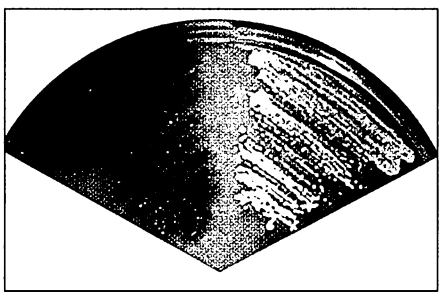
support the so-called "protein-only" hypothesis, a vocal minority insists that it is not yet proven—and that an as-yet-unidentified microbe, such as a virus, may team up with the prion protein to devastate the nervous system (*Science*, 22 October 1999, p. 660). New findings in yeast, reported in this issue and in the January issue of *Molecular Cell*, may now provide additional comfort for the prion proponents, although skeptics are unlikely to be convinced.

Prion doubters find the hypothesis hard to swallow because it holds that the proteins can infect tissues and reproduce themselves, thus violating long-standing dogma that a DNA- or RNA-based genome is necessary for such autonomous behavior. During the 1990s, geneticist Reed Wickner at the National Institutes of Health in Bethesda, Maryland, suggested that some bizarre patterns of yeast inheritance might be explained if these single-cell organisms also harbored prions. Soon afterward, University of Chicago geneticist Susan Lindquist's team showed that such so-called "yeast prions" do exist and that they appear to behave similarly to mammalian prions. They cause changes in the yeast's biochemical properties that can be passed on to daughter cells when the yeast cells divide, apparently independently of any infectious microbe or the yeast's own genome (*Science*, 2 August 1996, p. 580).

In the current work, reported on page 661, Lindquist and postdoc Liming Li created an artificial prion by fusing part of a yeast prion protein called Sup35 to a normal cellular protein from the rat. Like known yeast prions, this chimeric protein altered the biochemical properties of yeast cells in ways that could be inherited by progeny cells. "The astonishing thing is that the prion property can be transferred to a totally different protein," says neuropathologist Adriano Aguzzi of the University of Zurich, Switzerland. Indeed, this view is bolstered by a second paper, published in the current issue of *Molecular Cell*, in which the Lindquist lab has identified a new yeast prion, a protein called Rnq1, and shown that a segment of this protein also confers prionlike activity.

The new experiments build upon previous work showing that Sup35, like prion proteins that infect mammals, exists in two states: a normal, soluble form, and an abnormal, insoluble conformation. When the abnormal protein contacts its normal counterpart, it can trigger the structural change that converts it to the insoluble form, thus causing the prions to clump. In nerve cells, this causes permanent damage. In yeast, normal Sup35 helps control the yeast's genetic machinery, telling it when to stop translating messenger RNA into proteins. But when abnormal Sup35 dominates, this translational control is lost.

Researchers have found that a specific segment of Sup35 and Ure2, a yeast prion identified by Wickner, is needed for the proteins to act as prions. Lindquist and Li attached this "prion domain" from Sup35 to the rat glucocorticoid receptor, which controls the transcription of DNA into RNA—an entirely different function from Sup35's. In its soluble form, the new protein, called NMGR, could still induce transcription, as demonstrated by its ability to turn on a gene coding for the enzyme β-galactosidase. But when switched to its prion form, the hybrid protein was no longer capable of turning on the gene. Most importantly, this inactivated phenotype could be transmitted in a heritable fashion between mother and daughter yeast cells.



CREDIT: L. LI AND S. LINDQUIST

Switcheroo. The artificial prion protein can exist in an active form in which it turns on the β-galactosidase gene (indicated by the blue color of the yeast) and an inactive form in which it doesn't (white yeast cells).

To find the new yeast prion, Lindquist and graduate student Neal Sondheimer searched gene databases for sequences sharing the characteristic features of the prion domains of Sup35 and Ure2, which both have large amounts of the amino acids glutamine and asparagine. They hit upon a protein they called Rnq1. Although Rnq1's function is as yet unknown, it can exist in normal and abnormal states like other prions. In addition, when the team substituted Rnq1's prion domain for that of Sup35 and introduced the altered protein into yeast, it had the same biochemical properties as Sup35, thus proving, the authors say, that the prion domains are alone responsible for perpetuating the prion behavior.

"The new experiments provide an almost incontrovertible argument in favor" of the protein-only hypothesis, at least in yeast, Lindquist says. "One has to come up with some very implausible scenarios to explain all of this with a virus." But some researchers argue that the new work may not be relevant to mammals. Yale University neuropathologist Laura Manuelidis, a leading prion skeptic, says Lindquist's work with yeast has put the prion within a "more acceptable, experimentally testable paradigm." But she notes that the yeast prion model "has nothing to do with infectious disease."

Despite these criticisms, researchers agree that genetically engineered prions might help resolve the debate over the protein-only hypothesis in mammals, particularly if pure prion preparations unassociated with any suspected virus or other microbe could re-create prion diseases in test animals. So far, attempts to do this with genetically engineered versions of the human prion protein PrP have failed, although this might be due to difficulties in coaxing the protein into the exact conformation necessary for infectivity.

But in work reported in the October 1999 issue of *Nature Cell Biology*, the Lindquist group did succeed in expressing mouse PrP in both yeast and cultured nerve tumor cells and getting it to convert to an abnormal form close to that adopted by naturally occurring mouse prions. The team is now testing whether these transgenically produced prions can infect mice. "If they can put in a pure or recombinant PrP protein, made in a virus-free cell, and get something that replicates infectivity in mammals, then I would be convinced [that the protein-only hypothesis] is correct," says Manuelidis. "I've been waiting 20 years to see that experiment."

–MICHAEL BALTER

SCIENTIFIC PUBLISHING

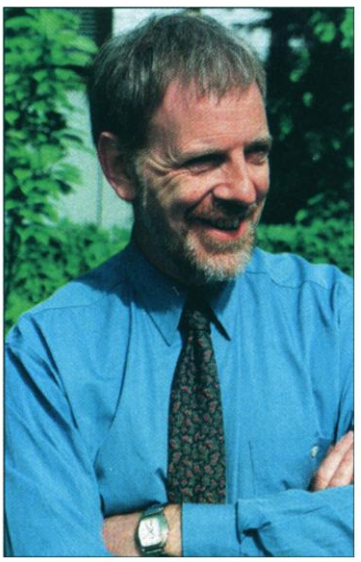
Publishers Discuss European E-Print Site

While U.S. organizers were putting the finishing touches on a new Web site known as PubMed Central, a group of scientists and publishers met in Heidelberg last week to plan a European counterpart called "E-Biosci." The U.S. project, due to go online within a week, is billed as a free archive of biomedical papers. It catalyzed the European initiative but will not be exactly the same. E-Biosci, according to organizers, is likely to require tougher peer reviewing for nonpublished articles and may allow publishers to charge some fees for access to their papers.

The prime mover behind E-Biosci, Frank Gannon, executive director of the European Molecular Biology Organization (EMBO), believes it is "quite feasible" for the European site to begin operating this year, but he acknowledges that no final plan has been agreed on, and long-term funding has not yet been secured.

The 30 key European players who met on 19 January in Heidelberg to discuss E-Biosci did not set its exact contours. Participants in the meeting included representatives of EMBO, European science publish-

ers, European research organizations, and national science ministries. "Everyone agreed that something has to be done, and quickly," said Gannon, an Irish molecular biologist. "But follow-up meetings will be needed to decide the best way of solving the problems." Gannon says organizers must now find long-term financing, iron out technical problems, and drum up support from the European Union (E.U.) and national research agencies.



Prime mover. EMBO's Frank Gannon aims to launch Web site this year.

The biggest challenge appears to be reaching agreement among Europe's scientific publishers. Stefan von Holtzbrinck, managing director of Macmillan's Nature Publishing Group—which publishes *Nature* and five related journals—told *Science* that he supports the idea of such a Web site, but that E-Biosci should give publishers the option of charging fees for access to their publications. "We would not go with any venture that would require you to make all of your content cost-free from day one," he said. Another European science-publishing executive, who asked not to be named, said he expects that several European publishers "will participate in some way"—perhaps by giving cost-free access to certain journals or articles that were published more than a year earlier. Martin Richardson, publishing director of the Oxford University Press, said he supports "the idea of setting up a European archive" and noted that Gannon and other E-Biosci organizers "are trying very hard to come up with a proposal that will be acceptable to publishers."

Gannon said E-Biosci may not insist on entirely free access: "We and PubMed have the same aims. But I think that PubMed will not be able to offer the complete literature," because publishers may not be willing to share text for free, "and I don't think that E-Biosci will be completely free." Gannon was pleased by what he called "the positive input" from the publishers represented at the meeting: Macmillan, Elsevier Science, Springer Verlag, Oxford University Press, and Blackwell Science.

One way in which E-Biosci probably will differ from the U.S. Web site is in limiting the posting of unpublished papers. Gannon said that everyone at the Heidelberg meeting agreed that unpublished drafts and preprints "would have to be seriously peer reviewed," not simply screened, before being put on the site. PubMed Central, in contrast, may have an adjunct site called "PubMed Express" that will include unreviewed papers. And another European Web site, a private venture called "BioMed Central," is planning to make draft papers available. Vitek Tracz, chair of the Current Science Group in Britain, issued a press release last week saying that the site, funded by advertising and service fees, would be launched in May (www.biomedcentral.com).

Financing is another major issue for E-Biosci. Last summer, EMBO agreed to allocate $511,000 to start the venture, but the project does not yet have any other long-term funding commitments. Although the E.U.'s research commissioner, Philippe Busquin, says he fully supports the concept, Brunno Schmitz—who represented the Research Directorate in Heidelberg—cautions that the European Commission at most "could only provide seed money" for E-Biosci. Gannon said other revenues might come from advertising, science trusts, or from national research councils.

Representatives of science councils in Scandinavia were among the most enthusiastic about E-Biosci at the Heidelberg meeting, with Finnish molecular biologist Marja Makarow calling the Web site "a very good project that should get started as soon as possible." But some worry that E-Biosci might hurt scientific societies, which rely on journals for revenue. The European Science Foundation (ESF) may sponsor a symposium on the impact of e-publications on public trust, patenting, and scientific societies. Said Tony Mayer, who heads the ESF secretary-general's office: "We support the E-Biosci concept, but we are concerned about the implications of e-publication in general on the scientific system."

Meanwhile, Gannon predicts that E-Biosci "will collaborate very actively with PubMed Central" as part of "a wider global effort" to make scientific publications more accessible on the Web. And the chief organizer of PubMed Central—David Lipman, director of the National Institutes of Health's National Center for Biotechnology Information (GenBank)—says he strongly supports the EMBO initiative and hopes that "Europe will participate as an equal partner."

–ROBERT KOENIG

ORGANIC CHEMISTRY

Cubic Compound Makes a Bigger Bang

Alfred Nobel would no doubt be intrigued by a feat of organic chemistry reported in this week's international edition of *Angewandte Chemie*: the synthesis of what may be the most powerful nonnuclear explosives ever made. If they can be produced in bulk, the new compounds would put dynamite—Nobel's patented invention—to shame.

The new explosives—heptanitrocubane and octanitrocubane—have been on the drawing board for more than a decade. Their inspiration was a compound with a molecular core consisting of a cube of eight carbon atoms studded with hydrogens, first synthesized in 1964 by Philip Eaton, an organic chemist at the University of Chicago, and his colleagues. Eaton and others later realized that if they could replace the hydrogens with reactive nitro groups—each containing a nitrogen and two oxygens—they'd have an ultradense, and therefore ultrapowerful, explosive.

But swapping nitros for the hydrogens proved a Herculean task. Eaton's team struggled with the synthesis for some 15 years until at last, in 1998, they found a reaction that tacked on all but the eighth nitro. Now Eaton—along with chemist Mao-Xi Zhang and crystallographer Richard Gilardi of the Naval Research Laboratory in Washington, D.C.—has discovered a more efficient way to construct the seven-nitro heptanitrocubane, as well as the magic mix of ingredients and conditions that tacks on the eighth to form octanitrocubane. "I think it's fantastic," says Leo Paquette, an organic chemist at Ohio State University in Columbus. "To get all the way to eight nitro groups is clearly a feat. I really had serious doubts that he'd ever get there. It's asking a lot of the molecule to squeeze all those nitro groups into a limited space."

That tightly packed structure gives the new nitrocubanes a density of about 2 grams per cubic centimeter (g/cm^3), a number closely tied to the explosive power of compounds. By contrast, the density of TNT is 1.53 g/cm^3, HMX—the most powerful conventional military explosive in regular use—is 1.89 g/cm^3, and Cl-20—another experimental explosive—is closer to the nitrocubanes at 1.96 g/cm^3. Eaton notes that other factors also play important roles in the power of an explosive, such as how completely the material combusts when triggered. But because the explosiveness of a compound grows as a square of the density, even small changes in this number can have a dramatic effect. Calculations suggest the new explosive may deliver up to 30% more bang than HMX.

CREDIT: EMBO