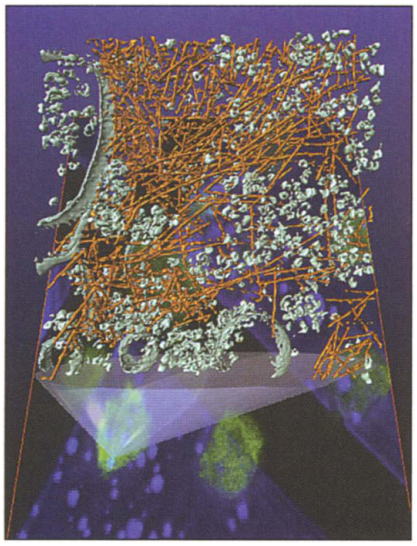
TECHNIQUES

A New Window on the Cell's Inner Workings

Biologists can take beautiful pictures of whole cells and, at the other extreme, outline the molecular structure of single proteins. But they have not had a good way to look at midsized objects within a cell—organelles in the range of 5 to 10 nanometers, such as actin filaments that cleave a cell in half or the protein-packaging Golgi apparatus. Now, researchers have opened a window on such structures with a technique called cryo-electron tomography (cryo-ET), which combines flash-freezing of whole cells with automated electron scanning to create three-dimensional images of intact organelles.

Cryo-ET bridges a "resolution gap," explains technique pioneer Wolfgang Baumeister of the Max Planck Institute for Biochemistry in Martinsried, Germany, noting that it does not require staining, fixation, or disruption of the cell membrane. "The traditional staining and drying techniques" used to examine cells for several decades have been known to introduce confounding structures, says biologist Holger Stark of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. "Using cryo-ET to study the cell in its natural environment will enable cell biologists to clearly distinguish between real structures and artifacts," Stark says.



Actin unveiled. Cryo-ET reveals part of the macromolecular structure of a cell in this composite illustration.

Baumeister's group reports on page 1209 a "major proof of concept," as cell biologist Dorit Hanein of the Burnham Institute in La Jolla, California, describes it. Baumeister's use of cryo-ET to image a network of actin filaments in the highly motile cells of the amoeba *Dictyostelium discoideum* enables the first structural measurements of actin's geometry in the intact cell. "I think it is really exciting, just wonderful," says Hanein. "We knew how actin and myosin interacted in the test tube, but that didn't answer the question of how they look in the cell." Seeing what cryo-ET can do to image actin in an undisturbed cell, she says, "really opens [our] eyes" to what might be possible as the technique improves further.

Cryo-ET works much the same way as medical tomography, such as computerized tomography and positron emission tomography scans, with penetrating beams of radiation used to create image slices. Whole cells are vitrified or flash-frozen and are then mounted on an apparatus that automatically moves through a range of tilt angles, enabling the cell to be sliced like a pie. At each angle, a thin region is exposed to a beam of electrons, yielding a two-dimensional image that is recorded as digital data. A computer assembles the 2D data into a composite 3D image. Then pattern recognition software uses the imaged structure to locate the corresponding organelle in another cell. Although the concept has been around for more than 3 decades, Baumeister explains, only recently have technical advances enabled researchers to obtain data quickly enough to prevent the electron beam from damaging cell structures.

Cryo-ET will make possible new analyses of cell structure. Baumeister's group, for example, hopes to create a 3D map of all the organelles in a eukaryotic cell and use it to create the first integrated link between atomic-level images (from nuclear magnetic resonance and x-ray crystallography) and cell-level images from light microscopy. Such studies will allow placement of atomic-scale-resolution protein structures within the spatial context of a whole cell, says microscopist Esther Bullitt of the Boston University School of Medicine. Cryo-ET will also help fill gaps in our knowledge of cell movement, says cell biologist Marcus Fechheimer of the University of Georgia, Athens. In the field of cell motility, genetic studies of molecular interactions have "far outpaced" research on "ultrastructure," he explains, but researchers have not been able to visualize how the cell architecture changes as a result of genetic tampering.

For structural biologist Kenneth Taylor of Florida State University, Tallahassee, "the biggest application at the moment for cryo-ET will be to determine the 3D structure of cellular organelles that cannot be easily isolated intact." The technique shows particular promise for imaging any organelle that can be found in the thin margins of the cell, he says, such as the Golgi apparatus, microtubule organizing center, cilia, and flagella.

The Baumeister lab has shown that cryo-ET is feasible, says Taylor, and, as the tech-

CREDIT: (TOP) O. MEDALIA *ET AL.*, *SCIENCE* **298**, 1209 (2002)

ScienceScope

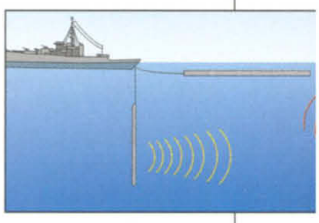
U.S. Signs Gene Pact In an about-face, the Bush Administration has signed a genetic resources treaty it once opposed. Last week in Rome, the United States signed the International Treaty on Plant Genetic Resources for Food and Agriculture, which calls for free exchange of the seeds stored in the world's publicly owned "gene banks."

Last year, U.S. officials said that they were "precluded" from signing the treaty because it restricts the patenting of genes from seed banks, a position that might conflict with U.S. law. In addition, the U.S. wanted the freedom to block seed transfers to nations, such as Cuba, that are subject to economic sanctions. But agricultural researchers and biotech and seed companies affected by the treaty argued that U.S. officials "should be at the table" when seed-transfer rules are drafted, said Peter Bretting, a manager of the U.S. Department of Agriculture's National Plant Germplasm System.

The Senate must ratify the treaty once Administration officials hammer out details and submit it for a vote.

Court-Ordered Silence A federal judge in San Francisco has temporarily blocked the U.S. Navy from deploying a new sonar system, siding with environmentalists and researchers who say its powerful sound pulses could harm whales and other marine mammals.

Navy engineers have spent decades designing the new SURTASS LFA sonar, which uses low-frequency sound to detect submarines hundreds of kilometers away. But plans to deploy the system have become entangled in controversy, as other types of military sonar have been linked to whale deaths (*Science*, 26 January 2001, p. 576). In July the Navy agreed to limit the sonar's use to offshore and nonpolar areas. But the Natural Resources Defense Council and other groups challenged the deal, saying that regulators had downplayed the sonar's threat.



On 31 October, Magistrate Judge Elizabeth Laporte agreed, ordering both sides back to court on 7 November to discuss ways to better balance environmental and military concerns. Observers say the case could mean greater scrutiny for other groups—from the oil industry to marine scientists—that use sound to probe the ocean.

nique gains adherents, it will undoubtedly become more accessible to a larger number of labs. "There will be an explosion in cryo-ET in the next 10 years or so," predicts microscopist Timothy Baker of Purdue University in West Lafayette, Indiana. "It will be a major player in cell biology, no doubt about it."

–ERICA GOLDMAN

U.S. BUDGET

Smithsonian Science: Vote of Confidence

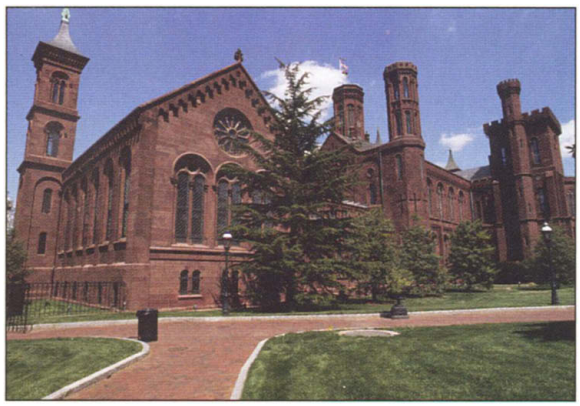
A year ago, the Smithsonian Institution, home to 16 museums and six research centers, was in danger of losing one-third of its 2003 research funds in a bruising encounter with the White House Office of Management and Budget (OMB). The Administration had decided that the National Science Foundation (NSF) should manage $35 million of the Smithsonian's portfolio. But scientists objected, the transfer to NSF was shelved, and last week two national panels concluded that the whole idea was a big mistake.

A quasi-federal agency established in 1846, the Smithsonian relies on the federal government for 57% of its annual expenses, including funds for research centers that carry out studies ranging from plant systematics to astrophysics. OMB last year wanted to shift funds for three of those centers to NSF: the Harvard-Smithsonian Center for Astrophysics (CfA), the Smithsonian Environmental Research Center, and the Smithsonian Tropical Research Institute (STRI). The budget agency argued that scientists at these centers should compete for grants like nongovernment researchers do and that NSF would do a better job of peer review.

When this proposal was leaked in December 2001, Congress, the Smithsonian, and independent scientists objected loudly. OMB backed down (*Science*, 7 December 2001, p. 2066). In return, the Smithsonian agreed to ask the National Research Council (NRC) and the National Academy of Public Administration (NAPA) to look into how its appropriation might best be spent.

Both panels released reports last week saying, in effect, "Don't fix what isn't broken." They endorsed the status quo because they "were just so impressed by" the Smithsonian's scientific research, says NRC panel member Anthony Janetos of the H. John Heinz III Center for Science, Economics and the Environment in Washington, D.C. He says research is "one of the real gems" of the institution. Like other panel members, Janetos thinks cutting the direct federal support would be devastating. The NRC report concluded that "it would probably lead to the demise of much of the Smithsonian's scientific research program."

Rather than give Smithsonian scientists an unfair competitive advantage, the NRC and NAPA panels argue, federal appropriations keep them on par with their academic colleagues. Three-quarters of the funding pays for salaries, and most of the rest pays for maintenance, administration, and other routine costs. Researchers still need to get some outside funding to do their work. The fact that Smithsonian researchers compete successfully for outside grants—they won 325 in 2001—indicates the excellence of the science they do, the reports conclude. "The staff is doing very well competing on the outside," says Cornelius Pings, former president of the Association of American Universities and head of the NRC committee. And that should allay OMB's fears about the quality of the work, he adds. However, NRC did call for more extensive, periodic reviews of research by outside experts.



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Research relief. Two panels advised that Smithsonian science budgets should remain intact.

The panels also uncovered some problems. "The numbers associated with scientific research at the Smithsonian were a little confusing," says J. William Gadsby, NAPA director of management studies. "We had difficulty sorting things out." For example, figures provided by Smithsonian leaders often didn't match those provided by the science centers themselves. David Evans, the Smithsonian undersecretary for science, agrees it is a problem and hopes that a newly installed accounting system will remedy it.

The reports "are ringing endorsements" of Smithsonian scientists, says CfA's director, Irwin Shapiro. Adds STRI's director, Ira Rubinoff: "Hopefully this will allow us to go on and do our work." Even so, they and their colleagues must wait to see whether the White House follows these recommendations in its 2004 budget.

–ELIZABETH PENNISI

With reporting by Andrew Lawler.

ScienceScope

Heavy Objections Some public health advocates want the Bush Administration to remove a controversial researcher from a lead-poisoning advisory panel. But the Administration isn't budging.

More than 60 groups last week asked Health and Human Services (HHS) Secretary Tommy Thompson to remove William Banner of St. Francis Hospital in Tulsa, Oklahoma, from the panel, which advises the Centers for Disease Control and Prevention (CDC) on how to prevent childhood lead poisoning. They note that Banner has testified on behalf of lead paint producers in legal proceedings, arguing that blood lead levels up to seven times the current federal standard don't harm children (*Science*, 25 October, p. 732). That record makes Banner's appointment "an egregious slap in the face to sound science informing the CDC," says Eileen Quinn, deputy director of the Alliance to End Childhood Lead Poisoning.

The appointment of pediatric hematologist Sergio Piomelli of Columbia-Presbyterian Medical Center in New York City, who believes that the current federal blood lead standard is too strict, has also ruffled feathers. Piomelli told *Science* that a lead industry representative called to say, " 'We would like to nominate you,' and I said, 'Sure.' " But he stresses that he fought the lead industry for years to remove lead from gasoline.

HHS spokesperson Bill Pierce says both appointees are "highly qualified." Critics promise to keep a close eye on future appointments to the 20-member panel.

Unhappy Wait French scientists will have to wait at least another year to see if the conservative government will fulfill a campaign promise to increase the nation's research budget. Despite a petition signed by more than 5000 researchers—including Nobel laureates Georges Charpak and François Jacob—the National Assembly voted 5 November to decrease the 2003 budget by 1.3% over current levels. The same day, research minister Claudie Haigneré announced that she plans to ask for a 4% boost in 2004.

Chemist Henri-Edouard Audier of the École Polytechnique near Paris, who launched the petition campaign, is not impressed. "Madame Haigneré only made this announcement after we sent the petition to the press," he says, adding that French scientists intend to "keep up the pressure for the entire next year."

Contributors: Richard Stone, Martin Enserink, Dan Charles, David Malakoff, Dan Ferber, and Michael Balter