimed at understanding the functions of mammalian CLC proteins. Contact: Dr.Anselm Zdebik (a.zdebik@ucl.ac.uk), Closing Date: 31.7.08**
- **Many other UCL career opportunities can be found at : http://www.ucl.ac.uk/hr/vacancies/adverts/job-list.html.**

"We are dying to get more foreigners here. We are starting an international postdoctoral program where we are paying more competitive salaries."
—Mariano Barbacid

The most storied research organization in Germany is the Max Planck Society, which encompasses 78 institutes, centers, and laboratories employing some 4,400 scientists and 11,300 students and fellows. "People who have been group leaders at a Max Planck Institute have been very successful in their careers," says Aufderheide, either moving on to university professorships or moving up to the director level at Max Planck.

Of the 270 directors at Max Planck, 27 percent have foreign passports and 40 percent have come from abroad (including returning Germans). Recent hirings have increased the international representation at Max Planck further. "This is very atypical for Germany."

Not that universities or other research institutions wouldn't want to increase the number of foreigners, but there are barriers. "For students, the numbers have risen a lot during recent years," says Konze-Thomas, to about 10–20 percent. But faculty profiles have not changed much. University professors in Germany have heavy teaching loads compared to other EU countries, as much as nine hours per week, says Konze-Thomas. And they teach in German.

Funding of research comes from the DFG, from private research institutions like the Max Planck Society, and from the universities themselves. On the other hand, the teaching mission of universities is dependent solely on Germany's member states; thus, Bavaria is responsible for its universities and Lower Saxony is responsible for its own. "We have huge differences in the regional funding of universities," says Konze-Thomas.

United Kingdom

The UK has long been a leader in Europe in biomedical research, both in terms of funding and output, and it shows no sign of slowing down. "Public funding for science has actually increased year on year for at least the last 10 years," says **Chris Watkins**, translation theme leader for the UK's Medical Research Council (MRC), the primary government funding agency for biomedical research. "Last fall's spending review was a very good one for science and a very good one for the MRC. Our budget went up 30 percent," including a £543 million allocation from the government for the 2007-2008 fiscal year.

Watkins lists the many reasons why a researcher would want to come to the UK. "Clearly, we have a very strong research environment. And when you look at the figures of our citation impact, we really do punch above our weight," he says, noting that the UK stands second only to the United States in terms of worldwide publications and citations. He also cites government investments in infrastructure and the commitment to support translational and clinical research.

The UK also is ahead of the game when it comes to academic-industrial partnerships and promoting commercialization of research findings. "When the government talks about science, it always talks about science and innovation," says Watkins. The MRC has its own technology transfer division to work with the intellectual property of its intramural program, which includes three research institutes and about 50 units and centers, altogether employing some four thousand people. In the last fiscal year, revenue from licensing added another £46 million to the MRC coffers, all of which gets funneled back to support research.

Young researchers can find opportunities to have much more independence much earlier in their careers in the UK, says **Mark Walport,** director of The Wellcome Trust based in London. The Wellcome Trust awards postdoctoral fellowships with four years of funding. "It enables them to go anywhere in the world. This is empowering because they can choose where to do the research; it's their funding," says Walport, who notes that other funders, like the Royal Society, have good fellowship schemes as well. The Wellcome Trust also awards principal fellowships analogous to the Howard Hughes Medical Institute in the United States.

In addition to supporting individual investigators, The Wellcome Trust supports research schemes in which they see a need for complementary funding. "We're not there to replace the funding of government," says Walport. "We're there to provide synergy." These initiatives include supporting interdisciplinary research, particularly by incorporating the physical sciences into biomedical research, supporting clinical pharmacology in the development of new medications, and bringing together geneticists and epidemiologists to develop a better understanding of genetic variation.

Expanding Horizons

The good news is that going abroad to work in science is smiled upon from all fronts. Whether it's to go to graduate school, to do a postdoctoral fellowship, or to land a more permanent position, most people agree that the experience can broaden one's world, both personally and scientifically.

While personal factors may direct scientists to look at one country over another, it's worth trying to understand the greater research climate in a country. Language, pay, and research opportunities in a specific lab may be immediate concerns, but they are only a small part of the picture. Larger scale issues such as growth in funding, intellectual property rights, and openness to collaboration across different sectors all have the potential to affect a career in ways that might be good, or bad, news.

Jill Adams is a freelance writer living in upstate New York.

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DEPARTMENT OF BIOLOGY
UNIVERSITY OF COPENHAGEN

Nine Associate Professorships

The Dept. of Biology, University of Copenhagen, seeks nine associate professors starting January 1st, 2009, or as soon as possible thereafter. Appointees are expected to pursue innovative and internationally competitive research programs in one of the areas specified below. International postdoctoral experience, collaborative work in multidisciplinary programs, a track record in dissemination, and the ability to attract external funding will be considered assets. Successful candidates will be expected to significantly contribute to undergraduate and graduate teaching at the Dept. and must therefore document relevant expertise in teaching and student supervision. Deadline for applications is Wednesday October 1st, 2008 at 12.00 noon. Please find the job ads in full at: http://www.ku.dk/english/vacant_positions/.

With more than 200 scientists, the Dept. of Biology at the University of Copenhagen is the largest academic institution in the biological sciences in Denmark. The Dept. has research programs in all major areas of biology, including expertise on all types of model organisms: their structure, function, physiology, ecology and evolution at levels from molecules in the cellular universe, via organismal biodiversity and species interactions, to evolutionary and ecological processes in the biosphere. This expertise is integrated with Departmental educational programs which focus on research-based teaching of more than 2,000 students, and outreach at the highest academic levels. The Dept. attracts significant external funding, which currently covers ca. 50% of activities. Information about the Department can be found at http://www1.bio.ku.dk/english/.

Animal Behaviour (position 211-0185)

The appointee is expected to pursue a research program within the field of vertebrate social behaviour. An experimental approach is required and should ideally be inspired by field work so that the position complements and strengthens ongoing research programs. A description of the current activities in the Animal Behaviour Group can be found at www.bio.ku.dk/animbehav. Inquiries concerning the position can be made to Torben Dabelsteen, Head of the Animal Behaviour Group, E-mail: tdabelsteen@bio.ku.dk.

Evolutionary Ecology (position 211-0186)

The appointee is expected to pursue a research program within the field of invertebrate social evolution, combining experimental and field approaches with a solid understanding of evolutionary theory and quantitative methods. An overview of current research at the Centre for Social Evolution can be found at www.bi.ku.dk/cse/. Inquiries concerning the position can be made to Jacobus J. Boomsma, Director of the Centre for Social Evolution, E-mail: JJBoomsma@bio.ku.dk.

Genome Stability (position 211-0181)

The appointee is expected to pursue a research program that can strengthen and complement current research in the Cell Cycle and Genome Stability group, which studies mechanisms for how levels of DNA building blocks cause mutations (see www.bio.ku.dk/ccgs for further information). Experience with fission yeast molecular genetics and multi cellular model systems will be considered assets. Inquiries concerning the position can be made to Olaf Nielsen, Head of the Cell Cycle and Genome Stability Group, E-mail: onigen@bio.ku.dk.

Immunology (position 211-0180)

The appointee is expected to pursue a research program that can strengthen and complement ongoing research on immune system signal transduction (cytokine receptor signaling, immune regulation and cancer research) within the Cell and Developmental Biology Section (see http://www.immi.ku.dk/no-group/NOhomepage.htm). A potential to establish collaborations with medical groups working on inflammatory diseases and cancer will be considered an asset. Inquiries concerning the position can be made to Niels Ødum, Head of the Immune System Signal Transduction Group, E-mail: n.odum@immi.ku.dk.

Molecular Biology of Cilia (position 211-0179)

The appointee is expected to pursue a research program addressing basic questions relating to the molecular mechanisms of ciliogenesis and the cell biology of cilia. The position should strengthen and complement ongoing research on the assembly and function of motile and primary cilia (see www.bio.ku.dk/english/research/cu/cilia/). Documented expertise with mammalian and at least one other, simpler model system, and the potential to develop interactions with research on more general biological and biomedical problems related to cilia will be considered assets. Inquiries concerning the position can be made to Else Hoffmann, Head of the Section for Cell and Developmental Biology Section, E-mail: ekhoffmann@bio.ku.dk.

Molecular Microbiology (position 211-0183)

The appointee is expected to pursue a research program, addressing basic questions in bacterial metabolism and evolution and combining a variety of approaches from biochemistry, molecular biology, physiology, genomics and/or computational biology. The position is intended to strengthen and complement ongoing departmental research on Molecular Microbiology, which is currently done in the Molecular Microbial Ecology group (www.bi.ku.dk/microbiology/splejsen/) and the Danish Archaea Center at the Department (http://dac.molbio.ku.dk/). Inquiries concerning the position can be made to Olaf Nielsen, Deputy Head of Department, E-mail: onigen@bio.ku.dk.

Plant Molecular Biology (position 211-0178)

The appointee is expected to pursue a research program in plant molecular biology that can strengthen and complement current research in the Department (see details at www.imbf.ku.dk/mundy). Knowledge of plant immune and stress responses will be an advantage, and the research programme should have the potential to extend the use of plant models to understanding processes in other organisms. Documented expertise in *Arabidopsis* molecular genetics and functional genomics, experience with at least one other model system, and the potential to establish collaborations with colleagues working on other biological and biomedical models will be considered assets. Inquiries concerning the position can be made to John Mundy, Head of the Plant Molecular Biology Group, E-mail: mundy@my.molbio.ku.dk.

Structural Bioinformatics (position 211-0182)

The appointee is expected to pursue a research program in computational prediction of 3D protein structure. The position is placed at the Bioinformatics Centre (see: www.binf.ku.dk/) and should strengthen and complement the development and use of probabilistic models of macromolecular structures to predict the structure and dynamics of proteins and RNA. The candidate should be capable of interdisciplinary work involving statistics, biophysics, and informatics. Inquiries concerning the position can be made to Anders Krogh, Head of the Bioinformatics Centre, E-mail: krogh@binf.ku.dk.

Terrestrial Plant Ecophysiology (position 211-0184)

The appointee is expected to pursue a program within the Section for Terrestrial Ecology studying ecophysiological processes from the leaf/root to ecosystem level in natural and semi-natural ecosystems. The position should expand existing research programs in Physiological Plant Ecology, which involve research on carbon, water and nutrient relations in terrestrial ecosystems and the effects of global change and anthropogenic stress factors in Arctic and temperate ecosystems (see: www.bio.ku.dk/forskning/to/). Inquiries concerning the position can be made to Rasmus Kjøller, Head of the Section for Terrestrial Ecology, E-mail: rasmusk@bio.ku.dk.

Michelin-funded Visiting Research Opportunities in Materials Science

Ecole Supérieure de Physique et de Chimie Industrielles (ESPCI) - Paris, France

ESPCI invites applications for **several research positions in Materials Science, at the Visiting Professor, post-doctoral and/or PhD levels**. Beginning on September 1st, 2008, the offer will remain valid for an extended period of time.

These full-time positions are funded by **Michelin** (**www.michelin.com**), the worldwide leader in the tire and rubber industry. **Michelin** allocates nearly 4% of its revenue to R&D (600M€ in '07). Six thousand researchers and process engineers throughout Europe, North America and Asia work towards sustaining Michelin's leadership position through a committed policy of innovation in the areas of materials, products and manufacturing processes.

ESPCI (**www.espci.fr**) is a leading "Grande Ecole" in France training scientists and engineers at the graduate level, as well as a world-renowned research institution with a distinguished history that counts among its current and former faculty Pierre and Marie Curie, Pierre-Gilles de Gennes and other notable Nobel laureates. **ESPCI** hosts seventeen laboratories conducting research in physics, chemistry and biology, with a strong emphasis on the scientific fields related to these positions.

The appointments will provide the successful candidates with opportunities to perform collaborative research on the following topics: polymers and polymer-filled composite materials; methods to describe them across multiple scales; methods to characterize nanometer-size structures and the corresponding mechanical properties at the macroscopic scale; physical-chemical features of interfaces and near-surface domains and adhesion issues; aging; modeling of the visco-elastic behavior; tribology; and rheology.

In addition, the successful candidates will be requested to participate in research programs involving one or more laboratories at ESPCI and/or research teams from Michelin, and to deliver scientific lectures covering his/her core area of expertise. These lectures may be geared towards students and research scientists at ESPCI, as well as research teams at Michelin.

The duration of the appointment may range from 1 month to 12 months. Professors at the full, associate and tenure-track levels as well as senior research fellows are particularly encouraged to apply. Financing will be commensurate with the candidate's credentials and research program and includes competitive salary and possibly accommodation, as well as excellent medical benefits.

Post-doctoral candidates and/or PhD students trained in the research topics above, or associated with a visiting Research Professor, may join the program, through a dedicated grant or in partnership with one of the laboratories at ESPCI.

Prospective candidates may send their CV, together with 3 letters of recommendation, by mail, e-mail or fax to François Fuseau, General Secretary, ESPCI, 10 rue Vauquelin 75231 Paris Cedex 05, France; **francois.fuseau@espci.fr**; +33 14 33 14 222. Review of applications will begin immediately and will continue indefinitely.

Umeå University announces...

At Umeå University, there is world-leading research within several areas. We offer an attractive range of academics and quality study environments. Umeå University's campus provides an inspiring milieu for the 4,000 employees and 29,000 students that have chosen us. We stand united before exciting challenges and tremendous opportunities.

16 Post-doc positions (2 years) at the Faculty of Science and Technology

The Faculty of Science and Technology has decided to further strengthen its staff of younger researchers, and therefore searches 16 post-docs for immediate employment.

Intelligence and Cognition. **Ref no 315-2172-08**

Tree Automata Theory for Computational Language Technology. **Ref no 315-2173-08**

Evolutionary Ecology. **Ref no 315-2174-08**

Spatial Population Dynamics. **Ref no 315-2175-08**

Biomechanical and Biophysical Properties of Bacterial Pili and Fimbria. **Ref no 315-2176-08**

Computational Organic Electronics. **Ref no 315-2177-08**

Molecular Analysis of Cell Division Reinitiation in Plants. **Ref no 315-2178-08**

Mechanisms Driving Plant-Specific Planar Polarity in Arabidopsis. **Ref no 315-2179-08**

Biogeochemical Cycling of Environmental Pollutants. **Ref no 315-2181-08**

Plant Proteases. **Ref no 315-2182-08**

Mathematical Statistics for Studies of Environment and Climate Change. **Ref no 315-2183-08**

Mathematics, **Ref no 315-2184-08**

Anaplastic Lymphoma Kinase (Alk) Function in vivo. **Ref no 315-2185-08**

Tumor Biology: Role of the Myc Oncogene in Cancer. Ref no **315-2187-08**

Modeling, Control and Automation for Forestry Cranes. **Ref no 315-2188-08**

Semantic Face Image Annotation and Retrieval on Large Databases. **Ref no 315-2189-08**

For more information: **www.jobb.umu.se**

Cambridge Research Institute

The Cambridge Research Institute (CRI) is a state-of-the-art Cancer Research UK core-funded facility that opened in 2007 at the Addenbrooke's Hospital medical campus of the University of Cambridge. The juxtaposition of the CRI to the medical school and nearby world-class institutes provides an exciting medical scientific environment that fosters the investigation of basic cancer biology and the development of novel clinical applications for cancer patients. The CRI is the nucleus of the Cambridge Cancer Centre (www.cancer.cam.ac.uk), which brings together researchers in many disciplines from across the University and associated Institutes and local biotech; and it is also linked to the clinical services and research of the hospital.

We are developing a set of integrated programmes in selected epithelial cancers (currently lung, pancreas, prostate, breast and ovary) that span from normal biology to clinical application. We wish to recruit clinical and non-clinical scientists whose interests will impact on these programmes (and potential programmes in other epithelial cancers) at any point in the basic to clinical spectrum.

TRANSLATIONAL POST-DOCTORAL FELLOWS

Ref: 7774

CRI has created translational post-doctoral training fellowships to stimulate the development of clinician-scientists for an academic career in cancer medicine. Fellows will participate in the Institute's clinical and scientific programmes in selected epithelial cancers (currently lung, ovary, pancreas, prostate and breast). You will have completed a Ph.D. and possess clinical qualifications in either oncology or in relevant organ site specialities (e.g. respiratory medicine or gastroenterology), or in oncological surgery, pathology or radiology. You will be provided with full salary, access to core scientific services plus support for yourself and a technical assistant for up to four years, and will be a full member of the most appropriate laboratory at CRI. We envisage that up to 10-20% of the fellow's time will be spent in the clinic. You will be expected to develop a line of basic and clinical research alongside the laboratory head, with the expectation that such research may form the basis of your future career path. Mentorship will be provided by the laboratory head and the Institute Director, and other appropriate Group Leaders as needed.

TENURE AND TENURE-TRACK GROUP LEADER POSITIONS

Ref: 7775

Clinical and Non-Clinical Scientists

We wish to recruit up to 3 Group Leaders, with a particular focus in:

(1) Proteomics

(2) Quantitative biology

(3) The biology of epithelial neoplasia

You will be expected to lead an independent research programme that contributes to the overall goals of the Institute. All posts carry a significant core package of salaries and support, which continues for the term of the appointment. Tenure-track appointments are for up to 7 years. Honorary clinical appointments can be negotiated for clinical appointees.

To apply for either position please visit: http://jobs.cancerresearchuk.org/ using the relevant reference number.

Closing date for both positions is: 15th August 2008.

Please be advised that interviews for both positions are planned for late October/early November.

Please direct informal enquiries to Ann Kaminski in the first instance: ann.kaminski@cancer.org.uk

For further information please visit: www.cambridgecancer.org.uk

CANCER RESEARCH UK

Registered charity no 1089464

the Beatson
institute for cancer research

Development of an Independent Research Programme in Cancer Biology

The Beatson Institute for Cancer Research, one of Europe's leading research Institutes, has now moved to a new state-of-the-art building in parkland on the northwestern edge of Glasgow and provides a dynamic, supportive and well-resourced environment for its scientists. We are seeking applications for Group Leaders to develop an independent research programme in cancer biology. We would, in particular, welcome applications from scientists working in the fields of regulation of protein stability, spatial assembly of multi-protein complexes or the development of three-dimensional tissue culture and mouse models for cancer. The resources available include research posts from our Cancer Research UK core grant with space for expansion for further staff on external funding.

Further information on the Beatson's research activities, infrastructure and facilities is available on our website www.beatson.gla.ac.uk Applications, including a one to two page statement of research interests and goals, CV, list of publications and the names of three referees, should be sent to Professor Karen Vousden, Director, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland (email k.vousden@beatson.gla.ac.uk).

Postdoctoral Scientists

We are currently seeking highly motivated and dedicated postdoctoral researchers to work in a variety of projects within the Institute. Group Leaders at the Institute welcome applications from prospective postdoctoral fellows. Posts are offered initially for three years with the possibility, if successful, of a further two. Salaries start from £25,500 and more with relevant experience. If you are interested in the work we do and would like to find out if there are any suitable positions available please logon to our website at www.beatson.gla.ac.uk/research and contact individual Group Leaders directly by following the links. Current vacancies include:

Prof Laura Machesky (e-mail l.machesky@beatson.gla.ac.uk)
A study of cell migration and invasion in 3D environments, both in reconstituted systems and in vivo.

Prof Frank Kozielski (e-mail f.kozielksi@beatson.gla.ac.uk)
The structure and function of cancer-related proteins.

Prof Hing Leung (e-mail h.leung@beatson.gla.ac.uk)
Aberrant signalling in prostate carcinogenesis.

Prof Brad Ozanne and Dr Jim Norman (e-mail b.ozanne@beatson.gla.ac.uk, j.norman@beatson.gla.ac.uk)
Investigation of the interaction of Rho-family GTPases and integrins during invasive tumour cell migration.

Applications for the above posts with CV and names of two referees should be sent to the relevant group leader.

CANCER RESEARCH UK
Charity no: SC006106

The University of Edinburgh

A reputation for excellence built over 400 years, 8000 staff and a vibrant, forward-looking culture make the University of Edinburgh one of the top employers in the city. Critical to our continued success is the significant number of staff employed in supporting roles. So, whatever your skills, if you want to be part of an organisation shaping tomorrow's world, we can offer you a rewarding and interesting future.

Research Group Leader

The Wellcome Trust Centre for Cell Biology at the University of Edinburgh require outstanding young scientists for independent Research Group Leader positions, at the level of Career Development Fellows.

The Centre is affiliated with the Institute of Cell Biology in the School of Biological Sciences and is equipped with state-of-the art facilities and instrumentation for cell biological research, including core support in microscopy, bioinformatics and proteomics. Current research strengths in the Centre are in areas of chromosome biology (chromatin structure, epigenetics, and kinetochore function/checkpoints), subcellular organisation (nuclear envelope, cytoskeleton, and mitotic mechanism) and RNA metabolism (mRNA and rRNA processing), with an emphasis on model systems. We are particularly interested in new investigators studying RNA metabolism and also those using higher eukaryotic systems and/or biochemical approaches to cell biology, but we encourage applications from all areas.

Most group leaders within the Wellcome Trust Centre for Cell Biology are supported by independent research fellowships, and you will secure such independent funding within a year of arrival. Ideally, you should have postdoctoral experience, making you eligible to apply for five or six year early career fellowships from the major UK funding bodies, such as the Wellcome Trust.

Applications, containing a CV (including names of three academic referees) and a brief research outline/proposal, should be sent to Professor Adrian Bird (Director), Wellcome Trust Centre for Cell Biology, University of Edinburgh, Michael Swann Building, The King's Buildings, Edinburgh EH9 3JR, United Kingdom. Applications may also be sent as Word (.doc) or PDF files by email to Professor Bird's secretary, (christine.struthers@ed.ac.uk).

Applicants should ask their referees to send letters of reference by the deadline (below). Informal enquiries may be made to christine.struthers@ed.ac.uk Further information about the Centre can be found at www.wcb.ed.ac.uk Further information about Wellcome Trust Career Development Fellowships can be found at www.wellcome.ac.uk/node2129.html

Closing date: 25 August 2008. Interviews are likely to be held late September or early October 2008.

Committed to Equality and Diversity

The University of Edinburgh is a charitable body, registered in Scotland, with registration number SC005336.

www.jobs.ed.ac.uk

Special Programme for Research and Training in Tropical Diseases (TDR)

A global research programme on infectious diseases of poverty, in which disease endemic countries play a pivotal role

Following the launch of its new strategy and the biggest reconfiguration in its 30 year history, TDR is recruiting the following positions. All positions are based in Geneva, Switzerland. Successful applicants will be staff members of the World Health Organization. For more information consult the WHO/TDR website: **www.who.int/tdr**.

Applications must be made on-line through the WHO website. The deadline for receipt of applications for all positions is **1 August 2008**. Candidates from disease endemic countries and those who have worked in such countries are encouraged to apply.

Research for Neglected Priorities

► **Coordinator (Research) P6:** Lead the research functions of TDR and oversee the management of nine research business lines. (HQ/08/TDR/FT579)

► **Scientist (Leader – Antimalarial Policy and Access and Senior Adviser Malaria Research) P6:** Lead TDR malaria research with special responsibility for antimalarial policy and access. (HQ/08/IER/FT530)

► **Scientist (Chemotherapy for Helminths and other NTDs and Senior Adviser NTD Research) P6:** Lead neglected tropical disease research with special responsibility for drug development and evaluation. (HQ/08/IER/FT456)

► **Scientist (Leader – Integrated Community-based Interventions) P5:** Lead and develop innovative and efficient strategies for providing community-based interventions to poor populations. (HQ/08/IER/FT457)

► **Scientist (Leader – Visceral Leishmaniasis Elimination) P5:** Lead and research for Visceral Leishmaniasis elimination focusing on the Indian sub-continent. (HQ/08/IER/FT455)

► **Adviser (Leader– Diagnostics Research) P6:** Lead quality assured diagnostics research. (HQ/08/TDR/FT580)

► **Scientist (Quality Assured Diagnostics) P5:** Manage clinical, scientific and administrative matters related to diagnostics projects. (HQ/08/TDR/FT496)

► **Technical Officer (Quality Assured Diagnostics) P4:** Participates in clinical, scientific and administrative aspects of diagnostics projects. (HQ/08/TDR/FT462)

► **Scientist Drug (Drug Discovery Biology) P5:** Manage the biological components of TDR drug discovery activities. (HQ/08/TDR/FT491)

► **Technical Officer (Drug discovery and database management) P3:** Provide database management in support of drug discovery. (HQ/08/NPR/FT463)

► **Technical Officer (HIV/TB Co-infection) P4:** Provide scientific and technical support for TB/HIV research. (HQ/08/TDR/FT474)

Empowerment

► **Coordinator (Empowerment) P6:** Coordinate the development of leadership in health research and decision making in disease endemic countries. (HQ/08/TDR/FT493)

► **Technical Officer (Quality Management) P4:** Provide technical expertise on quality assurance issues and training needs. (HQ/08/TDR/FT465)

► **Technical Officer (Research Capacity Strengthening) P4:** Provide scientific expertise for research activities that promote empowerment. (HQ/08/TDR/FT471)

► **Technical Officer (Clinical Coordination) P4:** Participate in the coordination of TDR clinical trial activities. (HQ/08/TDR/FT464)

Stewardship

► **Scientist (Biomedical Science) P5:** Provide specialized expertise by identifying priority needs and major research gaps in infectious diseases of poverty. (HQ/08/TDR/FT472)

► **Scientist (Public Health) P5:** Provide expert advice to develop an evidence and analysis-driven process for the identification of priority needs and major research gaps in infectious diseases. (HQ/08/TDR/FT473)

Portfolio Policy and Development

► **Scientist (Portfolio Management) P5:** Develop and advance quality management and strategic monitoring and evaluation of the programme. (HQ/08/TDR/FT475)

► **Scientist (Portfolio Policy) P5:** Develop and advance policy and technical interfaces of collaboration with external organizations. (HQ/08/TDR/FT477)

Programme Related Support

► **External Relations Officer (Governing Bodies Manager) P5:** Manage and coordinate the external relations, governance and resource management activities of TDR. (HQ/08/TDR/FT492)

► **Programme Manager P5:** Lead and coordinate all aspects of administrative, human resources, budgetary, financial and contract management in TDR. (HQ/08/TDR/FT494)

► **Programme Officer P4:** Provide expert advice and support on budgetary and financial matters with particular attention to donor agreements. (HQ/08/TDR/FT482)

► **Technical Officer (Graphic Designer) P3:** Design and lay out of scientific materials; brochures, pamphlets, reports, posters, newsletters and other regular communication vehicles. (HQ/08/TDR/FT490)

► **Editor (Web Editor) P3:** Develop and maintain the TDR website. (HQ/08/TDR/FT495)

World Health Organization

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by UNICEF/UNDP/World Bank/WHO

CIC energiGUNE

Research Leader

Electrochemistry for Energy Storage and Fuel Cells

Permanent Position

Location: Vitoria-Gasteiz, Basque Country, Spain.

The recently founded ***Fundación energiGune*** (energiGune Foundation, Vitoria-Gasteiz, Basque Country, Spain) for research into energy is looking to hire a research leader in the area of Energy Storage and Fuel Cells.

The ***Fundación energiGune***, co-financed by private sector funds and the Department of Industry and Energy of the Autonomous Regional Basque Government, operates within the framework of recently approved long-term research plans.

This permanent position involves designing the research laboratory and setting up the research team. Applicants will have proven and wide-ranging experience and leadership in the fields of electrochemistry, material science and catalysis, connected to developing advanced batteries, electrochemical condensers and fuel cells. A good understanding of the processes occurring in micro and nanostructure electrodes is expected, in order to improve performance and reduce degradation.

Qualifications requirements include a PhD in Chemistry, Physics, Chemical Engineering, or Material Sciences (with an emphasis on electrochemistry) and knowledge of processes associated with nanostructure materials for electrodes and electrolytes.

We are looking for a motivated and experienced research leader with initiative to develop an ambitious research programme. Applicants should be open to interdisciplinary collaboration with other research centres.

Preference will be given to candidates with experience in leading research teams focused on the afore-mentioned fields of interest in research bodies of recognised prestige. Applicants should be fluent in English, and knowledge of Spanish and/or Basque would be useful but not mandatory. All applicants are invited to submit a detailed biography.

Applications should be sent to: **cicenergigune@ikerbasque.net**
Deadline: **15 october, 2008**

www.cicenergigune.com

MAMINA European Network

Macro, Micro and Nano Aspects of Machining- MAMINA
(FP7 – PEOPLE – Initial Training Networks)
6 three-year PhD-projects and 4 one-year projects

The MAMINA project will combine the work of 19 European universities, research institutions and industrial companies to analyse and improve the machinability of 3 high performance alloys that are widely used in industry. The chip formation process will be studied in detail in experiments and simulations from the nano to the macroscopic scale. Different approaches will be made to improve metal cutting operations of the investigated alloys. The results will be transferred to real industrial applications.

The MAMINA network currently offers 10 positions with project duration up to 3 years for early stage researchers from the fields of theoretical and experimental physics, material science and mechanical engineering starting from August to November 2008.

At the Technische Universität Braunschweig, Germany, new alloys with improved machinability will be developed, produced, investigated and tested in industrial applications within the PhD-project Development of Free-machining Alloys (ESR 1). The cutting process will be simulated in the PhD-project Finite-Element-Simulation of the Cutting Process (ESR 2). In the project Investigation of High-performance Alloys (ESR S1) the chemical, physical and mechanical properties of high-performance alloys will be investigated.

All projects are carried out in different European countries including work at industrial partners of the consortium. Besides the research activities, the education will be completed by the attendance of a unique training programme including participation in international schools and conferences.

Please visit the MAMINA webpage **www.mamina.eu** for detailed description of all individual projects and for online application.

Grant for Postdoctoral Positions in Sweden

This grant enables researchers with doctorates (PhDs or equivalent) to work at Swedish higher education institutions or research establishments. The programme spans up to two years. Research areas: Natural Sciences, Engineering Sciences, Medicine, Humanities, Social Sciences and Educational Sciences.

Call for applications opens early July.
Submission deadline is August 25, 2008.

Further information at www.vr.se

Vetenskapsrådet

UNIVERSITY OF HELSINKI

The Institute for Molecular Medicine Finland (FIMM, www.fimm.fi) was established in 2007 as an international research institution, characterized by its partnership with the European Molecular Biology Laboratory (www.embl.org) along with Nordic nodes for molecular medicine in Norway and Sweden. At the national level, FIMM is a unique joint venture of the University of Helsinki, the Hospital District of Helsinki and Uusimaa (HUS), the National Public Health Institute (KTL) and VTT Technical Research Centre of Finland. FIMM headquarters are located in a brand new state-of-the art Biomedicum Helsinki 2 research building on the Meilahti medical campus in Helsinki.

We are seeking outstanding candidates as a

Research Director of the FIMM Genome and Technology Center

for a five-year period starting January 1, 2009.

- The Research Director will oversee technology development and research service operations at the national Finnish Genome Center, with an existing staff of about 20 people. We value candidates with strong leadership skills and visions on technological and scientific priorities in the post-genome era and a track record in high-profile publications in human genetics, functional genomics, bioinformatics, systems biology and/or translational research.
- Detailed information about the Research Director's position is available at website www.fimm.fi/ > Open positions.
- Applications should be addressed to the Board of FIMM and sent electronically to the Institute for Molecular Medicine Finland (FIMM) at fimm-email@helsinki.fi to arrive **no later than September 5, 2008, at 3.45 p.m.** (at 1.45 p.m. GMT).

Helsinki, July 3, 2008
Administration Office

HUS KTL VTT EMBL

www.helsinki.fi/university

HELMHOLTZ ZENTRUM BERLIN für Materialien und Energie

berlin

The Helmholtz-Zentrum Berlin für Materialien und Energie GmbH (HZB)

Member of the Hermann von Helmholtz-Gemeinschaft Deutscher Forschungszentren e.V.
and the
Technische Universität Berlin (TUB)
invite applications for a joint appointment in the field

„Materials Research for Photovoltaics"

as

Head of Department

"Materials Research"
in the Division Solar Energy Research of HZB and

University Professor (W3)

at the Fakultät II "Mathematik und Naturwissenschaften"
of the Technische Universität Berlin.

Solar Energy Research at the HZB focuses on the development of thin-film solar cells. It builds upon a rather unique variety of analytical methods, a basis which will get even stronger by the merger of the HZB and BESSY to the Helmholtz-Zentrum Berlin für Materialien und Energie, scheduled for January 1, 2009. Presently, research at the HZB is mainly covering inorganic absorbers based upon compound semiconductors or poly-crystalline silicon, supplemented by activities aiming for new materials and novel concepts and process technologies.

HZB and TUB aim to promote research and academic education towards innovative, in particular organic and/or nano-structured absorbers and corresponding solar cell concepts. We therefore search for a distinguished physicist, chemist or materials scientist with an outstanding international reputation and expertise in the field of organic semiconductors and/or nano-structures of functional systems relevant for photovoltaics. He/she is supposed to develop a long-sighted research program for the HZB-department "Materials Research", with a special focus on materials science supporting the development of innovative photovoltaic devices. We expect him/her to be committed to the general program of teaching and examining of students at the TUB, to inspire the scientific work in his/her department and to encourage internal and external collaborations.

Applications should include a curriculum vitae, a list of publications and previously taught courses, a statement of research and teaching interests, and up to five selected publications and are to be sent until 31 August 2008 to Prof. Dr. Michael Steiner, Scientific Director, HZB, Glienicker Straße 100, 14109 Berlin, Germany. Who for further information may also be contacted by phone (+49 (0) 30 8062 2762) or e-mail steiner@helmholtz-berlin.de.

To accelerate the process, applicants are kindly requested to send their application materials both in written form as well as electronically via e-mail. Application materials will not be returned. Therefore, you are requested to send only copies of all documents. Applicants must meet the legal requirements for appointments of professors in accordance with § 100 of the "Berliner Hochschulgesetz". Habilitation or documented evidence of equivalent scientific qualifications is required.

HZB and TU are equal opportunity employers, committed to the advancement of individuals without regard to race, colour, religion, sex, age, national origin, ethnicity, disability or any other protected status. HZB and TU seek to increase the proportion of female faculty members. Thus qualified women are particularly encouraged to apply.

This appointment will be decided in close co-ordination with the appointment „Charge Carrier Dynamics in Solar Cells".

HELMHOLTZ ZENTRUM BERLIN für Materialien und Energie

Freie Universität Berlin

The Helmholtz-Zentrum Berlin für Materialien und Energie GmbH (HZB)
Member of the Hermann von Helmholtz-Gemeinschaft Deutscher Forschungszentren e.V.
and the
Freie Universität Berlin (FU)
invite applications for a joint appointment in the field

„Charge Carrier Dynamics in Solar Cells“

as

Head of Department

“Charge Carrier Dynamics”
in the Division Solar Energy Research of HZB and

University Professor (W3)

in the Department of Physics of the FUB

Solar Energy Research at the HZB focuses on thin-film photovoltaics. A large variety of analytical methods including experiments at the synchrotron source BESSY II and at the HZB research reactor BER II offers excellent prerequisites to control the interdependence of the properties of a material and its inner structure. The situation will be further improved when HZB und BESSY merge to the Helmholtz-Zentrum Berlin für Materialien und Energie, by January 1, 2009.

A crucial task for any type of solar cell is the detailed understanding of how the optically induced charge carriers are separated and move through the complex structures of the cell. The new department “Charge Carrier Dynamics” should integrate the relevant expertise already existing at the HZB and develop and provide new experimental techniques, in particular those using synchrotron radiation. A better understanding of the interdependence of structural and electronic properties should help in identifying the processes, which are relevant for limiting the efficiency of a solar cell.

To head the new department, we search for a distinguished physicist, chemist or material scientist with an international reputation based upon relevant contributions to thin-film photovoltaics. Expertise in spectroscopic methods, in particular those utilizing synchrotron radiation, will be especially appreciated. The successful candidate is expected to develop a convincing future-oriented research program within the frame sketched above.
We expect the successful applicant to be committed to the general program of teaching and examining of students at the Freie Universität (the compulsory amount of teaching will be 2 hours per week per semester), to inspire the scientific work in his department and to foster internal and external collaborations.

To be appointed, he/she must meet the requirements of the Berlin Higher Education Act (§ 100 BerlHG, more detailed information available on request). HZB and FU are equal opportunity employers, committed to the advancement of individuals without regard to race, colour, religion, sex, age, national origin, ethnicity, disability or any other protected status. HZB and FU seek to increase the proportion of female faculty members. Thus qualified women are particularly encouraged to apply. Handicapped applicants will be given preference over others of equal qualification.

Applications should be received until 31 August 2008 and should be addressed to Prof. Dr. Michael Steiner, Scientific Director, HZB, Glienicker Straße 100, 14109 Berlin, Germany, who for further information may also be contacted by phone (+49 (0) 30 8062 2762) or e-mail (steiner@helmholtz-berlin.de).

This appointment will be decided in close co-ordination with the appointment „Materials Research for Photovoltaics”.

Associate Director Translational Medicine and Drug Development - Geneva

Medicines for Malaria Venture

Medicines for Malaria Venture (MMV) was established in 1999 as a partnership between the public and private sectors to discover, develop and deliver new antimalarial drugs at prices affordable to developing countries, with a view to ultimately eradicate this terrible disease.

MMV is based in Geneva as an independent not-for-profit Swiss Foundation. It has an entrepreneurial modus operandi and has established a new business model through which it selects and manages its R&D portfolio.

We are looking for a talented Associate Director Translational Medicine and Drug Development to join our scientific staff and contribute to the impact of our scientific programmes.

Primary duties

- Assume responsibility for project from candidate declaration to end of phase I.
- Formulate development project plans in collaboration with clinical development departments.
- Ensure that translational projects meet TPP requirements.
- Assist the Expert Scientific Advisory Committee in selection of development projects; proactively create new partnerships developing new medicines.
- Review assessment reports to support candidate selection.
- Collect and provide information to support project progress to phase and human exposure.
- Ensure that IMP is manufactured to GMP specifications.
- Ensure that animal studies and clinical trials are conducted under GLP / GCP standards and are sufficient to achieve product registration.

Essential qualifications

- PhD and/or MD with experience in biochemistry, toxicology, parasitology/ infectious diseases background is an asset.
- Project management and leadership experience in preclinical and early clinical development; at least 5 years of experience that is directly related to the duties and responsibilities specified.
- Knowledge of regulatory framework (GMP, GCP and GLP).

For a detailed job description please visit **www.mmv.org** Interested applicants should send their complete file before July 31st 2008 to **jobs@mmv.org**

Director Clinical Science - Geneva

Medicines for Malaria Venture

Medicines for Malaria Venture (MMV) was established in 1999 as a partnership between the public and private sectors to discover, develop and deliver new antimalarial drugs at prices affordable to developing countries with a view to ultimately eradicate this terrible disease.

MMV is based in Geneva as an independent not-for-profit Swiss Foundation. It has an entrepreneurial modus operandi and has established a new business model through which it selects and manages its R&D portfolio.

We are looking for a talented Director Clinical Science to join our scientific staff and contribute to the impact of our scientific programmes.

Primary duties

- Understand the regulatory environment: Know and respect the regulations surrounding the primary activities of the clinical development projects.
- Management of Drug Safety: Insure that the clinical teams' handling of all AE's and SAE's is according to ICH standards.
- Medical and Scientific: Provide medical input to ensure the scientific quality of the clinical programs.
- Resource Utilization: Operate within time and budget constraints of the clinical program.
- People Development: Work in collaboration with the Medical Director to assist the clinical development team to enhance skills of all members of the team.
- Effective Communication: Communicate project related information including the planning and execution of meetings and presentations.

Essential qualifications

- MD, or MD/PhD with at least 3 to 5 years of experience directly related to the duties and responsibilities specified.
- Knowledge of randomized controlled clinical trials principles, methodology, and procedures.
- Knowledge of adverse medical event investigation, analysis, and reporting procedures and standards.
- Knowledge of FDA and EMEA regulatory requirements and ICH/GCP guidelines is preferred.

For a detailed job description please visit **www.mmv.org**. Interested applicants should send their complete file before July 31st 2008 to **jobs@mmv.org**

molecular systems biology

EDITOR

MOLECULAR SYSTEMS BIOLOGY

Nature Publishing Group (NPG) and the European Molecular Biology Organization (EMBO) jointly publish **Molecular Systems Biology**, a scientifically outstanding journal that covers all aspects of the interdisciplinary field of systems biology at the molecular level. The journal is faced with rapidly increasing numbers of high quality submissions and we are thus looking for a new EMBO in-house scientific editor.

This is an exciting and challenging opening. The editor will work together with the existing EMBO editorial team, and share responsibility for maintaining its high scientific standards. This will involve intense interactions with prominent researchers together with a high level of responsibility and visibility.

Specific responsibilities will primarily include managing the peer-review process and making decisions on acceptance or rejection. Additionally, the editor will also be engaged in commissioning and editing Reviews, News & Views and Editorials and will work closely with the Journal's Senior Editors and Nature Publishing Group to implement the journal's editorial policies and strategies.

This is a great opportunity to continue to work in science and to be intensively exposed to high quality research in the rapidly developing new disciplines of Systems- and Synthetic Biology.

Candidates should have a strong scientific background, a PhD, post-doctoral experience and several publications. They should have a strongly developed interest in Systems Biology, a thorough knowledge of molecular biology and a broad interest in diverse areas of the life sciences. A good working knowledge of English is essential, as are also good communicative skills and an ability to work well in a team.

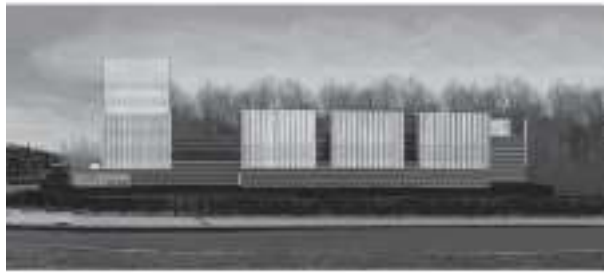
An initial contract of 3 years will be offered to the successful candidate. This can be renewed, depending on circumstances at the time of review.

npg EMBO

CIC nanoGUNE

nanoscience cooperative research center

The big challenge of the small



The Nanoscience Cooperative Research Center, CIC nanoGUNE Consolider, located in San Sebastián (The Basque Country, Spain), will open the doors of its own building with state-of-the-art facilities for nanoscience research after summer 2008.

www.nanogune.eu

ALBERT-LUDWIGS-
UNIVERSITÄT FREIBURG

The University Medical Center Freiburg, Germany seeks to recruit an outstanding physician scientist for the position of a

W 3-Professor for Rheumatology and Clinical Immunology (Succession of Professor Dr. H.-H. Peter)

As head of the Division of Rheumatology and Clinical Immunology the candidate will be in charge of teaching, research and patient care in the field. The Division runs a large outpatient clinic as well as an inpatient facility for inflammatory rheumatic diseases and adult immunodeficiencies. The candidate is invited to join ongoing research activities within a Collaborative Research Group for "Immunodeficiency" (SFB620) and a Focus on "Infectious diseases and Immunology" supported by several clinics and institutions of the Medical Faculty and the Max Planck Institute for Immunobiology.

The candidate must possess an MD degree, a board certificate in Internal Medicine and Rheumatology, experience in Clinical Immunology (e.g "Fachimmunologe" of the German Society for Immunology) and a postdoctoral lecturing qualification (Habilitation or equivalent). Furthermore the candidate is expected to have outstanding credentials in research, teaching and patient care. She/he must have impeccable interpersonal, managerial, and supervisory skills as well as extensive experience in coordinating clinical, educational, and research programs.

The duties and responsibilities in the field of patient care will be subject to a separate employment contract with the University Medical Center Freiburg. The Faculty of Medicine and the University Medical Center intend to reorganize patient care and medical research in new interdisciplinary centers. In this context the Division of Rheumatology and Clinical Immunology will be fully integrated into an innovative Excellence Center for Chronic Immunodeficiency (CCI) supported by the Federal Ministry of Education and Research (BMBF).

The W 3-position is tenured according to § 50 para. 1 State University Law (Landeshochschulgesetz) and available on October 1st, 2009.

The University of Freiburg is an equal opportunity employer. Applications of women are strongly encouraged. Handicapped candidates with equivalent qualifications will be given preference as well.

For application forms please send an E-Mail to dekanat-professuren@uniklinik-freiburg.de. Completed applications along with all pertinent documents should be sent to the Dean of the Medical Faculty, Prof. Dr. med. Christoph Peters, Albert-Ludwigs-University, D-79085 Freiburg (Phone: ++49-761 270-7235/7234, Fax: ++49-761-270-7236) no later than August 31st, 2008.

CHALLENGE THE FUTURE

Junior & Senior Faculty for New Dept. of Bionanoscience

Faculty: Applied Sciences
Term of contract: Tenure track or tenured

An entirely new Department of Bionanoscience will be established at TU Delft, dedicated to research at the interface between nanoscience, synthetic biology, and cell biology. It will study single cells in all their complexity down to the molecular level, from both fundamental scientific and application points of view. The new activities will extend the current nanoscience research themes at the Kavli Institute of Nanoscience (www.ns.tudelft.nl) and the life sciences and biotechnology themes at the Kluyver Centre (www.bt.tudelft.nl). The current biophysics core group (www.ns.tudelft.nl/mb) is very internationally oriented and has an excellent record in single-molecule nanoscience. Excellent facilities are already available, and the planning for a new, state-of-the-art building for the Biotechnology, Chemical Technology, and Bionanoscience departments is in its final stages.

We aim to attract about 15 top scientists at all levels from junior to senior faculty in a variety of disciplines. Research subjects can cover, but are not limited to, the following topics: 1) Biology and biophysics at the level of the single cell, 2) Molecular/structural biology and biochemistry, 3) Synthetic biology, 4) Theoretical biology and biophysics, 5) Single-molecule biophysics and high-resolution microscopy, 6) Nanoprobes, nanomedicine, and bionanoapplications.

We look for researchers with an outstanding track record in the disciplines mentioned above, with a pioneering mentality, people who are at ease with scientific and engineering challenges and who through their research, management skills and teaching qualities can inspire co-workers and students alike. The successful candidates will be expected to establish a strong, externally funded research programme, and be committed to excellence in teaching at the undergraduate and graduate levels.

Information and application
For more information about the job and procedure you can visit our website: www.bn.tudelft.nl

www.bn.tudelft.nl

TU Delft
Delft University of Technology

NRF RESEARCH FELLOWSHIP

The Singapore National Research Foundation (NRF) invites brilliant, young researchers who are ready for their first independent research appointments to apply for the prestigious NRF Research Fellowship Awards.

✓ **Are you among the best in your research field?**
✓ **Are you ready to lead your first independent research team?**
✓ **Join the ranks of the elite NRF Research Fellows!**

Apply now if you have a PhD degree from a reputable university and work at the forefront of research in your field. A prior post-doctoral stint at a renowned university or research organisation would be a great advantage.

The NRF Research Fellowship provides:

- Complete freedom and independence to pursue your research direction in Singapore
- A 3-year research grant of up to US$1.5 million, with a possible extension for another 3 years
- A competitive salary
- The opportunity for joint appointment at the host university or research institution
- Freedom to select the host institution in Singapore

The NRF Research Fellowship is open to all talented scientists and researchers under the age of 39 years at the date of application, and within 10 years post-PhD. We welcome research in all disciplines of science and technology.

Please apply online at the following web-link before **30 September 2008**:

https://rita.nrf.gov.sg/AboutUs/NRF_Initiatives/NRF_RF_2008/default.aspx

Shortlisted candidates will be invited to Singapore to present their research work, meet local researchers and identify potential collaborators and host research organisations. Final selection for the awards will be made by the NRF Scientific Advisory Board co-chaired by Dr Curtis Carlson (President & CEO of SRI International) and Prof. Ulrich Suter (Professor of Polymer Materials, ETH Zurich).

For further queries, please email **karen_tan@nrf.gov.sg**

About the National Research Foundation

The NRF supports the Research, Innovation and Enterprise Council chaired by the Prime Minister to provide a coherent strategic overview of R&D policies and direction in Singapore. It manages a S$5 billion National Research Fund to develop R&D as a key driver in transforming Singapore into a knowledge and innovation based economy.

Singapore National Research Foundation
100 High Street, #03-02, The Treasury
Singapore 179434
Tel: +65-63329010
Website: www.nrf.gov.sg

ASSISTANT/ASSOCIATE PROFESSOR

DEPARTMENT OF MEDICINAL CHEMISTRY
COLLEGE OF PHARMACY, UNIVERSITY OF MINNESOTA
CANCER EXPERIMENTAL THERAPEUTICS

The University of Minnesota, Department of Medicinal Chemistry in the College of Pharmacy invites applications from outstanding candidates for a twelve-month, full-time, tenure-track Assistant Professor or tenured Associate Professor position. The University seeks applicants with a Ph.D. degree in medicinal chemistry or related fields and the potential to develop or a record of creating a nationally recognized, externally funded research program in medicinal chemistry with an emphasis on the development of experimental therapeutics for treating cancer. Department of Medicinal Chemistry at the University of Minnesota is equipped with extensive resources for drug discovery including chemical process development core, high throughput screening facility, X-ray crystallography, and modern bioanalytical facilities. The successful candidate will interface with basic scientists and clinicians in the Comprehensive Cancer Center at the University of Minnesota in an effort to enhance the discovery and development of experimental therapeutics and will participate in teaching professional students in the College of Pharmacy and graduate level courses in the Department of Medicinal Chemistry. To apply, please go to www.pharmacy.umn.edu/employment for application instructions and links. Review of completed applications will begin immediately and will continue until the position is filled. For specific questions regarding this position, please contact the Search Co-Chairs, Dr. David M. Ferguson (ferguson@umn.edu) and Dr. Natalia Tretyakova (trety001@umn.edu).

Details about the Department and the position can be found at:
www.pharmacy.umn.edu/medchem
For additional information, please visit the following links:
Institute for Therapeutics Discovery and Development:
http://www.pharmacy.umn.edu/medchem/ITDD.html
and Masonic Cancer Center: http://www.cancer.umn.edu/about/

UNIVERSITY OF MINNESOTA

The University of Minnesota is an equal opportunity educator and employer.

Regional Project Coordinator

United Nations Development Programme

The United Nations Office for Project Services seeks a highly qualified Regional Project Coordinator to implement UNDP's Sustainable Management of the Shared Living Marine Resources to the Caribbean Large Marine Ecosystem (CLME) and Adjacent Regions, based in Cartagena, Columbia. This United Nations Development Programme effort is funded by the Global Environment Facility and executed by the United Nations Office for Project Services.

The deadline for applications is 25 July 2008.

For details on this and other positions please visit the UNOPS website:
www.unops.org and see Employment Opportunities.

UNOPS

United Nations Office for Project Services

UNOPS helps its partners meet the world's needs for building peace, recovering from disaster, and creating sustainable development. UNOPS recruits staff for a broad range of complex and demanding projects around the globe.

NIAID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

TENURE/TENURE-TRACK POSITIONS IN VIROLOGY AND IMMUNOLOGY

Laboratory of Virology
Rocky Mountain Laboratories, Hamilton, Montana
National Institute of Allergy and Infectious Disease / National Institutes of Health
Department of Health and Human Services

The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR), Laboratory of Virology (LV), Rocky Mountain Laboratories (RML), NIH, DHHS, in Hamilton, Montana, seeks applicants for one or two tenure-track/tenured positions (assistant/associate professor equivalent) to conduct independent research on viral agents requiring high or maximum containment.

The LV conducts high-impact, innovative scientific research on viral agents requiring high or maximum containment, including filoviruses, bunyaviruses, arenaviruses, and flaviviruses with the goal of developing diagnostics, vaccines, and therapeutics. The research conducted in the LV includes studies of vector/reservoir transmission, pathogenesis, pathophysiology and host immune response of high containment viral pathogens. Candidates must be able to develop an independent research program, supervise staff and fellows, and collaborate with RML/DIR researchers working on other viral diseases.

One selected candidate is expected to implement and direct a vigorous, independent research program in molecular virology, antivirals, or vectors and virus transmission of agents requiring high or maximum containment. Fieldwork and ecological studies is a desirable component of these research programs.

The other selected candidate is expected to implement and direct a vigorous, independent research program in host immune responses and/or vaccines against viral agents requiring high or maximum containment. This program is expected to include studies of innate and adaptive immune responses in animal models.

Candidates for either position must hold a Ph.D., D.V.M, or M.D. degree and have a minimum of 3 years of relevant postdoctoral experience. Independent resources including space, support personnel, and an annual budget for services, supplies, and salaries are committed to the positions. Facilities at existing NIAID field sites in Africa and Asia may be available to the incumbents, and support for field work and ecological studies at new sites is possible. These are appointments under Title 42. Salary is dependent on experience and qualifications.

RML's state-of-the-art facilities include an operational BSL-3 facility, a BSL-4 laboratory and animal facility nearing completion that can accommodate work with both small animal and non-human primate models, and core facilities for genomics, electron microscopy, and flow cytometry. Other RML research programs focus on prions, retroviruses, numerous pathogenic prokaryotic organisms and pathogen transmission by arthropod vectors. RML is located in the scenic Bitterroot Valley of western Montana within easy access to some of the finest outdoor recreational opportunities in North America. Additional information on the positions can be obtained by contacting Dr. Marshall E. Bloom at **mbloom@niaid.nih.gov.**

Application Process: To apply, submit a curriculum vitae and bibliography, including a list of your five most significant papers and a 2-3-page description of a proposed research program via e-mail to Wanda Jackson at **NIAID.DIR.Search@niaid.nih.gov**. In addition, three letters of recommendation must be sent directly from the referees to Dr. Jeffery Taubenberger, Chair, NIAID Search Committee, c/o Wanda Jackson at **NIAID.DIR.Search@niaid.nih.gov** or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, Maryland 20892-1356. E-mail is preferred. Completed applications will be reviewed starting **September 1, 2008**. Please refer to ad #023 on all correspondence. Further information regarding LV is available at **http://www3.niaid.nih.gov/labs/aboutlabs/LV/** , information regarding the DIR laboratories is available at **http://www3.niaid.nih.gov/about/organization/dir/default.htm** and information about working at NIAID is available at **http://healthresearch.niaid.nih.gov**.

Applicants must be U.S. citizens, resident aliens, or nonresident aliens with or eligible to obtain a valid employment-authorizing visa.

Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases
Proud to be Equal Opportunity Employers

National Cancer Institute

CHIEF, CHEMICAL BIOLOGY LABORATORY

Application Deadline: September 15, 2008

NCI is seeking an outstanding, internationally recognized scientist to serve as Chief of the Chemical Biology Laboratory (CBL) in the Center for Cancer Research (CCR). The position, which is the equivalent of an academic Department Chair, is a key component of a major initiative to build CCR's chemistry program at the Frederick campus (http://www.ncifcrf.gov/). The CBL Chief will play a leading role in developing an integrated program of chemistry, structural biology, and lead compound discovery that both promotes the application of chemical biology approaches across CCR's research portfolio and interfaces with the Division of Cancer Treatment and Diagnosis's Chemical Biology Consortium. In addition to institute-wide responsibilities, the CBL Chief will direct an extensive individual research program that will complement and augment CCR expertise in chromosome biology, immunology, HIV/AIDS, cancer biology and molecular oncology, areas in which its Centers of Excellence have been established. Supported with stable financial resources, the CBL will have access to a wide array of intellectual and technological assets, including high-quality technology cores dedicated to protein chemistry, natural products chemistry, biophysics, mass spectrometry, imaging, microscopy, proteomics and genomics, bioinformatics/biostatistics, and flow cytometry, in addition to clinical support.

The National Cancer Institute (NCI) is part of the National Institutes of Health (NIH) in the Department of Health and Human Services (DHHS), a federal government agency. CCR is the largest component of the NCI Intramural Research Program, providing an environment conducive to advancing translational research and collaborative interactions through investigator-initiated and interdisciplinary team science. Additional information on CCR research priorities can be found at: http://ccr.cancer.gov/.

In addition to a Ph.D. or M.D./Ph.D. degree in a relevant discipline, applicants should possess outstanding communication skills and documented leadership experience. Tenured faculty or industrial scientists of equivalent rank with a demonstrated commitment to chemical biology should apply. Salary will be commensurate with experience and accomplishments. Applications should include a description of research interests and leadership philosophy, career synopsis, and current curriculum vitae with complete bibliography.

Applications should be postmarked or received by email at cortnerj@mail.nih.gov by September 15, 2008. Send applications to: **Stuart Le Grice, Ph.D., Chair, Chemical Biology Laboratory Search Committee, c/o Janelle Cortner, Ph.D., Building 428, National Cancer Institute at Frederick, Frederick MD 21702.**

DHHS, NIH and NCI are Equal Opportunity Employers

Postdoc Position

A funded post-doctoral fellowship is currently available at the Center for Cancer Research (CCR), National Cancer Institute (NCI), Frederick, MD for a productive, highly-motivated, and energetic individual. The opening is for a project studying the relationship between T cell avidity and tumor immunity using novel murine models. A dynamic research environment and outstanding resources at NCI-Frederick are available for enthusiastic individuals. Requirements include an M.D., Ph.D., or equivalent degree and experience in Immunology research. Candidate must have excellent verbal, written, communication and organizational skills and an ability to handle multiple projects simultaneously. Experience with mouse models is preferred. More information on research projects can be found at **http://ccr.cancer.gov/staff/staff.asp?profileid=7740**. Interested individuals should send their CV and a letter of research interests to Dr. Andy Hurwitz: **hurwitza@ncifcrf.gov**.

Postdoc Positions

Two post doctoral positions are available immediately in the Laboratory of Cellular and Molecular Biology (LCMB) and the Laboratory of Cellular Developmental Signaling LCDS), Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS). The fellows in these positions will participate in a multi-disciplined program focused on understanding the function and regulation of Arf GTPase-activating proteins in cell adhesion and migration associated with development and cancer cell invasion and metastasis.

Position 1: Arf GTPase-activating proteins in Xenopus laevis development. This project will focus on the ARAP proteins, which are dual Arf GAPs/Rho GAPs that occur only in vertebrates. The ARAPs regulate cell adhesive structures, the actin cytoskeleton and trafficking of transmembrane receptors. The Xenopus system will be used to determine how the role of ARAPs in cell adhesion and migration affect morphogenetic processes in vivo.

Position 2: ARAPs and ASAPs control of focal adhesions and invadopodia in mammalian cells. Several Arf GAPs, including ASAP1, ASAP2 and ARAP2 regulate focal adhesions and control cell migration ASAP1 also associates with invadopodia, stuctures that mediate invasion of cancer cells. Biochemical and imaging techniques, including confocal and total internal reflection microscopy, will be used to determine molecular mechanisms underlying Arf GAP control of the formation and turnover of these structures.

Candidates interested in position 1 should send a cover letter, CV including bibliography and contact information for three references to: **Ira O. Daar, PhD, LCDS, NCI-Frederick, Building 560, Frederick, MD 21702, e-mail: daar@ncifcrf.gov**.

Candidates interested in position 2 should send materials to **Paul A. Randazzo, M.D., Ph.D, LCMB, NCI, Building 37, Room 2042, Bethesda, MD 20892, e-mail: randazzp@mail.nih.gov**. Salary is commensurate with research experience and accomplishments.

WWW.NIH.GOV

NIAID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Help Us Help Millions

POST-DOCTORAL FELLOW - LABORATORY OF IMMUNOLOGY, NIAID - RNA INTERFERENCE AND MICRORNAS

A postdoctoral position is available in the group of Dr. Stefan Muljo to study the role of the endogenous RNA interference machinery (e.g. microRNAs) in the immune system. Applicants should have a recent Ph.D. and/or M.D. degree and publications in peer-reviewed journals. Talented individuals with backgrounds in physics, statistics or computer science are encouraged to apply. The postdoctoral fellow is expected to be a highly motivated, creative and independent fast learner with a strong work ethic, excellent organizational, writing and communication skills, and critical thinking abilities. Must be able to design and execute experiments. The work will be carried out at the main NIH campus in Bethesda, Maryland (near Washington, DC) at the Laboratory of Immunology, National Institute of Allergy and Infectious Disease. There will be a stimulating working environment with access to a highly dynamic group of colleagues, state-of-the-art equipment, research core facilities, and excellent funding. The successful candidate will be expected to take a multidisciplinary approach towards systematically understanding how the molecular circuits in mammalian cells are wired - with particular emphasis on the network controlled by the RNAi system.

Qualified candidates should send their CV, statement of previous, current and future research interests and goals, and name & contact details of at least three referees to Dr. Stefan Muljo c/o Angela Devin via e-mail at devina@niaid.nih.gov or via mail at:

Dr. Stefan Muljo
Laboratory of Immunology, NIAID, NIH
9000 Rockville Pike, Building 10, Room 11N254
10 Center Drive – MSC 1892
Bethesda, MD 20892-1892

For more information about NIAID and to view additional job opportunities, please visit:
http://healthresearch.niaid.nih.gov/li

Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases
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NIEHS

Department of Health and Human Services
National Institutes of Health (NIH)
Director, National Institute of Environmental Health Sciences (NIEHS)

THE POSITION: The NIH is seeking exceptional candidates for the position of Director, NIEHS, to provide leadership to one of the preeminent centers for environmentally-related research in the world. The Director, NIEHS, also serves in a dual role as the Director, National Toxicology Program, and in this role reports to the Secretary, Department of Health and Human Services (DHHS). This position offers a unique opportunity for the right individual to provide strong and visionary leadership to an organization dedicated to reducing the burden of human illness and dysfunction from environmental causes by understanding each of these elements and how they interrelate. The Director will manage a high-level complex organization and must demonstrate integrity and fairness, adhering in work and behavior to the highest ethical standards, and upholding the highest standards of scientific research and/or business practices. Applicants must possess an M.D. and/or Ph.D. and have senior-level research experience and knowledge of research programs in one or more scientific areas related to environmental effects on human health and/or toxicology research. They should be known and respected within their profession, both nationally and internationally, as individuals of outstanding scientific competence. Salary is commensurate with experience, and full Federal benefits, including leave, health and life insurance, retirement and savings plan (401K equivalent) will be provided. A detailed vacancy announcement that includes application procedures is available at **http://www.jobs.nih.gov (under Executive Jobs)**. **NIEHS is located in Research Triangle Park (RTP), North Carolina**. Questions may be addressed to **Ms. Lynnita Jacobs at: SeniorRe@od.nih.gov**. CV and bibliography must be received by **11:59 p.m. Monday, August 4, 2008.**

HELIOS

Helios Staff Scientist

Berkeley Lab is a world leader in science and engineering research, with 11 Nobel Prize recipients, and 60 present members of the National Academy of Sciences. Berkeley Lab conducts unclassified research across a wide range of scientific disciplines and hosts four national user facilities. **www.lbl.gov**

The Helios Solar Energy Research Center (SERC) at Berkeley Lab invites applications for a Staff Scientist in the field of solar fuel generation by engineered materials (artificial photosynthesis).

This role oversees multi-disciplinary research directed towards the development of novel, nano-scale systems for the conversion of solar energy to fuel. Funding is provided and includes support for research staff.

Research areas of primary interest include:

- Innovative nanoscale photovoltaics tuned to support catalytic activities
- Development of improved catalysts to split water and or to reduce carbon dioxide
- Development of nanoporous or other platforms to support the components

The Staff Scientist may join an existing group project area or create an independent program and will be responsible for performing original and relevant research. The candidate will also be expected to directly supervise professional and technical support staff and prepare scientific data for publication.

Please visit **http://jobs.lbl.gov** and enter 21885 in the search field to view the job details and to apply.

Berkeley Lab is an Affirmative Action/ Equal Opportunity Employer committed to the development of a diverse workforce.

BECAUSE

We are focused on truly innovative science.

Postdoctoral Fellowships

For more than 30 years, Genentech has been at the forefront of the biotechnology industry, using human genetic information to develop novel medicines for serious and life-threatening diseases.

The Genentech Process Research and Development organization is offering Postdoctoral Fellowships in an academic setting to support promising university research that can be applied to advance its technologies for process development and for production of recombinant protein pharmaceuticals. Proposals for Fellowship support of any topic contributing to this goal are welcome, especially novel approaches to expression, purification, analysis, formulation and manufacturing technology. Applications are invited for standard awards of $80,000 for one year, renewable for a second year.

To apply and for complete details, please visit **careers.gene.com** and reference Requisition **#1000023452.** Use "Ad – Science" when a source is requested. Inquiries can be directed to Adelle Lohse at **lohse.adelle@gene.com.**

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Genentech
IN BUSINESS FOR LIFE

BROOKLYN COLLEGE
The City University of New York

ZICKLIN PROFESSORSHIP IN BIOLOGY

The Biology Department at Brooklyn College has received an endowment to help support a microbiologist. This position is available at the Associate or Full Professor level, depending upon qualifications. Applicants are expected to have a productive research record and a desire to teach at an urban institution in an expanding department. We expect the successful candidate to show leadership in developing a graduate specialization in microbiology, as well as to teach, and to establish a funded research program at Brooklyn College. Participation in the CUNY biology doctoral program is required, and participation in the CUNY doctoral program in biochemistry is also possible.

A Ph.D. in one of the biological sciences or a M.D. Degree and an established and continuing record of publications in peer-review journals. The successful candidate should have a well-defined independent research program that will generate external funding and provide research training for undergraduate and graduate students; a history of external funding; effective communication skills that will enable the candidate to teach at the undergraduate and graduate levels, and teaching experience in microbiology and/or related areas. Review of resumes will begin on 9/1/08 and continue until the position is filled.

Please send curriculum vitae, three letters of recommendation, and a letter of interest as one package to: Michael T. Hewitt, Assistant Vice President for Human Resource Services, Brooklyn College, 2900 Bedford Avenue, Brooklyn, NY 11210-2889.

For additional information please see our website at www.brooklyn.cuny.edu

BROOKLYN COLLEGE IS CUNY

An AA/EO/IRCA/ADA Employer

Vice-Chair for Research/Open Rank

The Department of Anesthesiology, University of Texas Health Science Center at San Antonio, Texas (UTHSCSA) invites nominations and applications for the position of Vice-Chair for Research (VCR). As the chief research officer for the department, the VCR is responsible for implementation of the research vision, the overall management of departmental research activities, and the administration of sponsored research. The VCR will engage in multidisciplinary collaboration within UTHSCSA—a Clinical and Translational Science Award (CTSA) grantee—and its affiliated institutions.

Qualifications for this position include an M.D., M.D.-Ph.D., or Ph.D. degree in an appropriate field of study. The successful candidate will have a national/international reputation as a distinguished scientist with an outstanding record of research accomplishments; a proven track record of directing a research enterprise; outstanding communication skills as evidenced by an ability to mentor junior faculty, scientists, residents, and students. The candidate must be a critical and strategic thinker and a visionary leader who can develop and enhance the research enterprise; and one who can demonstrate expertise in crafting interdisciplinary proposals and negotiating multi-faceted awards. One or more currently funded NIH grant(s) and experience in translational research is highly desirable. Given the excellent research infrastructure in neurobiology at UTHSCSA, research experience in pain medicine would be a plus.

For more information, please visit our website at **www.anesthesia.uthscsa.com**. To apply or nominate a candidate for the position of Vice-Chair for Research, Department of Anesthesiology, U.T. Health Science Center at San Antonio, please submit a current CV, supporting documents, and names and addresses of five references to: **J. Jeffrey Andrews, M.D, Chair, Department of Anesthesiology – MSC 7838, U.T. Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229.**

All faculty appointments are designated as security sensitive positions. The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer.

Faculty Position

Linde Center . Global Environmental Science . Caltech

Following the endowment of **the Ronald and Maxine Linde Center for Global Environmental Science**, the California Institute of Technology invites applications for faculty positions in Environmental Science and Engineering, with a focus on climate science. Terms of the initial appointment as assistant professor are four years and contingent upon completion of the Ph.D. Exceptionally qualified applicants may also be considered at the associate or full professor level.

Our focus is on candidates who have an outstanding research record and a strong commitment to teaching. We have the opportunity to make more than one appointment; areas of interest include (but are not limited to):

- Physical Oceanography
- Glaciology and Sea Ice Dynamics
- Cloud Physics and Dynamics
- Global-Scale Biogeochemistry

Please visit **www.ese.caltech.edu/climate** for instructions on how to apply. For information about the Linde Center, visit www.lindecenter.caltech.edu.

CALIFORNIA INSTITUTE OF TECHNOLOGY
Linde Center for Global Environmental Science
Caltech is an Equal-Opportunity/Affirmative-Action Employer.
Women, minorities, veterans, and disabled persons are encouraged to apply.

Professor and Chair of Biomedical Engineering
University of California – Davis

The College of Engineering at the University of California, Davis, invites applications for Chair, Department of Biomedical Engineering. The Department of Biomedical Engineering is the newest Department in the College of Engineering. Over the last 8 years, 18 new faculty have been recruited, with research expenditures now exceeding $10M per year. A new undergraduate major has been developed and the Department also plays an integral role in the interdisciplinary campus-wide Biomedical Engineering Graduate Group. The Department has existing strengths in the areas of cellular and molecular engineering, micro- and nanosystems, biomedical imaging, therapeutics, and computational and systems biology, with an integrating theme of molecular and/or genetically based approaches to the measurement and modeling of biological systems, and to the diagnosis and treatment of disease.

The college is seeking a senior distinguished scholar with an international reputation and an outstanding research record in Biomedical Engineering. Candidates should have a Ph.D. in Biomedical Engineering, or a closely related field, evidence of leadership ability, a commitment to excellence in teaching and a history of service to the field. Candidates whose research interests complement, yet clearly extend, existing research strengths are particularly encouraged to apply.

Interested candidates should submit all materials via the web-based online submission system (**www.http://bmejobs.gc.ucdavis.edu/**). Required materials include a curriculum vitae, a 1-page vision statement regarding the future of biomedical engineering, brief statements of their accomplishments and priorities in research and teaching, and the names and contact information for at least five evaluators who have agreed to write letters of reference. Inquiries can be directed to the chair of the search committee at **bme-chair@ucdavis.edu**. The deadline for full consideration is **September 8, 2008** although applications will be accepted until the position is filled.

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, individuals with disabilities and veterans.

EASTERN ILLINOIS UNIVERSITY

Eastern Illinois University invites applications for **Chair, Department of Biological Sciences**, 12 mo position beginning July 1, 2009. The Chair is responsible for administration of all instructional programs in Biological Sciences. The Department includes 25 tenured/tenure track faculty and large undergraduate and graduate programs. Qualifications include a Ph.D. in Biological Sciences or a related field with a teaching, research and service record commensurate for tenure and the rank of full professor. Candidates must have a strong commitment to undergraduate and graduate programs and the advancement of faculty/student mentoring and research programs.

For more information on this position and application instructions, see the website at:

www.eiu.edu/~civil/employment.htm

AA/EOE

The University of Georgia

Post-Doctoral and Research Technician Positions
Human Embryonic Stem Cell Biology

The University of Georgia announces a new 5 year Program in "Basic Human Embryonic Stem Cell Biology" funded by the National Institutes of Health, National Institute of General Medical Sciences. The Program will be dedicated to understanding basic hESC biology, early human development and establishing new technologies for the generation of therapeutically useful cell types. The Program, directed by Dr. Stephen Dalton, is seeking applications from post-doctoral fellows and experienced research technicians to participate in projects from the following areas relating to hESC biology: • cell signaling and self-renewal • mechanisms of cell fate commitment • cell cycle control • epigenetic regulation • glycobiology of hESCs • endoderm and pancreatic differentiation • multipotent cardiogenic progenitors • technology development

The Program will be closely affiliated with the Southeast Stem Cell Consortium (**www.sestemcells.uga.edu**) and will be highly interactive with other stem cell, cancer and developmental biology Programs and Centers in the Southeast region.

Applications including a letter of interest, CV and contact information for at least 3 references should be sent by Email to **Dr. Stephen Dalton, Paul D. Coverdell Center for Biomedical and Health Sciences, The University of Georgia, Athens, GA, USA** at **sdalton@uga.edu**.

EEO/AA

Dean of the College of Agricultural Sciences, Colorado State University

Colorado State University (CSU) seeks a visionary Dean for its College of Agricultural Sciences. The College is poised for major University investments and expansion of faculty to supply world class science in support of industries on the forefront of 21st century agriculture. Applicants must have an earned doctorate and a distinguished record of performance consistent with appointment as a tenured Full Professor.

Applications will be accepted until the position is filled. For full consideration, applications should be submitted electronically by **October 15, 2008**. Applicants should send a letter expressing interest and qualifications for the position; a separate two-page philosophy of education, research, leadership and management; curriculum vitae; and the names, e-mail addresses, addresses, and phone numbers of five references to: **Lance Perryman, Dean, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1601; lance.perryman@colostate.edu; phone 970.491.7051**.

General information about CSU and the College of Agricultural Sciences can be accessed at **www.colostate.edu** and **www.agsci.colostate.edu/strategicplan/intro.htm**.

CSU is an EEO/AA Employer.

WE BELIEVE

THE CURE WILL BE FOUND RIGHT HERE

Faculty Position: Melanoma Research

The Donald A. Adam Comprehensive Melanoma Research Center at the Moffitt Cancer Center and Research Institute is seeking laboratory-based faculty members with a Ph.D., M.D. or M.D.-Ph.D. with an interest in melanoma research. The prospective candidates will be appointed at the Assistant, Associate or Senior Member level, and it is expected that they would establish an independent funded laboratory research program concentrating on translational melanoma investigation in the fields of genetics, signal transduction, microenvironment, apoptosis or the cell cycle.

An outstanding start-up plan is available, as well as a highly competitive salary package with excellent lab space. A specific attraction is the opportunity to interact with ongoing well funded research programs in translational immunology/ immunotherapy, drug development, population science and molecular oncology. The Comprehensive Melanoma Research Center will bring together clinicians, basic and translational scientists at Moffitt to aggressively pursue new ideas in the etiology, treatment and prevention of melanoma. At the Moffitt Cancer Center, significant growth in basic and translational research, in laboratory space resources and faculty recruitment will occur in the next decade as a high priority.

Faculty of the Moffitt Cancer Center are eligible for academic appointments at the University of South Florida College of Medicine. Academic rank is commensurate with qualifications and experience.

Please reference position no. MRI01. Interested candidates should send curriculum vitae and a brief statement of major academic interests in one single .pdf document to Kathleen.jordan@moffitt.org, or apply online moffittcareers.org.

MOFFITT CANCER CENTER

Moffitt Cancer Center provides a tobacco-free work environment, is an Equal Opportunity, Affirmative Action employerand a drug-free workplace.

Tenure-track or Tenured Position in Geochronology, Petrology and Geodynamics

The Department of Geological and Environmental Sciences seeks an outstanding scientist to lead a vibrant research program in the broad areas of geochronology, petrology and geodynamics in order to address large-scale petrologic and tectonic processes in the Earth's crust and mantle. Our preference is to make an appointment at the junior or mid-career level, but applications from scientists at all career levels will be considered. The successful applicant will build on newly established and long-standing strengths in geochronology, tectonics, and isotope geochemistry within the Department, interface with solid-earth processes, crustal evolution, seismology and other areas in the School of Earth Sciences, and teach at the undergraduate and graduate level. We especially welcome applications from scientists who integrate geochemical/petrological and/or physical/ computational approaches to problem solving.

The Stanford School of Earth Sciences houses a full range of isotope geochemistry/geochronology/thermochronology facilities. These feature the Stanford-USGS SHRIMP-RG ion microprobe and associated TIMS laboratory; a new multi-collector ICP-MS and high-resolution ICP-MS facility supported by newly commissioned clean labs; new $^{40}Ar/^{39}Ar$ and (U- Th)/He, and fission-track thermochronology laboratories containing multi-collector and single-collector mass spectrometers and state-of-the-art extraction lines; and cosmogenic radionuclide laboratories. In addition, an electron microprobe, a scanning electron microscope with EDAX and cathodoluminescence imaging, and sample preparation and mineral separation laboratories are available. Related facilities include stable isotope laboratories, ICP-AES and GC-MS capabilities, high-pressure experimental capabilities including a diamond-anvil cell laboratory, and the recently established Center for Computational Earth and Environmental Science.

Stanford University is an equal opportunity employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as from others who would bring additional dimensions to the University's research and teaching missions.

Please apply online in electronic format (.pdf only) with the following application material: cover letter, curriculum vitae, a statement outlining research and teaching experience and interests, and the names and addresses (including e-mail addresses) of three potential referees, at http://pangea.stanford.edu/jobs/. Select the Geochronology, Petrology and Geodynamics faculty position.

Questions can be directed to Elizabeth Miller (elmiller@stanford.edu)

We will begin reviewing applications September 30, 2008. Deadline for receipt of applications is November 30, 2008.

POSITIONS OPEN

FACULTY RECRUITMENT
The Biodesign Institute

We are seeking an outstanding candidate for a nontenure-track faculty position beginning in the 2008-2009 academic year. The position is available at the **ASSISTANT, ASSOCIATE, or FULL PROFESSOR** level. The individual will be expected to conduct research in the Center for BioEnergetics at Arizona State University (ASU) as part of an interdisciplinary team. Research in the center involves mitochondrial energy production and is focused on function and dysfunction in the respiratory chain. By understanding changes in functions associated with diseases of the respiratory chain, molecular strategies appropriate for treatment of such diseases can be defined. The goal of the research is to understand the molecular nature of respiratory chain diseases and to discover potential therapeutic agents that can be used to treat these diseases.

Qualifications required: Doctoral degree in biochemistry, cell biology, or related areas and record of research and teaching experience appropriate to rank.

Desired qualifications: Experience in the study of mitochondrial energy production and diseases.

Application deadline and procedure: Deadline/closing date is August 29, 2008. Applicants must send a letter of application indicating the specific position applied for, current curriculum vitae, names and addresses of four references, copies of three recent publications to: **Faculty Position, Center for BioEnergetics, The Biodesign Institute, Arizona State University, 1001 S. McAllister Avenue, Tempe, AZ 85287-5001** or to **e-mail: gina.dunphy@asu.edu** with Research Faculty job posting in the subject line.

ASU is a major university widely recognized as a rapidly emerging research institution in the United States. The Biodesign Institute at ASU is focused on innovations that improve health care, provide renewable sources of energy and clean our environment, outpace the global threat of infectious disease, and enhance national security. The goal is to find solutions to complex global challenges and accelerate these discoveries to market. More information about the Biodesign Institute may be found at **website: http://www.biodesign.asu.edu.**

Arizona State University is an Equal Opportunity/Affirmative Action Employer. A background check is required for employment.

The University of Virginia's Department of Chemical Engineering seeks applications for multiple **RESEARCH ASSOCIATE/RESEARCH SCIENTIST** positions available in the area of computational modeling of heterogeneous and homogeneous catalysis, electrocatalysis, surface reactivity, and aqueous metal interfaces. Ph.D. in chemistry, physics, or chemical engineering and experience in the areas of quantum mechanical calculations, kinetic Monte Carlo simulation, or molecular dynamics as applied to modeling surface chemistry and catalysis are required. The work is in collaboration with experimentalists in academia and industry. Applicants must apply online at **website: https://jobs.virginia.edu** and search by Position Number F5021. Applicants must complete a Candidate Profile and attach a complete resume, cover letter, and contact information for three references. *The University of Virginia is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION

Postdoctoral position in cellular and molecular immunology to study mechanisms contributing to immune regulatory effects of helminth infection on type 1 diabetes. Highly motivated researchers having experience using mouse models to investigate the immunology of infectious disease and/or study immune regulatory mechanisms are preferred.

Please submit curriculum vitae and three letters of reference to: **e-mail: gausewc@umdnj.edu; William C. Gause, Ph.D., University Professor, UMDNJ-New Jersey Medical School, Department of Medicine.** UMDNJ is a member of the University Health Systems of New Jersey. *UMDNJ is an Affirmative Action/Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

POSITIONS OPEN

RESEARCH CHEMISTS – GERIATRIC ENDOCRINOLOGY AND METABOLISM
Veterans Health Administration
Washington, D.C., Veterans Affairs Medical Center

The Washington, D.C., Veterans Affairs Medical Center seeks two outstanding candidates (GS-14 and GS-13) for positions as full-time Research Chemists in geriatric endocrinology and metabolism in the laboratory of the Associate Chief of Staff for Research and Development (ACOS R&D), in the Research Service of the Washington, D.C., VA Medical Center campus. Applicants must possess a Ph.D. and/or M.D. degree.

The successful candidates will have research skills in molecular biology, genetics/genomics, muscle, fat, and/or vascular biology related to obesity, diabetes, exercise physiology, nutrition, and/or gonadal steroid and growth hormone axis physiology. She/he will be expected to conceptualize, write, and conduct new laboratory-oriented research protocols, and to compete successfully for research funding from federal and/or private agencies. A strong publication record in laboratory research is required. Interdisciplinary research collaborations in endocrine-metabolic, geriatric, cardiovascular, and exercise rehabilitation research will be conducted at the Washington, D.C., VA Medical Center, and in partnership with the Baltimore VA Medical Center's Geriatric Research Education and Clinical Center (GRECC), Claude Pepper Older Americans Independence Center, NIH Clinical Nutrition Research Unit, Diabetes Research and Training Center, and Rehabilitation Research Center.

The GS-14 will serve as the principal laboratory-based scientist conducting basic research related to geriatric endocrinology and metabolism under the supervision of the ACOS R&D, and will serve as the Laboratory Director. The GS-13 will also serve as a senior laboratory-based scientist conducting basic research in geriatric endocrinology and metabolism and will, with the Laboratory Director and ACOS R&D, co-supervise the laboratory's other scientists, fellows, students, and other trainees.

The Research Service at the Washington, D.C., VA Medical Center provides contemporary laboratory, translational, and clinical research facilities in addition to a collegial and nurturing working environment.

Rank and salary will be commensurate with experience. Candidates should forward a resume, one-page letter of interest and the names and contact information of three references to: **Human Resources Department (05), 50 Irving Street NW, Washington, DC 20422, Attention Ms. Cheryl Williams. E-mail: cheryl.williams3@va.gov; telephone: 202-745-8000 ext. 7333.** Academic inquiries should be addressed to: **Dr. Marc Blackman, e-mail: marc.blackman@va.gov; telephone: 202-745-8000 ext. 8478.**

This announcement will remain open until filled.

The Washington, D.C., VAMC does not discriminate in employment on the basis of race, color, religion, sex, national origin, political affiliation, sexual orientation, marital status, disability, age, membership in an employee organization, or other nonmerit factor. The Washington, D.C., VAMC provides reasonable accommodation to applicants with disabilities where appropriate.

POSTDOCTORAL POSITION available to study mechanisms of DNA replication, DNA repair, and the cell cycle in mammalian cells and fission yeast. Applicants should have a strong background in molecular biology and/or biochemistry. Send curriculum vitae and contact information of two references to: **Dr. Eishi Noguchi, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA. E-mail: enoguchi@drexelmed.edu.** See **website: http://homepage.mac.com/enognog/Noguchi%20Lab.html.**

POSITIONS OPEN

NATIONAL SEARCH for the BIOLOGY CHAIRPERSON

Washington University in St. Louis invites applications for the position of Chair of the Department of Biology in Arts and Sciences. The Department's 29 faculty members conduct internationally recognized research across a wide spectrum of the life sciences. The Department has a strong commitment to teaching at both the undergraduate and graduate levels and has gained a national reputation for bridging research and teaching. Occupying 120,000 square feet of teaching and research space, the Department operates within a total yearly academic budget of over 20 million dollars. The Department, along with science departments from Arts and Sciences, the School of Medicine, and the School of Engineering, is strongly committed to interdisciplinary and cross-departmental teaching and research programs at both the undergraduate and graduate levels. Growth opportunities exist particularly in areas at the interface of biology with other scientific disciplines. The Biology Chair position is a full-time, tenured position at the rank of **FULL PROFESSOR.** Candidates should hold the Ph.D. degree with a nationally/internationally recognized record in teaching and research, and should have demonstrated leadership abilities and administrative skills. Review of applicants will begin October 1, 2008, and will continue until the position is filled. Applicants should submits complete curriculum vitae, a statement of research interests and educational and administrative philosophy, and the names/addresses of five references to: **Dr. Henry L. Roediger, III, Biology Chair Search Committee, P.O. Box 1125, Washington University, One Brookings Drive, St. Louis, MO 63130-4899.** (E-mail address is **e-mail: chairsearch@biology.wustl.edu.)** Electronic submissions are encouraged.

Washington University is an Affirmative Action/Equal Opportunity Employer.

POSTDOCTORAL FELLOW

A Postdoctoral position is available in the Department of Molecular and Comparative Pathobiology at the Johns Hopkins University School of Medicine to study molecular and cellular role of platelets in vascular inflammation. Current research is focused on novel pathways we have identified that mediate platelet activation and thrombosis. Our laboratory uses a combination of molecular, cellular, and in vivo animal mouse models. Candidates should have a Ph.D., M.D./Ph.D., or D.V.M/Ph.D. and a strong background in vascular biology. Please send a cover letter detailing both your previous scientific work experience and your interest in this position, curriculum vitae, and contact information for three references to: **Craig N. Morrell, D.V.M., Ph.D., Assistant Professor, Department of Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine, BRB 853, 733 N. Broadway, Baltimore, MD 21205,** or by **e-mail: cmorrell@jhmi.edu.** *Johns Hopkins University is an Equal Opportunity, Affirmative Action Employer with a strong commitment to racial, cultural, and ethnic diversity. Nominations and applications from women and individuals from a broad spectrum of backgrounds are encouraged.*

COLUMBIA UNIVERSITY
Department of Pathology and Cell Biology

The Department seeks highly qualified individuals for faculty positions in surgical pathology, anatomic pathology, and research. Appointments can be at the **ASSISTANT, ASSOCIATE, PROFESSOR, or ASSOCIATE RESEARCH SCIENTIST** level, depending on experience and qualifications. Clinical positions require Board certification and a license in New York State prior to the start of service. Research positions require a record of publication in leading journals and a statement of research directions.

Applicants should submit curriculum vitae and the names of three references to: **C. Kitzinger, Department of Pathology and Cell Biology, Columbia University, 630 West 168th Street, New York, NY 10032.** *Columbia University takes Affirmative Action toward Equal Employment Opportunity. Women and minorities are encouraged to apply.*

FACULTY POSITIONS DEPARTMENT OF PHYSICS THE UNIVERSITY OF TEXAS AT AUSTIN

The Department of Physics at The University of Texas at Austin is seeking candidates for tenure-track assistant professorship positions in physics starting in September 2009. In special cases, appointments at more senior levels will be considered. Successful candidates will assume full teaching responsibilities for undergraduate and graduate courses in the Department of Physics and are also expected to conduct vigorous research programs. Research areas of current highest priority for the Department are Biophysics Experiment and Fundamental Theory/Cosmology. Outstanding candidates in other areas of departmental focus will also be considered. Excellent English language communication skills are required. Applicants must have a Ph.D. (or equivalent) and a demonstrated potential for excellence in teaching and research.

Interested applicants should send a curriculum vitae, a list of publications, a statement of research interests, a research plan, and should arrange for at least five letters of recommendation to be sent to: **Prof. John T. Markert, Chair, Department of Physics, The University of Texas at Austin, 1 University Station C1600, Austin, TX 78712-0264**. Review of completed applications will begin in **October, 2008**.

The University of Texas at Austin is an Equal Opportunity/Affirmative Action Employer.

UNIVERSITY OF WYOMING

Haub School and Ruckelshaus Institute of Environment and Natural Resources
University of Wyoming, Department 3971, 1000 E. University Ave., Laramie, WY 82071-2000.
Tele: (307) 766-5080

THE UNIVERSITY OF WYOMING invites applications and nominations for the newly created **WYOMING EXCELLENCE/SPICER DISTINGUISHED CHAIR in ENVIRONMENT AND NATURAL RESOURCES**. We seek an individual with an exceptional record of teaching and/or equivalent practitioner experience, public outreach and scholarship in environment and natural resources management and policy, with an emphasis on conflict resolution and collaborative processes.

The successful applicant will be expected to establish a strong, funded research program, as well as teach at the graduate and undergraduate levels. The Chair will provide leadership and vision for interdisciplinary curricula at the graduate and undergraduate levels in the area of environment and natural resources, conflict resolution and collaborative processes. The position will be a joint appointment with the Haub School of Environment and Natural Resources and another appropriate UW department appropriate for the background of the successful candidate. The Haub School is an interdisciplinary program that seeks to transcend disciplinary boundaries and examine complex environmental and natural resource issues from the full range of perspectives. The Strong candidates may come from a number of backgrounds, such as law, economics, business, natural resources, etc.

Minimum qualifications include: an earned doctorate or other terminal degree; a distinguished record of scholarship commensurate with an appointment at the rank of Associate or Full Professor in one of UW's academic departments; strong research credentials at the intersection between conflict resolution/collaborative processes and environment/natural resources issues.

Preferred qualifications include: experience as a practitioner of collaborative process and conflict resolution; demonstrated expertise in public outreach.

Interested applicants are requested to submit electronically: a letter of application; curriculum vitae; statement of research and teaching philosophy; teaching evaluations (if applicable); contact information for three professional references to: **Chair, Spicer Chair Search Committee, c/o Nancy Hoffer, Haub School of Environment and Natural Resources, nhoffer@uwyo.edu.** The search committee will begin reviewing applications on **October 1, 2008** and will continue until the position is filled.

Persons seeking admission, employment or access to programs of the University of Wyoming shall be considered without regard to race, color, religion, sex, national origin, disability, age, veteran status, sexual orientation or political belief.

POSITIONS OPEN

FACULTY POSITION
Albany Medical College
Center for Neuropharmacology and Neuroscience

The Center for Neuropharmacology and Neuroscience (CNN) of Albany Medical College invites applications for a tenure-track faculty position at the **ASSISTANT or ASSOCIATE PROFESSOR** level. We seek a highly motivated individual with a strong record of research productivity and a desire to participate in graduate and medical education. The applicant's research should complement and/or enhance existing programs in the CNN as well as in collaborating clinical departments (e.g., neurology and psychiatry). We are particularly interested in building a program focused on the electrophysiology of neurodegenerative disorders but will also consider candidates having expertise in substance abuse research, research on neuropsychiatric disorders, and neural stem cell biology. A Ph.D. or M.D./Ph.D. degree and three years of postdoctoral experience are minimal requirements for appointment at the Assistant Professor level. Applicants for Associate Professor should have appropriate experience and a nationally recognized and funded research program. The Albany area offers diverse cultural and recreational attractions with easy access to Boston, New York City, and the Adirondack, Catskill, and Berkshire Mountains. For further information about the CNN, please visit our **website: http://www.amc.edu/Research/CNN/.**

Applicants should send curriculum vitae, description of research interests, and three letters of recommendation by September 15, 2008, to:

Stanley D. Glick, Ph.D., M.D.
Director, Center for Neuropharmacology and Neuroscience
Albany Medical College, MC-136
47 New Scotland Avenue
Albany, NY 12208

Or **e-mail: glicks@mail.amc.edu.**

An Equal Opportunity/Affirmative Action Employer: women and minorities are encouraged to apply.

ASSISTANT/ASSOCIATE PROFESSOR
Department of Pharmacology
The University of Toledo

The Department of Pharmacology, College of Pharmacy, at the University of Toledo is inviting applications for two tenure-track positions available at the Assistant/Associate Professor level. Requirements include the Ph.D. in pharmacology, pharmaceutical sciences, or a related field and productive postdoctoral experience. Candidates with experience in the area of experimental therapeutics are encouraged to apply. Responsibilities for the position will include teaching undergraduate and graduate level courses in pharmacology or related areas. Current areas of research considered to be priorities for the health sciences include: neurodegenerative diseases, cancer, organ transplantation/immunology, cardiovascular/diabetes, and orthopedics. The successful candidate will be expected to develop and maintain an active, externally funded research program that complements existing research strengths within the Department and/or College. A competitive salary and research startup package will be provided.

Interested individuals are encouraged to submit curriculum vitae with a letter describing teaching philosophies and research goals and to arrange for three letters of reference to be sent to: **Miles Hacker, Ph.D., Chair of the Search Committee (PCN 998133 and 996440), Department of Pharmacology, #607, The University of Toledo, College of Pharmacy, 2801 W. Bancroft Street, Toledo, OH 43606. E-mail: miles.hacker@utoledo.edu.** Applicant review process will begin on August 31, 2008, and will continue until the position is filled.

The University of Toledo is an Equal Access, Equal Opportunity, Affirmative Action Employer and Educator. Women and minorities are encouraged to apply.

POSITIONS OPEN

INSTITUT PASTEUR

POSTDOCTORAL FELLOWSHIPS
Institut Pasteur, Paris, France

Come work in Paris at the Institut Pasteur, a world-renowned, private, biomedical research organization! We invite applications from outstanding Fellowship candidates to any of 138 laboratories within our 10 departments. Areas include: developmental and cell biology, epidemiology, immunology genomes, genetics, microbiology, neuroscience, structural biology, parasitology, mycology and virology. Deadlines vary; see website for details. Annual package is $70,000 for three years. *U.S. citizenship required.*

E-mail: pasteurus@aol.com. Website: http://www.pasteurfoundation.org.

MANAGER of BUSINESS DEVELOPMENT

Harlan, Sprague, Dawley, Inc., is a global provider of preclinical research tools, research models, and services. We currently have an opening for a Manager of Business Development to manage sales and business developers to drive profitable growth and provide service excellence to customers within the international research and toxicology chemical development market. This position reports to headquarters in Indianapolis, Indiana, and requires extensive travel and work throughout the United States. Must have a Ph.D. or equivalent in pharmacology, toxicology, or related discipline and three years of experience in an international company within the global chemical, preclinical development, and regulatory safety testing industry as a business development manager, marketing manager, or related occupation. This experience must include two years of experience, which may have been gained concurrently, with all of the following: (1) preparing and implementing a specific service business development strategy; (2) managing and being accountable for an individual annual sales target of $5,000,000 or more; (3) managing, prioritizing, and building successful key global accounts and account plans; and (4) managing and developing sales forecasts and pricing strategy. Must also have experience as a study director in a contract research organization. To apply, please send resume to **e-mail: nmccarty@harlan.com.** Please reference CRO-MBD in subject line. *Equal Opportunity Employer.*

POSTDOCTORAL POSITION
NEURO-ONCOLOGIC NEUROSURGERY
Thomas Jefferson University/Jefferson Medical College

The Department of Neurological Surgery at Thomas Jefferson University is seeking a motivated individual to investigate vaccine candidates in animal glioma models as part of a multidisciplinary group studying brain tumor immunity in patients and in animal models.

The successful candidate will possess a Ph.D. in immunology with an interest in neuroimmunity and/or tumor vaccine development as well as expertise in the analysis of T cell function.

Interested individuals should forward their curriculum vitae, along with a list of three references, to:

David W. Andrews, M.D., FACS
E-mail: david.andrews@jefferson.edu
D. Craig Hooper, Ph.D.
E-mail: douglas.hooper@jefferson.edu
909 Walnut Street, Third Floor
Philadelphia, PA 19107
Telephone: 215-503-1774
Fax: 215-923-7745

Thomas Jefferson University/Jefferson Medical College is an Affirmative Action/Equal Opportunity Employer. Women and people of diverse racial, ethnic, and cultural backgrounds are encouraged to apply.

POSITIONS OPEN

POSTDOCTORAL POSITIONS
Molecular and Cellular Mechanisms in Cancer

The University of California, San Francisco (UCSF) has Postdoctoral research training positions available in multiple areas of cancer research including cancer genomics, angiogenesis, tumor microenvironment, cancer immunology, experimental therapeutics, biological regulations of cancer, developmental biology, biostatistics, and genetics. These positions are supported by an NIH training grant that includes 30 principal investigators at three UCSF campuses. The multiple disciplines of the faculty members provide opportunities for rigorous training in basic cellular and molecular mechanisms in cancer and scientific perspectives on cancer incidence, survival, and the realities of patient care. Postdoctoral fellows receive NIH-level stipends based on experience and travel funds to participate in scientific meetings. Potential applicants may review the participating faculty and their research programs at the **website: http://anatomy.ucsf.edu/werbwebsite/T32grant.htm.**

To qualify, applicants must be U.S. citizens or permanent residents and have received a Ph.D., M.D., or M.D./Ph.D. Prospective applicants should contact one or more participating faculty members regarding possible training options and research interests. The completed application should include the applicant's curriculum vitae, at least two letters of reference, and a one- to two-page research plan written by the applicant. Please submit questions and applications to **e-mail: werb.admin@ucsf.edu.**

MARKETPLACE