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NCRR Reporter

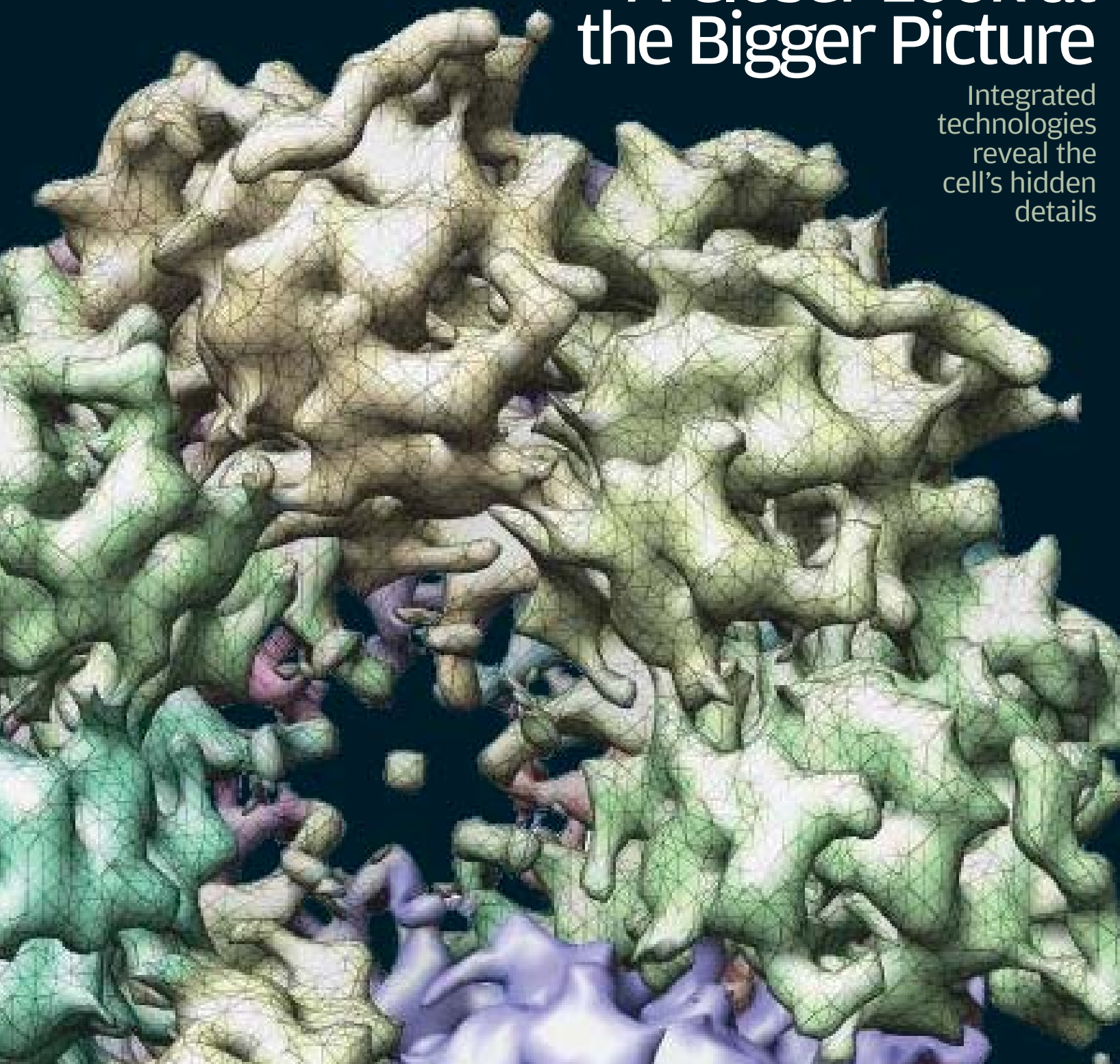
SUMMER 2004

CRITICAL RESOURCES FOR YOUR RESEARCH

U.S. Department of Health and Human Services

A Closer Look at the Bigger Picture

Integrated technologies reveal the cell's hidden details



FROM THE DIRECTOR

Enhanced Resource Sharing

Progress in biomedical research depends on prompt and broad access to sophisticated tools and resources. To ensure the widest availability, NCRR strongly encourages the sharing of resources. When resources are available to only a few scientists within one or a small number of venues, there is a great risk that fruitful avenues of research may be compromised. And in times of budget constraints, high-cost technologies and resources have to be accessible to as many users as possible to effectively leverage federal dollars and enhance research.

Our recent Strategic Planning process reminded us once again of the importance of ensuring that researchers are aware of NCRR-supported centers, instrumentation, networks, technologies, and repositories—all of which enable resource sharing. Feedback from investigators who participated in the Strategic Planning process indicated that our communication vehicles—including the *NCRR Reporter*—should enhance investigators' awareness of available research resources, their capabilities, and how to access them. The new design of the *Reporter*—debuted in this issue—is one step toward that goal.

Greater emphasis will be placed on critical resources, and the front section of the magazine will focus on the resources themselves, providing information on the most current resources for either research or services. To better meet the needs and interests of our readers, the *Reporter* also features several new types of articles, including "Quick Takes," which describe the latest Web sites, research tools, and other useful information from NCRR-supported resources. In addition, the new "Funding Matters" department will highlight various grant mechanisms and show how they've enhanced individual careers or enabled novel research advances.

Of course, the redesign is a "work in progress" that will evolve as we receive feedback from our readers. After you see a few issues with this new approach, please share your ideas with us about how to enhance the design, usefulness, or readability of the *Reporter*. We endorse sharing in all its forms.

Judith L. Vaitukaitis, M.D.
Director, NCRR

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NCRR Reporter

This quarterly publication of the National Center for Research Resources fosters communication, collaboration, and resource sharing in areas of current interest to scientists and the public.

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On the Cover: This close-up view of the molecular machine GroEL reveals previously unseen crevices and details. By combining technologies in unique ways, scientists at NCRR's advanced microscopy resources uncover molecular structures that are important to human health.
IMAGE COURTESY OF STEVEN LUDTKE, BAYLOR COLLEGE OF MEDICINE

QUICK TAKES

New tools and updates from NCRR Resources

New *Drosophila* "Knockouts"

Fruitfly strains with genetic insertions and deletions are now available.

A common method for determining gene function is to delete, or knock out, the gene and look for changes in the organism. For this type of research, many scientists use the fruitfly *Drosophila melanogaster* because it has a short life cycle and is easy to maintain in large numbers. Most chromosomal deletions in this animal have been generated through irradiation or chemicals, but the resulting strains are genetically diverse, complicating the process of analyzing gene function. Now, the Bloomington Drosophila Stock Center at Indiana University, in collaboration with Exelixis, Inc., a genomics-based drug discovery company, is making available genetically identical, or isogenic, *D. melanogaster* strains with small molecularly defined deletions. Together, the deletions cover more than half of the genome. As such, it is the most complete collection of molecularly defined genetic deletions in a multicellular organism. Additional fly stocks carry DNA sequence insertions in *D. melanogaster* genes, with which scientists can create their own customized deletions.

Some strains are currently available from the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu), with additional insertion strains to be distributed by Harvard University later this year. When available, announcements will be placed on the FlyBase Web site (http://flybase.bio.indiana.edu) and in *Nature Genetics*. ■

IMAGE BY ELAINE MENG, UCSF (RIGHT)

Tulane Provides Adult Stem Cells

The Center for Gene Therapy at Tulane University is offering academic researchers standardized preparations of human, rat, and mouse mesenchymal stem cells (MSCs) for nonclinical use. These adult stem cells, derived from the connective tissue framework of bone marrow, appear to have the capacity to differentiate into many cell types, including bone, cartilage, and neurons. In the future, MSCs might provide treatments for diseases—such as arthritis, osteoporosis, and neurodegenerative conditions—that require replacement of missing cells.

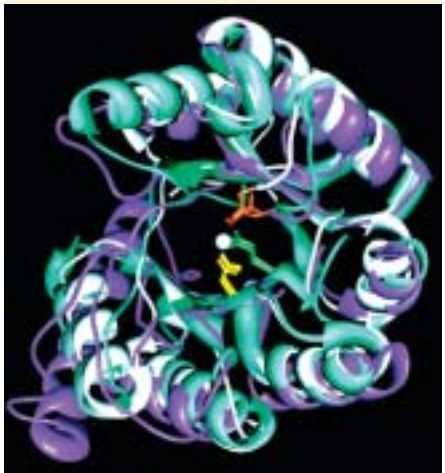
In 2003, NCRR funded a center grant to the Center for Gene Therapy to prepare, test, and distribute human MSCs. Cells are now being made available for in vitro or animal studies but not for administration to patients or for commercial purposes. Requests for cells should be directed to Peggi Wolfe, Center for Gene Therapy, Tulane University Health Sciences Center, phone: 504-988-7752; fax: 504-988-7710; e-mail: wolfe@tulane.edu. ■

Protein Modeling Made Easier

Although genome sequence data have been accumulating rapidly, data about the structures of the proteins produced from these genomes have not kept pace. To help close the gap, computer scientists have developed comparative modeling programs that compare proteins with unknown structures to similar "template" proteins with structures that have been determined experimentally.

All available protein structures derived through comparative modeling are included in a database called MODBASE, developed at the University of California, San Francisco (UCSF). Recent upgrades to MODBASE now allow protein models and their templates to be displayed using the CHIMERA Molecular Modeling System, developed by researchers at the NCRR-supported Resource for Biocomputing, Visualization, and Informatics at UCSF. This change simplifies the visualization process and enables the user to apply CHIMERA's rich set of analysis tools to studying the protein model. MODBASE and associated resources can be accessed at http://alto.compbio.ucsf.edu/modbase-cgi/index.cgi. ■

CHIMERA compares the structures of different enzymes.



A Closer Look at the Bigger Picture

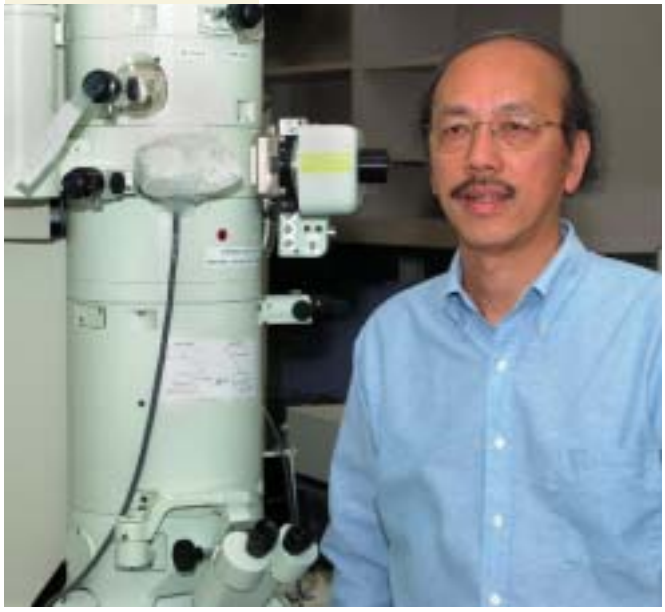
Integrated technologies examine the middle ground between atomic details and whole cells. **BY VICTORIA L. CONTIE**

When it comes to studying the inner workings of the cell, context is everything. Scientists today have well-established techniques for isolating and examining tiny cellular components, like proteins and other biological molecules, at atomic-scale detail. Other technologies can visualize the cell's overall architecture and its large internal structures. But the areas in between—including multiprotein complexes like viruses, molecular machines, and filaments—are key to understanding how cells operate in health and disease. Ideally, these complexes should be studied in their native environment—inside the cell—to truly understand how they function. But techniques for studying this middle ground, between molecular structures and the whole cell, present substantial challenges that researchers have just begun to address.

NCRR supports several national research resource centers across the country, where scientists are working to enhance and integrate a variety of technologies that can capture the three-dimensional (3-D) structures of multicomponent protein assemblies and discover where and how they function in the cell. "Using integrated technologies, researchers can examine not just individual molecules, but macromolecular machines and protein complexes that are working in concert, or communicating with one another, and establish their various locations and interactions within the cell," says Amy Swain, a Health Scientist Administrator in NCRR's Division for Biomedical Technology Research and Research Resources.

Molecular-scale machines play an important role in human health, since they include essential cellular components like the ribosome, which manufactures protein chains by latching onto and maneuvering protein-building molecules through its internal, movable assembly line. Another molecular machine, known as a flagellum, is a whiplike propeller that gives mobility to infectious bacteria and other single-celled organisms. The detailed workings of these and other molecular assemblies can now be scrutinized via integrated technologies.

Leading these efforts are several advanced microscopy resources, all



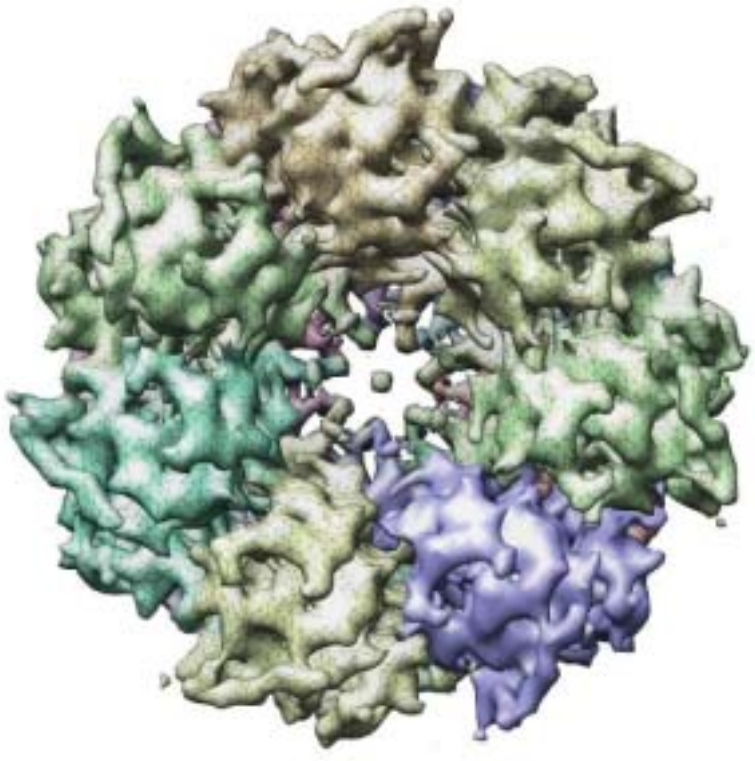
Wah Chiu uses electron cryomicroscopy to study the structures of large molecular complexes and viruses.

of which are available for collaboration and use by the scientific community (see "To Gain Access" on page 7). Scientists at these NCRR resources specialize in developing electron microscopy (EM) techniques, novel software, and other tools to create detailed 3-D reconstructions of macromolecular assemblies that are too complex to be solved by conventional high-resolution techniques, such as X-ray crystallography or nuclear magnetic resonance spectroscopy.

ACCENTUATE THE POSITIVE

Because every technology has particular strengths and limitations, researchers can mix and match new and mature technologies in unique ways, to maximize the benefits and offset the limitations of each. This integration of technologies—sometimes known as a hybrid approach—is essential for understanding the inner workings of the cell, because no biological process involves only one protein in isolation. "We need to look at a particular protein as a component of a biological functioning unit," says Wah Chiu, principal investigator of the NCRR-funded National Center for Macromolecular Imaging, located at Baylor College of Medicine in Houston.

Chiu enlisted such complementary techniques in his structural studies of GroEL, a molecular machine that assists in the proper folding of proteins. He and his colleagues combined the power of an electron cryomicroscopy (cryo-EM) technique known as single-particle analysis with an X-ray-based technique called solution scattering, which can correct for artifacts introduced by the microscope. By combining these two technologies, the researchers could obtain a more accurate picture of the structure of GroEL. "Solution scattering provides complementarity. It also allows you to study proteins and biosystems in a more natural state, in solution," says Hiro Tsuruta, a senior research associate at the NCRR-supported Synchrotron Radiation Structural Biology Resource in Stanford, California, where the solution-scattering data were collected.



Microscopy and synchrotron resources revealed the intricacies of GroEL, a molecular machine that aids protein folding.

Overall, the 3-D reconstruction of GroEL is consistent with

Seeing With Electrons

Biologists have used electron microscopy (EM) for more than half a century to obtain images of cells and tissues at higher resolution than is possible with ordinary light microscopes. Structures of cells and large parts of cells can be visualized by visible light, because they fall within the size range of the wavelength of visible light. In contrast, structures of molecules can be visualized using X-rays, which can have very short wavelengths of 1 angstrom or less.

EM helps to bridge the resolution gap in examining cellular structures, because the wavelengths of electrons used in an intermediate-voltage electron microscope are much shorter than wavelengths of visible light. The shorter wavelengths allow EM to probe and reveal molecular, rather than atomic details of a sample.

EM resolution comes at a cost, however. Wet samples cannot be viewed in an EM, because the vacuum of the instrument would cause water to boil; specimens are therefore either frozen solid or altered in some way to replace cellular water. Moreover, the beam of electrons used for imaging can damage the sample, particularly when the specimen receives enough dose to deliver highly detailed images. Thus, studies of biological structures must strike a balance between image resolution and dose to the specimen, striving to get as much useful information about cellular structure as possible. Integrated technologies help to overcome some of the current limitations of EM techniques. ■

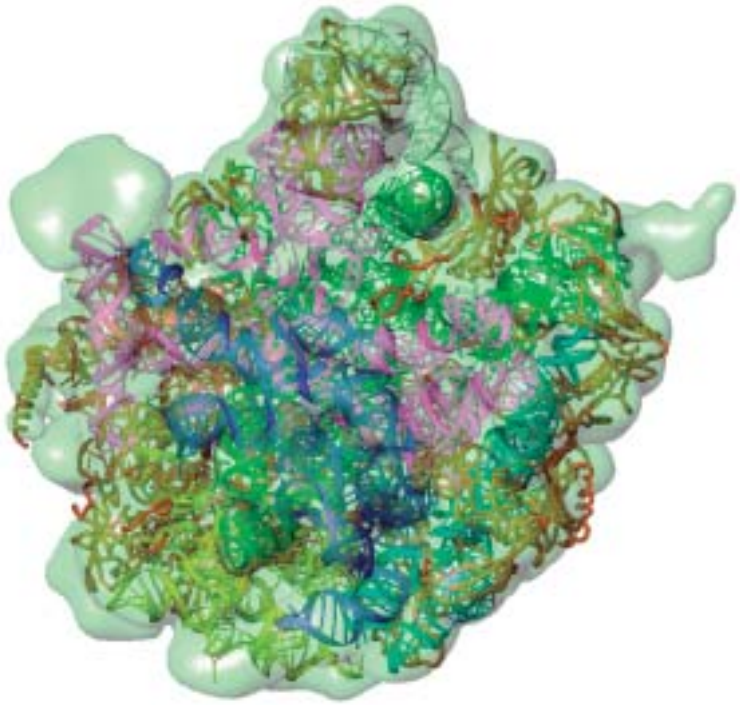
> Researchers can mix and match new and mature technologies in unique ways, to maximize the benefits and offset the limitations of each.

previously published data, Chiu notes, "yet we discovered that our structures do not look identical to the X-ray crystal structures." The new model revealed seemingly flexible regions that could be important in the regulation of the folding activity of this machine. "We believe that our structures correspond to a more natural, solution-based structure of the molecule," says Chiu, whereas the X-ray structure is based on proteins packed in a crystalline environment.

REGARDING THE RIBOSOME

The single-particle cryo-EM technique used by Chiu and others was originally developed in the early 1990s by Joachim Frank, principal investigator at the NCRR-supported Resource for Visualization of Biological Complexity, located at the New York State Department of Health's Wadsworth Center in Albany. The technique involves rapidly freezing millions of identical particles—for example, a virus or a multiprotein complex—and then recording 2-D projection images. Because the particles are randomly oriented before freezing, the cryo-EM "snapshots" capture the particles at multiple angles. Specialized software can then align and refine data from similar 2-D images, ultimately integrating them into a 3-D reconstruction of the entire particle. The more particles that are imaged, the better the statistical definition of the data, and the higher the resolution of the final 3-D model.

The internal complexities of the ribosome's large subunit were mapped by merging crystallography and cryo-EM data.



"We are using and constantly improving cryo-EM reconstruction of molecules in single-particle form, and we apply it to look at the way the ribosome works. This has always involved a hybrid approach, in which one or several X-ray structures are docked into the cryo-EM map," Frank says.

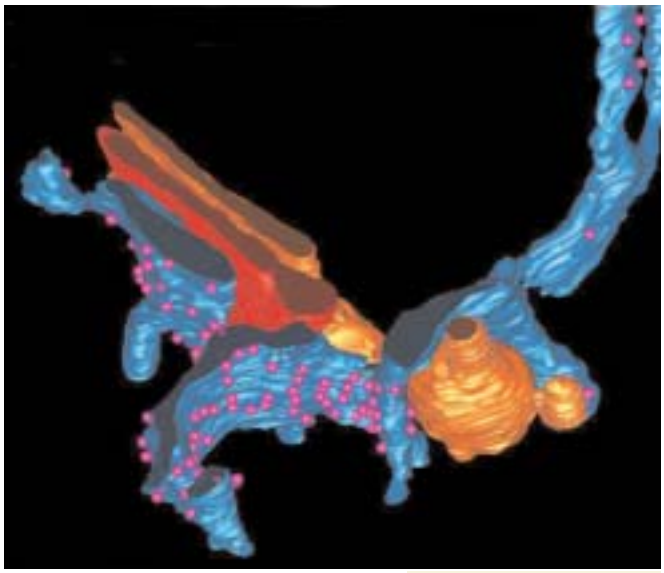
As the cell's protein-building factories, ribosomes are ubiquitous in all types of cells. They show up as thousands of tiny granules when cells are viewed with conventional EM. But over the past five years, hybrid techniques have revealed the striking complexities of this multimolecular machine, composed of more than 50 proteins and three strands of RNA.

"With cryo-EM and single-particle reconstruction, we have a means of showing the ribosome in different functional states, but not at atomic resolution," says Frank. "Today, the real challenge is to see the ribosome's dynamically changing structure as it goes about its business." Therefore, Frank and his colleagues have used computer programs to "mold" atomic-resolution crystal structures of presumably stable portions of the ribosome into larger-scale images of the whole complex obtained via cryo-EM.

One recent study showed details of how the ribosome structure changes during protein synthesis, as the smaller of the ribosome's two subunits rotates in a ratcheting motion to move RNA and related molecules through a cleft in the complex. The rotation changes the ribosome from a compact to a loose structure, accompanied by the reshuffling of many internal proteins. "Cryo-EM is the only way of looking at some of these structures," Frank says, especially when they have fragile intermolecular connections that might not be preserved via crystallography or other methods.

TOMOGRAPHY'S BROADER VIEW

Moving up to the whole-cell level, a technique known as EM tomography holds promise for revealing where and how molecules and complexes function within cells. The NCRR-supported microscopy resources in Albany, San Diego, and Boulder have



■ **Scientists at the University of Colorado used EM tomography to create a 3-D reconstruction that shows the cell's Golgi apparatus (gold and red), endoplasmic reticulum (blue), and ribosomes (purple spheres).**

been leading development of technologies and software for cellular EM tomography for more than a decade. "EM tomography is a bridging technique between whole-cell imaging with light microscopy and the high-resolution techniques like X-ray crystallography," says Daniela Nicastro, a scientist at the Resource for 3-D Electron Microscopy of Cells at the University of Colorado in Boulder, headed by J. Richard McIntosh. "It allows you to view molecular machines without removing them from the context of the cell," Nicastro says.

Like the computed tomography (CT) scans used in hospitals, EM tomography takes 2-D projection images, or "snapshots," of an object—such as isolated organelles or whole cells and tissues—from a variety of angles. Computers then reconstruct the corresponding 3-D images. More than 100 images of a single object may be obtained by tilting it around an axis that is perpendicular to the electron beam. The greater the number of tilted views and the greater the range of angles they cover, the better the detail in the 3-D reconstruction.

Nicastro is exploring the potential of a cutting-edge technique known as cryo-EM tomography, which involves flash-freezing macromolecular complexes, organelles, or whole cells to preserve them in a near-native state, without the need for chemical fixation. Major limitations of this technique include the difficulty of sectioning frozen specimens and the radiation damage caused by the electron beam as it takes multiple images of a single sample. Because cryo-EM tomography requires specialized equipment and expertise, only a handful of laboratories in the world are currently performing such studies, including the NCRR resources in Boulder and Albany.

Nicastro is testing the strengths and limitations of a hybrid approach that combines cryo-EM tomography with single-particle analysis. In her studies of whiplike cellular protrusions known as flagella, she uses tomography to create 3-D reconstructions of rapidly frozen flagella from sea urchin sperm. Nicastro then analyzes the dyneins—the molecular motors responsible for producing the whipping motion—in more detail by "extracting" tomographic subvolumes that contain dyneins, then correlating and averaging their structures to uncover the molecules' conformations within the cell. Although her findings are still preliminary, Nicastro has detected different groupings of dynein structures that seem to correspond to the straight, curving, and curved regions of the moving flagellum frozen in motion. "Eventually, I would like to see the different structural conformations of dynein that are related to the actual beating of flagella," she says.

Beyond microscopy, NCRR supports additional resource centers that develop key components of hybrid technologies, including mass spectrometry, two-hybrid techniques, and simulation and computation. "Clearly, hybrid technology is an area that is taking off, and NCRR will continue to be a key player in future developments," says NCRR's Amy Swain. "Technology development is the core of our Biomedical Technology Research Resources. It's what we do." ■

ADDITIONAL READING

■ Ludtke, S. J., Chen, D.-H., Song, J.-L., et al. Seeing GroEL at 6-D resolution by single particle electron cryomicroscopy. *Structure* 12:1129-1136, 2004.

■ Gao, H., Sengupta, J., Valle, M., et al. Study of the structural dynamics of the *E.coli* 70S ribosome using real-space refinement. *Cell* 113:789-801, 2003.

■ McIntosh, J. R. Electron microscopy of cells: A new beginning for a new century. *Journal of Cell Biology* 153:F25-32, 2001.

TO GAIN ACCESS: NCRR supports five advanced electron microscopy resources that are accessible, free of charge, to qualified scientists. Each of these resources includes advanced instrumentation and expert staff, who develop novel imaging technologies and related software packages that can be downloaded from the resource Web sites. To learn more about applying for access to these national research resources, visit the Web sites listed below.

National Center for Macromolecular Imaging, Baylor College of Medicine. Principal Investigator: Wah Chiu. http://ncmi.bcm.tmc.edu.

National Center for Microscopy and Imaging Research, University of California, San Diego. Principal Investigator: Mark H. Ellisman. http://ncmir.ucsd.edu.

National Resource for Automated Molecular Microscopy, The Scripps Research Institute. Principal Investigator: Bridget Carragher. http://nramm.scripps.edu.

Resource for the Visualization of Biological Complexity, Wadsworth Center, New York State Department of Health. Principal Investigator: Joachim Frank. www.wadsworth.org/rvbc.

Three-Dimensional Electron Microscopy of Cells, University of Colorado. Principal Investigator: J. Richard McIntosh. http://bio3d.colorado.edu.

For information about other biomedical technology research resources, visit NCRR's online directory at www.ncrr.nih.gov/ncrrprog/btdir/btdirectory.asp.

From Physician to Clinical Researcher

Grant program opens door to clinical research careers.

"As a resident, I saw that not enough was done for sickle cell patients when they came to the hospital," says Kenneth Ataga, a sickle cell specialist now at the University of North Carolina (UNC) at Chapel Hill School of Medicine. "We gave them pain medications and IV fluids and sent them home." The reason for the lack of better care quickly became clear to Ataga. "A lot has been done in the basic science of sickle cell disease, but there is a big need to translate these findings into practice," he says.

Ataga, 36, is doing just that, with the help of an NCRR Patient-Oriented Research Career Development Award. The grants, called K23s, are awarded to physicians or dentists who are committed to clinical investigations. The program supports grantees for three to five years as they train in advanced and experimental clinical research methods, explains David Wilde, a medical officer in NCRR's Division for Clinical Research Resources and director of the Center's K23 program. All grantees must work closely with a mentor who is affiliated with an NCRR-funded General Clinical Research Center (GCRC). Since 2000, NCRR has awarded K23 grants to more than 100 clinicians. Because the GCRCs provide an ideal environment for K23 grantees, NCRR has become one of the leaders in this NIH-wide program.

Ataga first considered applying for a K23 while doing a fellowship in hematology and oncology at UNC, where he has been since 1997. As with many K23 grantees, it was Ataga's mentor who encouraged him to apply for the grant. And, like many K23 grantees, he appreciates the time the K23 gives him to do his research while still seeing patients.

For his K23 research, which began in 2002, Ataga is investigating the relationship between blood vessel occlusion, the hallmark of sickle cell disease, and the extreme coagulability of the patients' blood. Sickle cell patients often have painful episodes when sickled red blood cells block circulation and deprive the tissues of oxygen. He and his colleagues recently began a randomized trial to determine the effect of the blood thinner warfarin on patients with sickle cell. Also as part of his K23 award, Ataga recently completed a pilot study that examined how sickle cell patients respond to eptifibatide (EPF), a drug that inhibits platelets and is often used for heart patients. "The results are encouraging," he says. In addition to his K23 research, Ataga is collaborating on a study of the long-term use of hydroxyurea, a drug that reactivates the body's fetal hemoglobin, which is resistant to sickling.

Kenneth Ataga, a sickle cell expert and K23 grantee, preforms clinical studies that help move discoveries from the lab bench to the patient.

Through seminars and work with his mentor, Ataga has become well versed in the rules and regulations that affect the performance of clinical trials, including the legal and ethical issues associated with research on human subjects. Ataga also has the opportunity to take classes on the basic science of sickle cell disease and cell biology.

Another K23 grantee, Jared Gollob, 40, faced a roadblock when he decided to add clinical research to his repertoire, after focusing for many years on basic science. "It is difficult to find a grant that supports researchers engaged in both basic science and clinical research," he says. His advi-

PHOTOGRAPH COURTESY OF DAN SEARS/UNC NEWS SERVICES

sor at the time, Michael Atkins of Beth Israel Deaconess Medical Center in Boston, encouraged him to apply for a K23, which Gollob received in 2000. Gollob's work at the laboratory has enriched his K23 experience, he says. "The K23 allowed me to design clinical trials that grew out of my laboratory research," he says.

Gollob has a regular reminder in his life of the value of translational research. Seven years ago, when she was just another one of his patients with stage 4 melanoma, the woman who became Gollob's mother-in-law participated in a trial of recombinant interleukin-12 (IL-12), a cytokine that boosts immune function. Unlike many stage 4 cancer patients, she beat the odds and remains cancer free.

For many grantees, the K23 is a steppingstone for an R01 grant, NIH's primary mechanism for supporting independent researchers.

Although Gollob's K23 grant proposal focused on IL-12, his research has evolved during the four years that he has had the grant. Now at Duke University Medical Center in Durham, North Carolina, Gollob is studying renal cell cancer and melanoma and is serving as director of the Biologic Therapy Program. In an effort to understand why interferon drugs are ineffective against melanoma, he is investigating how the drugs affect a signaling pathway involved in the malignant behavior of melanoma cells and stem cell growth.

A K23 grant allowed physician Jared Gollob to combine a love of basic science with clinical research, as he investigates treatments for cancer.

PHOTOGRAPH COURTESY OF DUKE MEDICAL PHOTOGRAPHY

When Gollob applied for his K23 grant, he was in a good position to receive the award. He was both a researcher and a clinician, and he was in an environment that would support his work. Also, his research proposal described a thorough investigation into a promising new drug. When reviewing applications for K23 grants, NCRR looks at the appropriateness of the applicants and their mentors, the support that their institutions will provide, their proposed course work, and their research proposals, says Wilde.

To boost their chances of getting a K23, prospective applicants need to set themselves up for success, advises Louise Markert, program director of the GCRC at Duke University Medical Center. That means getting some research experience before applying. "If you have a fellowship program, take an extra year or two to do research," she says. Also, put yourself in a supportive academic environment, even if it means moving to a new institution, Markert says. The Duke GCRC, for example, helps its K23 grantees with all aspects of their research.

For many grantees, the K23 is a steppingstone to qualify for a Research Project (R01) grant, NIH's primary mechanism for supporting independent researchers. To qualify for an R01, scientists must already have preliminary data on their particular research topic. A critical role for the mentor is to guide the K23 grantee through the R01 application process. "The K23 has allowed me to get to the point where I can actually apply for an R01," says Gollob.

For NIH, the goal of the K23 grant program is to ensure that a new generation of physician scientists is available to help move research discoveries out of the laboratory and into the clinic. "NCRR plans to track our K23 graduates to measure their success at winning research grants," says Wilde. "We anticipate they will carry with them a fairly high level of enthusiasm for clinical research," he says. ■

APPLY FOR FUNDING: K23 grantees spend at least 75 percent of their time on clinical research. The remainder may be used for teaching, clinical practice, or other research pursuits. Applicants for NCRR K23 grants must have academic appointments at institutions that host General Clinical Research Centers (GCRC). They must also work with mentors who are active GCRC investigators. Because other NIH institutes offer K23 grants, the decision to apply to NCRR depends in part on the mentors' affiliations. Additional information about K23 awards is available at http://grants.nih.gov/grants/guide/pa-files/PA-00-004.html.

For information about other NCRR funding opportunities in clinical research career development, visit www.ncrr.nih.gov/clinical/cr_crcd.asp.

Seeking the Secrets of Successful Aging

A newly found gene mutation increases longevity. **BY STEVEN STOCKER**

Harry was an artist, store window designer, and theater set painter who died recently at the age of 102. Harry's lifestyle was not exactly conducive to living to old age: he never exercised, was overweight for much of his life, and smoked cigarettes. What kept Harry alive and relatively disease free may have been his genes, says Nir Barzilai, professor of medicine and molecular genetics at the Albert Einstein College of Medicine in New York City.

Harry was a participant in the Longevity Genes Project, started by Barzilai in 1998. This project is one of two large aging-related studies that draw on the resources of the NCRR-funded General Clinical Research Center (GCRC) at Einstein. The second study, the Einstein Aging Study, is investigating cognitive decline during normal aging and the onset of dementia. For both projects, the GCRC provides essential services, including blood tests, magnetic resonance spectroscopy, and statistical analysis.

Barzilai's team seeks to identify genes that help people reach very old age with their health and cognitive functions largely intact. His study group currently includes about 300 Ashkenazi Jews, ages 95 and above. Because most Ashkenazi Jews descended from a small, inbred population in Eastern Europe, this group is genetically quite homogenous and hence useful for identifying mutations associated with disease or longevity. In addition to the over-95 group, or "centenarians," Barzilai and his colleagues are studying more than 300 of the centenarians' offspring, who have an average age of 68. For comparison, the study has two control groups, age-matched to the offspring, including spouses and other individuals who are unlikely to carry longevity genes.

Using DNA extracted from blood samples at the GCRC, the researchers found that the centenarians and their offspring were more likely than the control groups to have a mutation in the gene for cholesteryl ester transfer protein (CETP). This enzyme plays an important role in "reverse cholesterol transport," in which lipoproteins in the blood transport cholesterol from peripheral tissues to the liver. The liver then converts the cholesterol into bile acids that are excreted. Using blood samples processed at the GCRC, the investigators discovered that the CETP mutation appears to increase the size of two of the lipoproteins—high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The mutation also appears to increase HDL levels, at least in many of the offspring. Previous research has shown that elevated levels of HDL, the so-called "good cholesterol," are associated with a reduced risk for cardiovascular disease (CVD), whereas elevated LDL levels are associated with an increased risk.

With statistical and computer support provided by the GCRC Informatics Core, the scientists determined that people with large HDL and LDL particle sizes were less likely to have hypertension, CVD, and metabolic syndrome (a constellation of medical disorders that increases the risk for CVD). Also, the centenarians often were healthier than the spouses of their offspring and remained fairly healthy up until the end of their lives. This was despite the fact that none of the centenarians exercised, about a third were overweight, and some smoked. Centenarians who had higher-than-normal HDL levels also showed little cognitive decline, as shown by a standardized test of cognitive status in adults. Harry, for example, had a perfect score on the test at the age of 100.

Scientists are now working to determine the mechanism by which the CETP mutation might confer health benefits. Strikingly, Barzilai's group found that almost 25 percent of the cen-

Nir Barzilai seeks genes that help people live to a healthy old age.

Centenarians who had higher-than-normal HDL levels also showed little cognitive decline, as shown by a standardized test of cognitive status in adults.

tenarians were homozygous—that is, they had two copies of the mutant CETP gene—in contrast to less than 9 percent of the Ashkenazi control participants. People who were homozygous for the mutant gene also had lower-than-normal blood levels of CETP. Because this enzyme transfers cholesterol from HDL to LDL and related particles, low levels of CETP result in fewer cholesterol molecules being transferred from HDL, which makes the HDL particles larger.

In one sense, the findings contradict established notions about CETP, says Barzilai. "CETP is considered to be important in reverse cholesterol transport, which reduces the risk of atherosclerosis," he says. "If you think of it that way, then any reduction in the efficiency of this pathway would be bad." Barzilai points out that HDL appears to have many beneficial biological properties, in addition to helping to transport cholesterol out of the body. "There's underappreciated literature showing that HDL has anti-inflammatory, antioxidant, and anticoagulant effects," he says. These effects might protect against disease and the effects of aging and might be magnified by high HDL levels and large HDL particle sizes.

The other aging project now under way at the GCRC is the Einstein Aging Study, which follows 488 individuals over the age of 75 who were dementia free at baseline back in the early 1980s. This project is headed by Richard Lipton, professor of neurology, psychiatry and behavioral sciences, and epidemiology and population health. "We're interested in identifying biological markers of dementia and cognitive decline," says Lipton. "The participants in our study will be going to the GCRC for a clinical assessment that includes determining percent body fat and body mass index, the latter expressing adult weight in relation to height. Participants also will have blood drawn for a variety of assays that will be performed at the GCRC Core Laboratory, and the laboratory will help us establish cell lines for identifying genetic markers."

The GCRC also is assisting in a magnetic resonance spectroscopy study to identify metabolic markers in the brain that might predict the onset of Alzheimer's disease long before the development of clinical symptoms or even anatomical changes in the brain. In collaboration with Jullie Pan, associate professor of neurology and neuroscience and director of the GCRC Core

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Laboratory, Lipton's team will use the Core Laboratory's human magnetic resonance system, including a 4-tesla magnet that is among the strongest magnets available for human research.

Lipton says that he is investigating many of the same biological and genetic markers that Barzilai is examining in his centenarian studies. "We're finding that some markers of longevity are also markers for successful cognitive aging, and many of the traditional risk factors for stroke and cardiovascular disease are also risk factors for Alzheimer's disease," he says.

However, not all of Lipton's findings follow this pattern. For example, low diastolic blood pressure—measured when the heart is relaxed—is not a risk factor for stroke or CVD, yet one of Lipton's studies found that elderly individuals with low diastolic blood pressure had an increased risk of developing Alzheimer's disease. The study's lead author, assistant professor of neurology Joe Verghese, suggests that low blood pressure may increase the risk of Alzheimer's disease by decreasing blood perfusion in the brain. Verghese has received an NCRR Mentored Clinical Research Scholar Award, which provides funding to help physicians and dentists become independent clinical investigators.

Ultimately, these studies may lead to new screening tools that can identify people at risk for various aging-related disorders, such as dementia. Another benefit may be new strategies for disease prevention, such as drugs that increase lipoprotein particle size. One drug company has developed a CETP inhibitor that has been shown in a recent study to increase HDL and LDL particle sizes and increase HDL levels. This study was conducted at the GCRCs of the New England Medical Center in Boston and the University of Pennsylvania in Philadelphia.

If this drug is eventually approved for use in patients, Barzilai says he may take it himself, in hope that it would increase his lifespan. "Check back with me in 50 years and find out if I'm still alive," he suggests. ■

The research described in this article is supported in part by the NCRR Division for Clinical Research Resources, the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Institute on Aging.

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Healing the Spinal Cord

Because severed nerve cells in the mammalian spinal cord do not regenerate spontaneously, patients with traumatic spinal cord injuries are often paralyzed for life. No existing drug promotes spinal cord regeneration, but scientists at the Center for Study of Gene Structure and Function at Hunter College in New York City recently identified a strong candidate. The center is funded by NCRR's Research Centers in Minority Institutions Program, which enhances the research capacity and infrastructure at minority colleges and universities that offer doctorates in health sciences.

Marie Filbin and her colleagues treated adult rats that had neck injuries to the right half of the spinal cord by replacing the injured part with embryonic spinal cord tissue and then administering the drug rolipram for 10 days via a subcutaneous pump. Following a four-week rest, each rat was placed in a clear, open-top plastic cylinder and its rearing behavior videotaped. Uninjured rats reared in the tube and explored the wall with both paws, always placing the palm side of each paw on the wall. Injured rats that had not received rolipram avoided using the forelimb of the side of the injury or placed the backside of that paw against the wall. In contrast, injured rats that had been treated with rolipram usually placed their paws correctly and reached higher than untreated animals, indicating that they had greater control over both paw and forelimb movements.

> In the rolipram-treated animals, neuronal growth was increased more than a hundredfold and the amount of scar tissue was reduced.

After behavioral testing, the injured site was examined microscopically. In the untreated rats, very few neurons had regrown into the transplant; in the rolipram-treated animals, neuronal growth was increased more than a hundredfold. In addition, the amount of scar tissue, which is a physical barrier to spinal cord regeneration, was reduced in the rolipram-treated animals.

Originally developed as an antidepressant, rolipram inhibits the enzyme that degrades cyclic AMP, an intracellular messen-

ger chemical that stimulates nerve growth. One major advantage of rolipram is that it reaches the brain and spinal cord when delivered subcutaneously or orally. The results of this study suggest that rolipram may be a potential first treatment for spinal cord injury. (*Proc Natl Acad Sci USA* 101:8786-8790, 2004)

—STEVEN STOCKER

NCRR RESOURCES: The Center for Study of Gene Structure and Function at Hunter College (http://sonhouse.hunter.cuny.edu/genecenter) is one of 19 Research Centers in Minority Institutions (RCMI) within the United States and its territories. NCRR established the RCMI Program to strengthen the research environment in colleges and universities that have a 50 percent or greater enrollment of students who are underrepresented in biomedical sciences, and award doctoral degrees in the health professions or health-related sciences. Underrepresented students are African Americans, Hispanics, Native Americans, Alaskan Natives, Native Hawaiians, and Pacific Islanders. Each RCMI has a thematic scientific focus in one research area, such as neuroscience, immunology, or bioengineering; the research can be either basic or clinical. More information about the RCMI program is available at www.ncrr.nih.gov/resinfra/ri_rcmi.asp.

The Evolution of Learning

THE MECHANISMS INVOLVED in the formation of long-term memories—memories lasting days to weeks or longer—may have evolved from the cell's response to stress or injury, according to a study by James Schwartz at Columbia University, in collaboration with colleagues from Israel. The scientists found that long-term memory formation in a marine mollusk called *Aplysia* was associated with activation of an enzyme, polyADP-ribose-polymerase-1 (PARP 1), previously linked to DNA repair. The *Aplysia* for this research were obtained from the NCRR-supported National Resource for *Aplysia* at the University of Miami.

The researchers discovered that PARP 1 was activated in *Aplysia* nerve cells during two types of learning. In one experiment, the investigators administered four mild and harmless electric shocks to one side of each animal at 30-minute intervals. This procedure enhances the extent and duration of the animals' withdrawal from a weak touch—a process called behavioral sensitization. The scientists found that PARP 1 was activated in the neurons mediating this sensitization but only on the side of the body that received the electric shocks.

In another experiment, the researchers examined feeding reflexes of *Aplysia*, which usually ingest seaweed, their natural food, when it touches their lips. When the animals were offered seaweed wrapped in plastic netting, they attempted to eat the seaweed but were prevented from swallowing because of the netting. Eventually, the animals stopped responding to the seaweed stimulation to their lips. When the animals were offered netted seaweed 30 minutes or 24 hours later, they again responded initially to the food but then rejected it, now after fewer eating attempts than the first time, indicating a memory of the previous negative experience. By administering a PARP 1 inhibitor before initial exposure to netted seaweed, the researchers found that the unpleasant memory could be eliminated during reexposure 24 hours later but not 30 minutes later. Thus, the scientists conclude, PARP 1 activation is associated with long-term (24 hours) but not short-term (30 minutes) memory.

The sea slug *Aplysia* is used to study the mechanisms of long-term memory.

The researchers suggest that PARP 1 may be involved in loosening the chromatin proteins that wrap around DNA, thereby exposing genes and providing access for enzymes involved in transcribing memory-enhancing sections of the DNA. These findings give scientists a potential new target for developing memory-enhancing drugs and therapies. (*Science* 304:1820-1822, 2004.)

—STEVEN STOCKER

NCRR RESOURCES: The National Resource for *Aplysia* provides research investigators with laboratory-reared *Aplysia californica* plus their food source, the red seaweed *Gracilaria*. The Resource cultures animals under standard conditions from egg to adult, using procedures that produce large batches of siblings. This reduces interindividual variation for those researchers needing cohorts with a consistent genetic background. Animals of all ages and stages are available throughout the year. In addition, researchers may request special cohorts, procedures, or manipulations of animal groups. Every year, the Resource ships about 25,000 animals and produces about 12 tons of red seaweed. Resource investigators also conduct studies on various subjects involving *Aplysia*, including life history, animal husbandry, and disease prevention. More information about the Resource can be found at www.rsmas.miami.edu/groups/sea-hares.

PHOTOGRAPH COURTESY OF GENNY ANDERSON, SANTA BARBARA CITY COLLEGE

The Birth of Biocomputing Networks

A high-risk project launched the first online research community for biomedical scientists.

Chat rooms, virtual communities, and shared electronic information are the stuff of everyday life. But more than 30 years ago, shared computer networks were mostly limited to a handful of research locations across the country that were connected to the U.S. Department of Defense's ARPANET, the predecessor of today's Internet.

Fortunately, back in 1973, a forward-thinking research team at Stanford University helped to open up access to online computing networks through the creation of SUMEX-AIM, the first nondefense, nationally shared computer system connected to ARPANET. Founded by Nobel Prizewinning scientist Joshua Lederberg and his colleagues, SUMEX-AIM (Stanford University Medical Experimental Computer for Artificial Intelligence in Medicine) pioneered the remote sharing of computational power and novel software among biomedical researchers and greatly influenced later developments in artificial intelligence (AI). Today the offshoots of SUMEX-AIM are apparent in shared computer-related biomedical resources like GENEBANK, software archives like Info-mac Digest (www.info-mac.org), bioinformatics networks, and online collaborations.

"SUMEX-AIM was an example of a high-risk research project that paid off in many ways," says Tom Rindfleisch, who served as director of the NCRR-supported SUMEX-AIM resource during its 18-year existence. "We were embarking on something that was really very speculative, trying to build a national community of collaborators who were working on artificial intelligence and sharing computer technologies that were just getting off the ground."

Through SUMEX-AIM, research groups around the country were able to gain access to a computer system remarkably advanced for its day. "But to put things in perspective, our initial computer required a big roomful of equipment—about 2,000 square feet—and the computer's speed was about 1/50 the speed of today's typical desktop machines," says Rindfleisch.

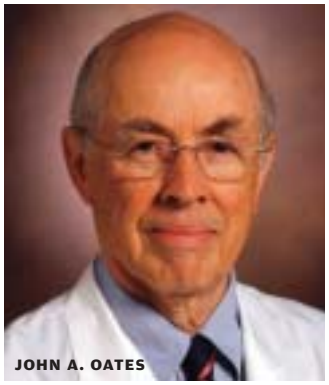
Among the many shared AI applications developed at the resource were an early rule-based expert system that analyzed mass spectra to determine molecular structure and a program that offered diagnostic advice to physicians by analyzing a database of about 700 disease profiles and 5,000 symptoms, signs, and laboratory measurements. "The problem-solving functions of AI have since found their way into a lot of different applications that are embedded in the general computing environment," Rindfleisch says.

Although AI applications and online collaborations are now common, the future of these emerging disciplines was doubtful in the early 1970s. "NCRR, then known as the NIH Division of Research Resources (DRR), played an absolutely pivotal role in recognizing the importance of this effort," Rindfleisch says. "DRR staff, study sections, and the advisory council were able to envision where this technology could go, and they were willing to take a risk and support this work."

SUMEX-AIM continued to operate and expand the scope of its collaborations—under the initial leadership of Lederberg, followed by principal investigators Edward Feigenbaum and then Edward Shortliffe—until the Internet paved the way for alternative networks, and SUMEX-AIM was discontinued. Today, NCRR remains a leader in supporting the development of computational networks, including the neuroscience-based Biomedical Informatics Research Network (see the Fall 2003 *NCRR Reporter*, pages 5-7) and NIH Roadmap initiatives like the National Centers for Biomedical Computing. "It's not a stretch to say that SUMEX-AIM was a forerunner for these types of collaborations," Rindfleisch says. "Bringing people together, to collaborate by sharing computer hardware and software, that's what SUMEX-AIM was all about."

—VICTORIA L. CONTIE

Joshua Lederberg helped pioneer the use of computer networks in biomedical research.


JOHN A. OATES

Oates Receives Clinical Research Award

John A. Oates, Thomas F. Frist Sr. Professor of Medicine and professor of pharmacology at Vanderbilt University, received the 16th Annual Award for Excellence in Clinical Research at the General Clinical Research Center (GCRC) Program Directors Meeting, held April 14-16, 2004, in Chicago. The award recognizes outstanding clinical investigators who have conducted studies in GCRCs within the previous decade.

Oates has published more than 400 papers on a wide range of diseases, including hypertension, colon cancer, and mastocytosis. Many of these studies made extensive use of the resources at the Vanderbilt University GCRC. Among his accomplishments is the development of a noninvasive diagnostic test for systemic mastocytosis, a disease in which mast cells accumulate in organs and tissues throughout the body. ■

NAS Members Received NCRR Support

Among the new members recently elected to the National Academy of Sciences (NAS) are scientists who have used or developed NCRR-supported resources for their research. NAS is a private organization that advises the federal government in matters of science and technology. Election to Academy membership is considered one of the highest honors that can be accorded a scientist or engineer.

Diane Griffin, Johns Hopkins University. Using the resources of the General Clinical Research Center (GCRC), Griffin investigated the immune system's role in various neurological diseases associated with HIV infection.

Nancy Hopkins, Massachusetts Institute of Technology. With NCRR funding, Hopkins developed a rapid technique for inducing mutations in zebrafish. The technique has helped to identify hundreds of genes involved in embryonic development.

Mark Keating, University of Utah. Using GCRC resources, Keating has characterized mutations responsible for various cardiovascular diseases and Williams syndrome, a developmental disorder.

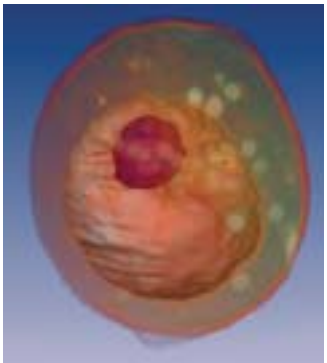
H. Eugene Stanley, Boston University. Assisted by the NCRR-supported Research Resource for Complex Physiologic Signals at Beth Israel Deaconess Medical Center, Stanley analyzes the physiological systems involved in circadian rhythms, heartbeats, and gait rhythms, seeking new measures that have diagnostic or prognostic utility.

Tilahun Yilma, University of California, Davis. In research aimed at producing an effective AIDS vaccine, Yilma has developed and tested potential vaccines at NCRR-supported National Primate Research Centers. ■

New Resource Views Cells with X-rays

Just as computed tomography provides detailed views of the inside of the body, the new field of cryo X-ray tomography is giving researchers high-resolution images of the inside of cells. To allow scientists to gain access to this new technology, NCRR and the U.S. Department of Energy established a new resource at the Lawrence Berkeley National Laboratory (LBNL) in Berkeley, California. At the **Resources for X-Ray Tomography of Whole Cells**, cell biologist Dr. Carolyn Larabell and physicist Dr. Mark Le Gros will build a transmission X-ray microscope that can produce three-dimensional (3-D) images of cells and their internal structures.

■ **Interior of yeast cell**



X-ray microscopy is an emerging technology that combines some of the best features of light and electron microscopy. X-ray microscopy uses low-energy, or "soft," X-rays from a synchrotron to image whole, hydrated cells at resolutions that are about four times better than those available from the best visible-light microscopes. Biological samples are rapidly frozen and need no further chemical alteration or staining to be imaged, unlike electron microscopy, which requires dehydration of the cells and elaborate preparation and staining. Another advantage of X-ray microscopy is that it can be used to localize thousands of labeled proteins and study their interactions within the cell. The new microscope is expected to be ready for use at the LBNL Advanced Light Source Synchrotron in 2006. ■

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Clinical Investigator Wins Presidential Honor

Stephanie Seminara, a faculty member of the reproductive endocrine unit at Massachusetts General Hospital (MGH) and assistant professor of medicine at Harvard Medical School, received a Presidential Early Career Award for Scientists and Engineers (PECASE). The annual award is the nation's highest honor given to professionals at the outset of their independent research careers. Seminara was among 57 federally funded investigators to receive a 2003 PECASE Award, presented September 9, 2004, at a ceremony on Capitol Hill.

Seminara was recognized for her ongoing genetic studies of a rare inherited disorder known as idiopathic hypogonadotropic hypogonadism, in which patients fail to undergo pubertal development. Her findings have implications for understanding normal reproduction and reproductive disorders. She receives primary funding for her research from the National Institute of Child Health and Human Development (NICHD), with additional support from the

STEPHANIE SEMINARA

NCRR-funded General Clinical Research Center at MGH. From June 1999 to April 2004, during the earliest stages of Seminara's career, NCRR supported her five years of mentored training in clinical research at the GCRC. "The five-year award from NCRR allowed me to develop and mature professionally, so I was well-prepared to write the proposal for my R01 grant from NICHD," says Seminara. "Without that training grant, I wouldn't be where I am today." (For more information about the GCRC-based Mentored Patient-Oriented Career Development Awards, see pages 10-11.) Seminara's ongoing studies of patients with reproductive disorders will continue to rely on the GCRC, which provides detailed analyses of each patient's biochemical phenotype. ■

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