U.S. Department of Health and Human Services
National Institutes of Health
National Center for Research Resources

National Center for Research Resources

NCRR Reporter

Spring 2004
Volume XXVIII, No. 2

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From the Director

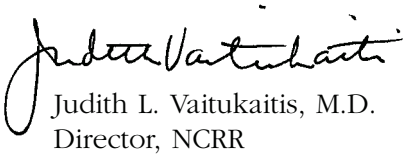
Novel Approaches and Solutions

NCRR's Biomedical Technology (BT) Research Resource Centers focus on the development and dissemination of leading-edge analytical technologies and apply these tools to problems spanning biology and medicine. Each year, nearly 6,000 biomedical investigators, with projects supported by NIH, other government agencies, and the private sector, collaborate with or use the services of the BT Research Resource Centers. These centers foster tremendous synergy by supporting technology development while providing biomedical investigators access to the resources, both driving innovation and maximizing scientific impact.

This issue features some of NCRR's most recent efforts to enhance the nation's capabilities related to cutting-edge biomedical technologies. The new Integrated Biomedical Technology Research Resources for Proteomics and Glycomics are developing novel methods to uncover the identities, structures, functions, and interactions of the thousands of proteins (proteomics) or carbohydrates (glycomics) found in cells. The need for continued development of more fully integrated approaches is no more imperative than in these two emerging fields, which must draw on expertise from diverse disciplines, including medicine, biology, analytical chemistry, and informatics.

NCRR also is building on its long-term investment in proteomics and glycomics through leadership of a complementary trans-NIH effort—National Technology Centers for Networks and Pathways. A part of the NIH Roadmap for Biomedical Research, this network of research centers will create new tools to describe the inherently dynamic interactions of proteins, which function most often in complex relationships rather than in isolation. This ambitious project will enhance research that defines the roles and functions of proteins, whether considered as networks of interactions or steps in biochemical pathways. Grant awards will be made later this year. The centers will develop instruments, methods, and reagents for quantitative measurements at subcellular resolution and very short timescales. Such truly interdisciplinary research will require a collaborative approach, with diverse teams drawn from multiple disciplines.

It is a hallmark of BT Research Resource Centers to work at the forefront of science, developing novel approaches for the solution of biomedically significant problems. These far-reaching efforts illustrate the key role of these centers: support to cutting-edge technologies and research tools; provision of flexible and diverse resources that can readily respond to unanticipated research opportunities; access to rare or unique resources critical for biomedical research and unlikely to find support elsewhere; and support for shared and accessible resources that significantly leverage federal dollars. We set our goals high in our quest to develop critical technologies, methods, and infrastructure. It is the only way to ensure investigators have the resources they need to address today's complex research problems.



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

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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Published for NCRR by the Research Resources Information Center (SSN: 0160-807X)

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Cover: Dr. Ljiljana Pasa-Tolic uses the 11.5-tesla Fourier transform ion cyclotron resonance mass spectrometer at Pacific Northwest National Laboratory, where NCRR-supported scientists have developed a rapid technique for identifying and quantifying all of the proteins in a biological sample. (Photo courtesy of Pacific Northwest National Laboratory)

NCRR Reports

Research findings with NCRR support

CF Linked to Fatty Acids

Recent studies have implicated prolonged inflammation as the primary cause of the lung failure seen in cystic fibrosis (CF). Now comes evidence that at least one trigger for this inflammation may be an imbalance in fatty acids, which are the main constituent of cell membranes. The findings may offer a new approach for treating CF.

About half of the 38 CF patients and 73 control-group volunteers who participated in the study were seen at the NCRR-supported General Clinical Research Center (GCRC) at Beth Israel Deaconess Medical Center. Tissue specimens from CF patients revealed abnormal levels of several fatty acids, including a reduction in docosahexaenoic acid (DHA), a compound that is converted to potent anti-inflammatory molecules in the body. The researchers propose that insufficient DHA may be at least one contributor to the excess inflammation seen in CF. Because the scientists had previously shown that high doses of DHA could correct fatty acid imbalances and reduce inflammation in a mouse model of CF, they propose that a related approach might hold potential for treating CF patients.

—*New England Journal of Medicine* 350:560-569, 2004.

Newborn Monkey from Ovarian Transplant

Up to 90 percent of women who undergo aggressive cancer therapy while of reproductive age become sterile because of treatment side effects. One remedy might be to remove and cryopreserve an ovary at the time cancer is diagnosed and then implant the ovary after the patient is cured of cancer. Toward that end, scientists have demonstrated that ovarian tissue transplants can support the development of live offspring.

The researchers removed ovaries from seven rhesus monkeys at the NCRR-supported Oregon National Primate Research Center and then transplanted some of each monkey's own ovarian tissue back into the body. Within months, the ovarian tissues resumed secreting sex hormones and, in some cases, produced eggs. The eggs were removed and fertilized with sperm, and the resulting embryos were implanted in the wombs of surrogate females. After five months, one of these pregnancies resulted in the birth of a healthy infant monkey. The next step is to attempt this procedure using frozen ovarian tissue, mimicking the same conditions that might eventually be used with women cancer patients.

—*Nature* 428:137-138, 2004.

DNA Origami for Nanoscale Devices

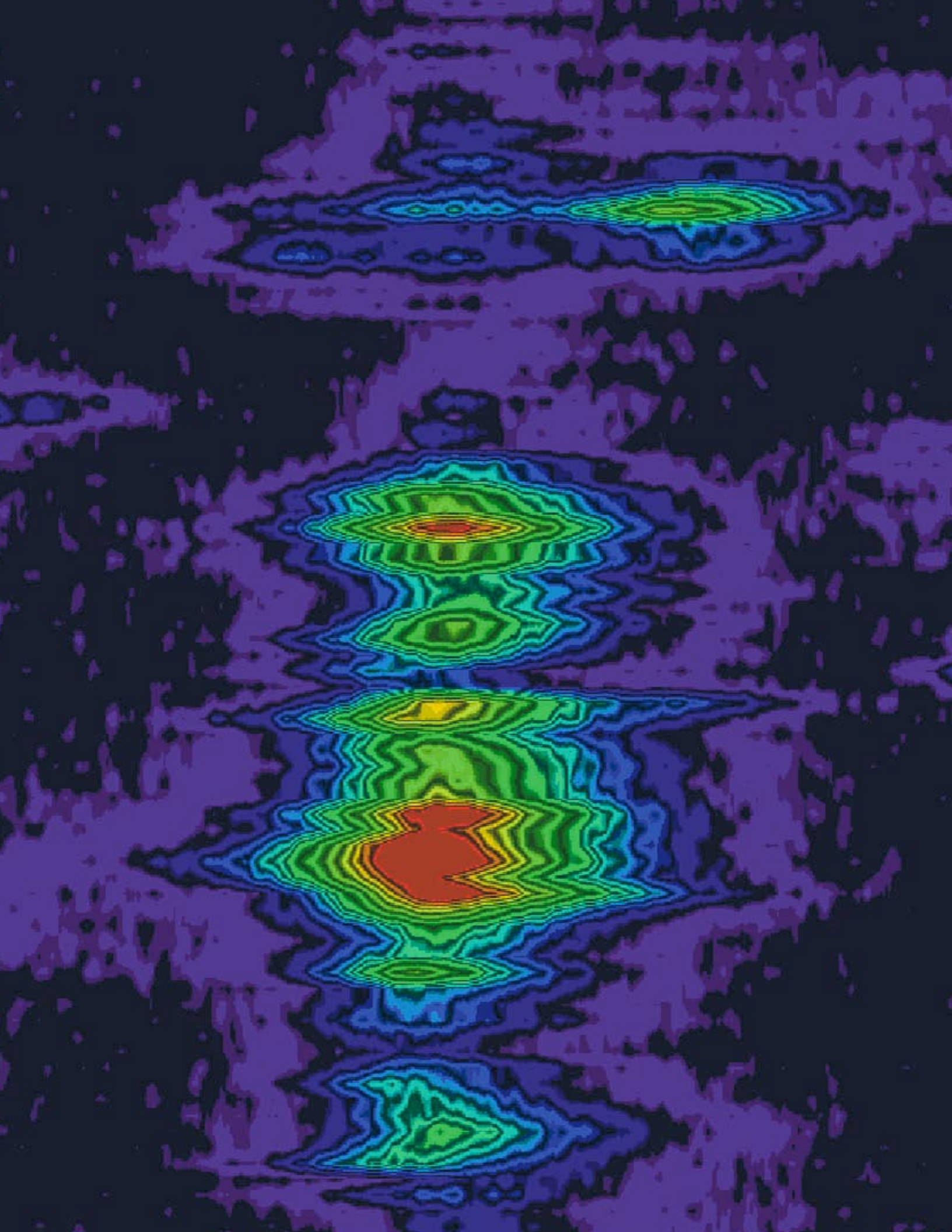
The next big thing in science may be really small things: microscopic motors, computers, and robots whose size will be measured in nanometers, or billionths of a meter. These nanodevices may eventually lead to novel tools for diagnosing and treating diseases, among other uses, but building these devices will require nanoscale building blocks constructed from materials such as biomolecules.

Now scientists have designed a single-stranded DNA molecule that, when heated and then cooled, folds into an octahedron, a three-dimensional (3-D) structure with eight triangle-shaped faces. To view the DNA octahedra, the researchers used a cryo-electron microscope at the NCRR-supported National Resource for Automated Molecular Microscopy, located at The Scripps Research Institute. Computer programs developed in Texas and California with NCRR funding were used to generate a 3-D reconstruction of the octahedron structure and then render detailed 3-D images of the structure.

Unlike other DNA molecules that have been designed to form 3-D objects, the octahedron-forming DNA can be mass-produced by inserting it into the genetic material of bacteria. The structure might eventually be used to form scaffolds that position other molecules at defined locations. Such structures might also aid assembly of nanoscale circuits for microscopic computers.

—*Nature* 427:618-621, 2004.

—Steven Stocker



Giving Proteomics a Push

by Steven Stocker

Now that genome sequencing has become almost routine, attention is shifting toward other components of the cell. Although genes direct cellular events, it is the proteins, carbohydrates, and other molecules that do the actual work. Because of their diverse chemistry and complex shapes, these molecules pose even greater technical challenges to scientists than do the nucleic acids that make up the genome. In fact, characterizing the cell's thousands of working molecules and their interactions is a task too complex to be accomplished via a single approach, technology, or institution.

NCRR has funded three new integrated research resource centers that bring together investigators with diverse expertise to develop novel, integrated methods and technologies for the study of proteins (proteomics) and carbohydrates (glycomics). A long-term goal is to develop tools and techniques that will be accessible to the broader scientific community, says Dr. Douglas Sheeley, the health scientist administrator who oversees the centers for NCRR's Division for Biomedical Technology Research and Research Resources. The three centers, each with a particular expertise, are located at the Pacific Northwest National Laboratory (PNNL) in Richland, Washington; the University of Michigan, Ann Arbor, which serves as headquarters for a multi-institutional effort; and the University of Georgia, Athens.

"Successful research in proteomics and glycomics requires three key ingredients: sufficient biomedical insight to understand the context of the experiment and prepare samples suitable for analysis, the technical skills to develop necessary analytical instrumentation and methods, and high-quality informatics systems to make sense of your data," says Dr. Sheeley. "These three domains are highly interdependent. An integrated resource center enables researchers with diverse skills in these areas to work together synergistically." According to Dr. Sheeley, this approach has already worked well for other NCRR-supported integrated centers, most notably the Yeast Resource Center at the University of Washington, where several important proteomics tools have been developed.

At PNNL's Proteomics Research Resource for Integrative Biology, Director Dr. Richard Smith and his colleagues are developing a rapid mass spectrometry (MS) technique for identifying and quantifying all of the proteins in a biological sample. PNNL's new high-throughput method is about two orders of magnitude faster than the more widely used MS technique known as tandem mass spectrometry. In addition, the new technique is close to two orders of magnitude more sensitive to low-abundance proteins, such as those that regulate gene expression.

In all forms of mass spectrometry, chemicals are electrically charged, or ionized, and the ions are propelled through a series of electric and magnetic fields, which separate the ions into a mass spectrum, much like a prism

This section of a virtual 2-D gel shows several protein spots from* Escherichia coli *that have been separated, from left to right, based on molecular weight and, from bottom to top, based on isoelectric point. With the sensitive new technique, scientists can separate and characterize the proteins in a biological sample.
(Image courtesy of Dr. Angela Walker, University of Michigan)

separates a beam of white light into a color spectrum. To characterize the proteins in a biological sample, the proteins are first treated with an enzyme to break them into peptides; the peptides are then separated using liquid chromatography and injected into a mass spectrometer. The tandem MS approach uses two sequential rounds of MS to obtain a mass and fragmentation pattern for each peptide, which can be compared with fragmentation patterns and predicted masses of peptides in a database to identify the source protein.

To characterize the proteins in a new biological sample, Dr. Smith first uses the tandem MS approach to identify peptides that can be used as markers, or tags, for particular proteins. He calls these peptides "potential mass and time" tags, because each has a unique amino acid sequence that results in a distinctive mass and chromatographic elution time. He then uses an extremely accurate MS technique called Fourier transform-ion cyclotron resonance (FTICR) to confirm the masses of each peptide with great certainty. When FTICR-MS identifies a peptide that has the same accurate mass and elution time as a "potential mass and time" tag already determined via tandem MS, the two peptides are considered with very high confidence to be one and the same. At this point the peptide is designated an "accurate mass and time (AMT)" tag and can be used as a protein marker.

The process of designating a peptide as a potential mass and time tag through tandem MS, and then validating it as an AMT tag via FTICR-MS, is necessary to provide confidence that the peptide is a reliable marker for a particular protein, says Dr. Smith. "Once you have AMT tags established for an organism, the tags can be used in subsequent experiments with that organism without further need to do tandem MS," he says. Because using FTICR-MS requires one step to the two required by tandem MS, scientists can achieve much greater throughput with AMT tags. And because FTICR-MS is an extremely sensitive MS technique, thanks in part to technology developed in Dr. Smith's laboratory, using AMT tags provides greater sensitivity for detection of low-abundance proteins. The AMT technique is a novel, powerful strategy for connecting the very high specificity of peptide sequencing for protein identification with an exquisitely sensitive and rapid detection method.

Scientists will have more versatile tools for exploring the complex activities of carbohydrates and proteins.

Dr. Smith and his colleagues currently are creating an AMT tag database for human blood plasma. The researchers have confidently identified about 3,200 distinct plasma proteins (excluding immunoglobulins), a number 10 times greater than had been reported previously. "This library of AMT tags for human blood plasma will be a tremendous resource for discovering biomarkers for essentially any disease state," says Dr. Smith. For example, abnormal levels of blood plasma proteins might be found to diagnose cancer long before tumors can be detected via X-ray images.

Another innovative technique for characterizing proteins is now being developed at the Integrated Proteome Technologies for Pathway Mapping resource in Michigan. The resource, directed by Dr. Philip Andrews, is associated with the Michigan Proteome Consortium, a statewide network of scientists and research facilities. The new "virtual 2-dimensional (2-D) gel" improves on conventional 2-D electrophoresis. As in the conventional method, a mixture of proteins is deposited on a gel strip and separated by isoelectric focusing, an electric field forcing each protein to migrate into narrow bands in the gel. However, with the new technique, instead of putting the strip at the top of a second gel to separate proteins by mass, the strip is analyzed directly by MS, band by band, to determine the mass of each protein with great accuracy. The many spectra are assembled into a virtual 2-D gel that can be displayed on a computer monitor and that contains much more information than a photo of a conventional 2-D gel.

Because the mass spectrometer is sufficiently accurate to distinguish between proteins with similar masses, scientists can detect post-translational modifications, or modifications made to cellular proteins after they have been synthesized. Like the FTICR technique, the virtual gel is highly sensitive to low-abundance proteins. But what the technique cannot yet do is identify proteins. "The mass of a protein is not enough by itself

to identify a protein," explains Dr. Andrews. "You also need information on protein structure, and for that you need to fragment the protein." The researchers currently are investigating different approaches for fragmenting the proteins in the gel, perhaps using a chemical process or even the mass spectrometer itself. "Ultimately, we hope to be able to identify the proteins, quantify them, and locate sites of post-translational modifications," says Dr. Andrews. "We also intend to automate the whole process."

At the Integrated Technology Resource for Biomedical Glycomics in Georgia, researchers focus on glycans, the carbohydrate structures in glycoproteins and glycolipids, and the roles they play in processes such as cell-cell interactions and signaling. In particular, the scientists are characterizing glycan changes that occur during differentiation of embryonic stem cells. "We are collaborating with a biotechnology company that is trying to influence the differentiation of stem cells in order to produce neuronal precursor cells for treating Parkinson's disease," says Dr. J. Michael Pierce, director of the resource center. "The researchers are interested in how cell-surface carbohydrates change during differentiation, because these molecules are logical biomarkers for monitoring differentiation." The goal is to be able to isolate desired cells at each differentiation step and then multiply these cells until a pure culture of the neuronal precursor cell is obtained.

To help accomplish this, Dr. Pierce and his colleagues will utilize "glycochips," which are DNA microarrays containing genes involved in glycan biosynthesis and recognition. Using the glycochips plus an array of real-time kinetic PCR analyses, the researchers will be able to identify genes that are switched on and off during each differentiation step. The researchers also will use mass spectrometry to study glycan structural changes during differentiation, and a bioinformatics component will integrate the resulting information and link with glycodatabases around the world.

According to NCRR's Dr. Sheeley, glycomics is a fast-emerging field that will greatly benefit from the novel technologies now being developed by Dr. Pierce's research group. "The technology for characterizing glycans lags far behind the technology for characterizing proteins," says Dr. Sheeley. "The glycomics resource at the University of Georgia is filling an important niche in this regard."

With the innovative methods and technologies now under development at all three integrated resource centers, scientists will have new and more versatile tools for exploring the complex activities of carbohydrates and proteins. Together with data on gene sequences and expression, proteomics and glycomics will provide researchers with a broad understanding of the cellular events that underlie human health and disease.

The integrated resources described in this article are supported by NCRR's Division for Biomedical Technology Research and Research Resources. For more information about the integrated research resource centers, consult NCRR's Biomedical Technology Research Resources Directory at www.ncrr.nih.gov/ncrrprog/btdir/btdirectory.asp.

Leveraging the Federal Investment in Biomedical Research

Solving complex problems in biomedical science requires integration and partnerships on multiple levels, from scientific collaborations to interagency leveraging of existing programs and resources. Therefore, NCRR sought to establish the new integrated biomedical technology research resources at institutions that already had suitable research infrastructure in place, usually supported by other funding agencies. For example, substantial investments from the U.S. Department of Energy (DOE) have long supported PNNL's proteomics research programs and related instrumentation. The Michigan Proteome Consortium, consisting of laboratories at three Michigan universities and a private research institute, was started with a grant from the state of Michigan. And the glycomics resource is located in the Complex Carbohydrate Research Center, established by the University of Georgia and funded by DOE, the National Science Foundation, and a Georgia public/private partnership, as well as NCRR. By leveraging the investment of federal dollars, NCRR helps to enhance the cost-effectiveness of biomedical research.

Research Highlights

Deconstructing Asthma

Asthma manifests in different ways. Some patients wheeze and cough in response to allergens or cold weather. Others experience shortness of breath or tightening of the chest. These and other various symptoms make asthma a challenge to effectively diagnose and treat. "We see asthma symptoms that often overlap with many other known diseases, like chronic obstructive pulmonary disease or cystic fibrosis," says Dr. Sally Wenzel, professor of medicine at the National Jewish Medical and Research Center and the University of Colorado Health Sciences Center in Denver. Some researchers have observed that early-onset asthma, arising before 12 years of age, is often triggered by allergic responses. But further attempts to definitively classify asthma have met with little success.

Now, in a five-year study conducted in part at the General Clinical Research Center (GCRC) at the National Jewish Medical and Research Center, scientists led by Dr. Wenzel have identified four clinically distinguishable categories, or phenotypes, of asthma based on age of onset and the presence or absence of inflammation-causing immune cells called eosinophils. The four categories are early-onset asthma with eosinophils in the airways, early-onset without eosinophils, late-onset asthma with eosinophils, and late-onset without eosinophils. Each group may represent a unique disease pathology.

The classifications could have important implications for understanding and treating asthma. Well-defined asthma subtypes may help physicians prescribe the best therapy for individual patients. And if the categories are found to involve unique biological pathways, they also could assist the hunt for elusive asthma genes, says Dr. Wenzel.

The work represents the first effort to define asthma phenotypes through a comprehensive evaluation of patient history, clinical outcomes, and immunopathophysiology. Inspiration for the study came when Dr. Wenzel observed that patients who developed asthma later in life had different symptoms than patients who developed it early, with some having asthma symptoms in the presence, others in the absence of eosinophils. "I felt it was important to determine if they had eosinophil-induced inflammation that did not respond to steroid treatment, or if the inflammation was treated and they still had the disease," she says. "That's what drove me to do the study, to see if differences could be identified in an objective manner."

The team observed 80 patients with severe asthma whose symptoms could not be controlled by steroid treatment. Clinical evaluation included bronchoscopies and laboratory tests conducted at or supported by the GCRC. Of the 80 study participants, 50 had early-onset asthma, with a mean age of onset of 2.6 years. The rest had late-onset asthma, arising at age 12 or older.

Measurement of eosinophils revealed a significant difference between early- and late-onset patients. Of the 30 late-onset patients, 19 had unusually high levels of eosinophils, whereas just 18 of the 50 early-onset patients had a similarly heightened count. That finding challenged the conventional wisdom that heightened levels of inflammation-inducing eosinophils are a primary cause of asthma symptoms—an assumption that had never been proven, says Dr. Wenzel. It wasn't the first crack in the eosinophil theory. Recent studies by other research teams had found that anti-interleukin-5 monoclonal antibodies, which specifically reduce eosinophil levels, offered patients no significant relief from asthma symptoms, casting further doubts on the primary role of eosinophils in asthma symptoms.

Clinical studies conducted by Dr. Sally Wenzel, shown here with a patient, found that individuals with asthma can be classified into four distinct phenotypes. *(Photo courtesy of the National Jewish Medical and Research Center)*

Nevertheless, Dr. Wenzel's team found that higher eosinophil levels, or eosinophilia, did seem to be associated with more severe asthma symptoms in both age groups, and patients with early-onset eosinophilia also had a higher frequency of near-fatal asthmatic attacks. The researchers also found that the pattern

of inflammation associated with eosinophilia differed between the two age groups. Early-onset patients tended to have increased immune cells (mast cells and lymphocytes) consistent with allergy-related inflammation, which supports previous observations that early-onset asthma is often allergic. Late-onset patients with eosinophilia had no evidence of this allergy-related inflammation.

> ***Well-defined clinical subgroups could also aid the search for asthma genes.***

"We were also surprised to find that many patients showed no signs of inflammation, generally considered a hallmark of asthma, yet they still had severe airflow limitation and many asthma symptoms," says Dr. Wenzel. The finding that severe symptoms arise even without apparent inflammation suggests that structural factors may contribute to symptoms; in these cases, inflammation-suppressing steroids would fail to prevent the root cause of the disease.

Late-onset patients with no significant inflammation also warrant a specific category. These individuals showed little sign of structural remodeling—such as thickening of fibrotic membranes—typically seen in early-onset disease. Dr. Wenzel and her colleagues suggest that this asthma subtype may result from some other condition, such as infection or gastrointestinal reflux.

Recognition of asthma subtypes could enhance patient treatment. The prominence of allergy-related inflammation seen in early-onset asthma suggests that physicians might recommend measures to counter specific allergens. In other cases, the continued presence of eosinophils even after standard steroid therapy implies that the inflammatory process remains ongoing. "With those patients, more aggressive treatment would be warranted with higher doses or with other anti-inflammatory agents," says Dr. Wenzel. On the other hand, patients with little or no eosinophilia should not be treated with more steroids, since there are no eosinophils to control, she adds.

Separating asthma patients into well-defined clinical subgroups could also aid the search for asthma genes, which has so far been inconclusive. "There have been some suggestive findings related to asthma phenotypes, but nothing has been definite," says Dr. Malcolm N. Blumenthal, director of the asthma and allergy program and a professor of medicine at the University of Minnesota. Dr. Blumenthal is involved in the multicenter Collaborative Study on the Genetics of Asthma (CSGA), which seeks to identify asthma susceptibility genes by studying familial asthma. The study receives primary funding from the National Heart, Lung, and Blood Institute, with additional support provided by the expert staff and research infrastructure of the GCRCs at the University of Minnesota, University of Chicago, and University of New Mexico. Nearly 300 families, representing several different ethnic groups, are participating in the CSGA, which has produced numerous papers that are helping to narrow the search for asthma-related genes. Recently, Dr. Blumenthal and his colleagues in the CSGA identified four chromosomal regions that are linked to susceptibility to atopy, defined as an allergic skin reaction that is a major risk factor for asthma.

Separating asthma patients into clinical subgroups may provide a better starting point for future studies. "Hopefully, such studies will help to identify more specific biological pathways," thereby making it easier to identify specific genetic risk factors, says Dr. Blumenthal.

Dr. Wenzel plans to investigate whether the same four classifications emerge in patients with moderate asthma. She also would like to identify markers that can further define the subgroups her team has identified. Through better understanding of clinical phenotypes and the molecular underpinnings of asthma, Dr. Wenzel expects that more individualized therapies for asthma patients will soon be uncovered.

—Jim Kling

Dr. Wenzel's research on severe asthma phenotypes is supported by the NCRR Division for Clinical Research Resources; the National Heart, Lung, and Blood Institute; and the National Institute of Allergy and Infectious Diseases.

For more information about NCRR's clinical research resources, see www.ncrr.nih.gov/clinical_rsrch.asp.

Additional Reading

1. Miranda, C., Busacker, A., Balzar, S., et al., Distinguishing severe asthma phenotypes: Role of age at onset and eosinophilic inflammation. *Journal of Allergy and Clinical Immunology* 113:101-109, 2004.
2. Blumenthal, M., Langefeld, C. D., Beaty, T. H., et al., A genome-wide search for allergic response (atopy) genes in three ethnic groups: Collaborative Study on the Genetics of Asthma. *Human Genetics* 114:157-164, 2004.

Bringing Life Sciences to Life

Say the word epidemiology in a pre-teen classroom and you're likely to draw blank stares. But talk about super sleuths and disease detectives, deadly ailments and backpack pain—and engage students in seeking clues and solving mysteries—then you've got a recipe for motivating fledgling scientists in middle school. The new "Detectives in the Classroom" curriculum, developed by researchers at Montclair State University in New Jersey, introduces students to epidemiology and, indirectly, to the science of making evidence-based decisions about important health issues.

The Montclair educators' efforts to take epidemiology off of its graduate school pedestal were made possible by funding from the Science Education Partnership Award (SEPA) program, which seeks to improve life science literacy throughout the United States. SEPA's approach is straightforward: support the development, implementation, and evaluation of innovative K–12 and community-wide life-science education programs. Since its inception in 1991, SEPA has supported more than 130 projects, and NCRR anticipates awarding approximately 12 to 15 new SEPA grants over the next two years. SEPA projects are generally collaborative, involving biomedical scientists and educators, as well as representatives of community groups, science centers, or museums.

Charlyne Jean jump-started her pre-med college career with a summer internship in 2001 at the University of Miami School of Medicine, where she studied the role of proteins in muscle contractions. *(Photo by Mickael Charles, Miami Museum of Science)*

"SEPA is an important part of NIH's public outreach efforts," says Dr. Tony Beck, who oversees the SEPA program for NCRR's Division for Clinical Research Resources. "The key to the SEPA program is its ability to engage and excite minority and underrepresented students about medical science and the inquiry-based scientific method. In doing so, the goal is to increase the number of these students who will pursue biomedical careers," he says.

The mission of SEPA has become more important over the years, as minority representation in science education has declined. The U.S. Department of Education's 2000 *National Assessment of Educational Progress* found that only 3 percent of African Americans and 7 percent of Hispanics scored at or above proficiency in science by 12th grade, and minority students remain underrepresented in engineering and science baccalaureate programs. To rectify such disparities, Montclair and other SEPA programs are involving minority and underrepresented students and their teachers in programs that create the excitement and challenge of biomedical research.

"We want to teach kids how epidemiologists ask questions and get answers," says Dr. Mark A. Kaelin, an associate professor in Montclair's College of Education and Human Services. He and his colleague Dr. Wendy Huebner, an epidemiologist for ExxonMobil Biomedical Sciences, Inc., developed their curriculum for middle school students in collaboration with teachers and a multidisciplinary advisory board. In 2002, they trained 10 middle school science teachers to teach the 30-hour curriculum, test it in their classrooms, and provide feedback on how to improve the course.

Each of the curriculum's five modules addresses a key question in epidemiology: During a disease outbreak, why do some people get sick while others remain healthy? Is there an association between the hypothesized cause and the disease? Is this association causal? What should be done when preventable causes of disease are found? Did the disease prevention strategy work?

To explore these questions, students participate in group discussions and experiments. In one lesson,

students assess the results of a hypothetical study of an acne medication by compiling data on exposed vs. unexposed individuals. Students create their own experiments as well, such as measuring the effects of backpacks on back pain. The curriculum avoids the "finger wagging" typical of health classes that focus on changing behaviors, and instead focuses on educating students about health sciences that underlie much of the health-related advice they hear, says Dr. Kaelin.

Last year, the Montclair team began to disseminate the curriculum to different venues, such as after-school and weekend programs, summer camps, and high schools. The entire curriculum is available on the Web at www.montclair.edu/detectives.

An important goal of the national SEPA program is to help science centers and museums teach inquiry-based thinking on health-related subjects. About one-fourth of SEPA-funded programs are based at science centers or museums, and Dr. Beck would like this number to grow. "Museum- and science-center-based programs reach a broad pool of the general public and complement the formal K-12 SEPA programs," he notes. One award-winning SEPA program—overseen by Dr. Judy Brown, senior vice president of programs at the Miami Museum of Science—has successfully enhanced science education among high school students for more than three years. Called Biomedical Training, Research, and College Prep (BioTrac), the program provides academic enrichment, hands-on activities, and mentoring to encourage underserved high school students to pursue careers in biomedicine. The museum staff partnered with the University of Miami and Miami-Dade County Public Schools to design and implement BioTrac.

As part of BioTrac's academic enrichment component, students conduct a community research project and meet weekly at the museum's laboratory to perform experiments and receive technology training. Students also participate in a six-week research internship in a laboratory at the University of Miami's School of Medicine. This experience—coupled with BioTrac's career awareness component, which offers college visits and meetings with college students majoring in the life sciences, as well as professionals working in the field—is key to the program's success. A recent survey of BioTrac participants attests to the program's effectiveness: Of the 43 BioTrac graduates, 42 entered college and more than half of them have gone into science, primarily biomedicine. This fall, BioTrac staff will offer the first three-day course on how to replicate the program at science museums, extracurricular science clubs, and elsewhere.

Beyond the schoolyard, the national SEPA program also seeks to educate the general public about clinical research. Of all the SEPA grantees, the record for covering the broadest terrain—literally—in reaching the general public may belong to the Imaginarium Science Discovery Center in Anchorage, Alaska. The SEPA project, overseen by Imaginarium Executive Director Mr. Christopher Cable, includes a Health Outreach Caravan that brings a dose of hands-on, interactive, and culturally relevant education to remote villagers.

Beyond the schoolyard, SEPA also seeks to educate the general public about clinical research.

In 2001, the first year of its SEPA grant, Imaginarium staff met with residents of seven rural Alaskan communities to discuss their needs for health information, explains Mr. Greg Danner, director of the Imaginarium's programs and exhibits. The following year, the Caravan traveled to eight remote communities, offering classes targeted to four different grade levels, a school-wide assembly, an evening festival for the local community, exhibits, hands-on activities, and science demonstrations. Future programs will focus on diseases and their causes, as well as lifestyles and health. After one year of field-testing, the programs are now available upon request to any Alaskan community. "We say we go anywhere, anytime," says Mr. Danner.

With projects in 31 states including Puerto Rico and Hawaii, NCRR's SEPA program provides a valuable science education tool for K-12 and for the general public via the SEPA science centers and museums. Moreover, "SEPA's scope of activity and eligibility requirements are flexible enough to make the program a valuable tool for supporting NIH initiatives, now and in the future," Dr. Beck says.

—Tina Adler

The Science Education Partnership Awards are provided by NCRR's Division for Clinical Research Resources. For more information about the SEPA program, see www.ncrr.nih.gov/clinical/cr_sepa.asp.

Critical Resources

A Resource for the Zebrafish Connoisseur

Whether teasing apart biochemical pathways or developing models for human disease, biomedical researchers enlist model organisms like the fruit fly or mouse in many of their investigations. Results from such studies usually apply to humans, because genes are remarkably similar in organisms ranging from single-celled microbes to higher vertebrates. But the most scientifically valuable and versatile model organisms form a somewhat exclusive group, with only a handful of creatures having undergone the extensive study and genomic analysis to qualify. One of the newest members of this select group is the zebrafish, *Danio rerio*, a modest but colorful tropical fish well known to fish store owners and hobbyists.

"The zebrafish is now universally accepted as one of the major model organisms," says Dr. Monte Westerfield, professor of biology at the University of Oregon. "The number of publications and researchers focusing on zebrafish has grown exponentially in recent years." As director of the NCRR-funded Zebrafish International Resource Center (ZIRC), Dr. Westerfield has witnessed, firsthand, the scientific community's increasing demand for zebrafish. ZIRC develops, preserves, and provides researchers with standardized zebrafish strains, mutant strains, research tools, and online support, including a zebrafish database. "Much of what we do is similar to large mouse facilities that supply research laboratories," says Dr. Westerfield. "During the last 12 months, we shipped fish to 240 U.S. labs and 65 international labs in 15 countries." The resource also supplies animals to the pharmaceutical industry, which is exploring the potential use of zebrafish in drug screenings.

The scientific value of the zebrafish lies in its position on the evolutionary ladder. As a vertebrate, the zebrafish is several rungs closer to humans than are fruit flies (*Drosophila*) or worms (*Caenorhabditis elegans*), yet the fish is also much more malleable than most mammalian animal models. Zebrafish currently are used in a wide range of studies, including research on blood diseases, fetal alcohol syndrome, the genetics of polycystic kidney disease, and developmental errors that lead to cleft palate.

Chosen partly for its hardiness, small size, and brief lifespan, the zebrafish possesses an additional trait that endears it to researchers: zebrafish eggs are transparent, allowing direct visualization of the embryo. "Scientists interested in embryonic development—that is, the genes involved in setting up the body and creating the organs—can look directly at how mutations in those genes affect development," says Dr. Westerfield.

Indeed, it may be in genetics that the zebrafish makes its biggest splash. A single fish lays hundreds of eggs at a time, providing ample resources for "forward genetic screens," in which researchers induce random mutations and then observe the offspring for changes in a biological process of interest. When a change occurs, researchers search for the mutation—and gene—involved in that process. Such experiments are key to understanding the role of individual genes. The screens are more difficult to perform using mice because they produce fewer offspring, notes Dr. Westerfield, and a facility that houses a few hundred mice can hold thousands of zebrafish. "Zebrafish enable us to do forward genetic screens very inexpensively," he says.

The growing number of mutant zebrafish strains, like those used for genetic screens, was a major impetus for establishing ZIRC. Throughout the 1980s and 90s, as the use of zebrafish began to increase, researchers maintained their own stocks and filled requests from other researchers for mutant strains, a time-consuming chore. By the mid-1990s, NCRR recognized the need for a centralized resource to house the growing number of mutant strains and responded by funding ZIRC in 1998. ZIRC's 10,000-square-foot, state-of-the-art facility was constructed with partial funding from NCRR's Research Facilities Improvement Program, and NCRR's Division of Comparative Medicine continues to fund ZIRC's ongoing operating costs.

It may be in genetics that the zebrafish makes its biggest splash.

Today, zebrafish researchers who have developed specialized strains can apply to ZIRC to request maintenance or preservation of the fish. Approved fish can be mailed directly to ZIRC where, after a stay in quarantine tanks, the fish are preserved either as live strains or as frozen sperm. Dr. Mary Mullins, associate professor of biology at the University of Pennsylvania School of Medicine in Philadelphia, is one of many scientists submitting zebrafish strains to the ZIRC resource. "We're trying to deposit most of our stock into the ZIRC," says Dr. Mullins, who has been studying zebrafish for more than 13 years. "It's going to help

Zebrafish have embryonic and brain structures similar to humans, which makes these northern India natives useful model systems.
(Photo courtesy of ZFIN)

enormously once all our strains are there. Especially if you have a big collection of mutants, you get a lot of requests from other researchers for strains."

Dr. Mullins uses zebrafish to study vertebrate embryo development, including the bone morphogenetic protein signaling pathway and maternal factors deposited in eggs. "We do a lot of video and time-lapse microscopy," she says. "We can learn an enormous amount by watching in vivo how axons grow and vasculature develops, how a particular gene might be involved in the normal development of a tissue or cell type, or what things go wrong in a mutant." Like a growing number of researchers, Dr. Mullins uses zebrafish engineered to carry a fluorescent "reporter gene" that aids visualization. Dr. Westerfield notes that "the number of submissions to the resource center, and the number of requests for these types of lines, is increasing dramatically."

When ZIRC first opened, one of the biggest challenges was to characterize and develop assays for common zebrafish diseases that had been known to wipe out entire laboratory stocks. "Because we monitor and control for these diseases, we're a specific-pathogen-free (SPF) facility," says Dr. Westerfield. "We provide fish guaranteed to be free of particular pathogens." In addition, outside investigators can send diseased fish to ZIRC for diagnosis and suggestions for treatment.

ZIRC also provides researchers with extensive online information sources. For example, *The Zebrafish Book* available on the ZIRC Web site provides advice on zebrafish care and experimental procedures. In addition, researchers can browse the online ZFIN database (http://zfin.org/zf_info/dbase/db.html) to learn which wild-type and mutant strains are available at ZIRC and then order online. ZFIN stores information on known zebrafish genes, such as sequence data; homology to human, mouse, and fly genes; protein function; gene expression; mutant phenotypes; as well as a complete listing of zebrafish research publications, researchers, and laboratories.

To maintain zebrafish lines, researchers must manage many fish tanks, a logistical headache because the standard 10-gallon glass aquarium is heavy, breakable, difficult to clean and move, and must be plumbed by hand. One of ZIRC's practical, but important, contributions is an integrated fish tank system based on a 1.5-liter tank, small and light enough to hold in one hand. "It's essentially a modular, plug-and-play system. You can plug in the water, air, and drain lines and literally have a hundred tanks running immediately." The product of several years of development, the system is now available from laboratory equipment suppliers.

With new equipment enhancing ease of use, and new technologies enhancing its versatility in the laboratory, the zebrafish has become an essential tool for biomedical research. "The biggest challenge is keeping up with the numbers," says Dr. Westerfield. "New genetic techniques are continuously being developed, and so new zebrafish lines are constantly being generated. This little research animal has so much to offer."

—William Oldendorf

For more information about the Zebrafish International Resource Center (ZIRC), visit the Center's Web site at http://zfin.org/zf_info/stckctr/stckctr.html, or contact Dr. Monte Westerfield at ZIRC, University of Oregon, Eugene, OR 97403-5274; phone: 541-346-4607; fax: 541-346-4548; e-mail: monte@uoneuro.uoregon.edu.

ZIRC is supported by NCRR's Division of Comparative Medicine. ZFIN, the model organism database for zebrafish, receives primary funding from the National Human Genome Research Institute, with additional support from the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, the National Institute of Dental and Craniofacial Research, the National Institute of General Medical Sciences, and NCRR. To learn more about other NCRR-supported comparative medicine resources, see www.ncrr.nih.gov/comparative_med.asp.

News from NCRR

Clinical Researchers Honored

Three investigators who conduct research using the resources of their institutions' General Clinical Research Centers (GCRCs) recently won awards that recognize the body of their research.

Dr. Merrill D. Benson—professor of pathology and laboratory medicine, of medical and molecular genetics, and of medicine at the Indiana University School of Medicine—won the Pasteur-Weizmann/Servier International Prize in Biomedical Research. At the university's GCRC, Dr. Benson studies diseases known as amyloidoses, in which fibrous protein deposits called amyloids accumulate in organs and tissues.

Dr. Joel D. Kopple, professor of internal medicine and public health at University of California, Los Angeles (UCLA), received the Robert H. Herman Memorial Award from the American Society for Clinical Nutrition. At the Harbor-UCLA Medical Center GCRC, Dr. Kopple studies nutrition and metabolism in relation to kidney disease.

Dr. Ronald S. Swerdloff—chief of the division of endocrinology, metabolism, and nutrition at Harbor-UCLA Medical Center—received the Distinguished Andrologist Award from the American Society of Andrology. Dr. Swerdloff relies on the GCRC for his studies that test potential male hormonal contraceptives and evaluate testosterone replacement therapies in men with hypogonadism.

Four Appointed to NCRR Advisory Council

The National Advisory Research Resources Council, which advises NCRR on policies and programs and performs second-level peer review of grant applications, has gained four new members. The new appointees are:

Dr. Kenneth G. Cornetta, Joe C. Christian Professor and chair of the department of medical and molecular genetics at Indiana University in Indianapolis. Dr. Cornetta is director of the university's National Gene Vector Laboratory (NGVL), which

produces retroviral vectors for gene therapy clinical trials and serves as the coordinating center for all five NGVLs. Dr. Cornetta's research focuses on gene therapy for cancer.

Dr. Cynthia E. Keppel, professor of physics at Hampton University in Virginia and director of the Center for Advanced Medical Instrumentation. Dr. Keppel applies detection and data acquisition techniques from nuclear and particle physics to medical technology, concentrating on nuclear medicine and radiation therapy.

Sheila Cohen Zimmet, director of research assurance and compliance for Georgetown University in Washington, D.C. Ms. Zimmet began her professional career as a neonatal intensive care nurse and then became a lawyer, focusing on clinical, bioethical, and biomedical research issues. She previously served on the Council from 1996 to 2000.

Dr. Stuart M. Zola, professor of psychiatry and behavioral sciences and director of the Yerkes National Primate Research Center at Emory University in Georgia. Dr. Zola researches neural mechanisms of memory formation, consolidation, and retrieval. He is perhaps best known for developing an animal model of human amnesia that conclusively identified brain structures important in memory function.

Rhesus cDNA Sequences and Microarrays

NCRR recently funded two projects to derive rhesus macaque cDNA sequences and design rhesus-specific microarrays. In the first project, Dr. Michael Katze, of the Washington National Primate Research Center, and

Dr. Shawn Iadonato, of Illumigen Biosciences, Inc., are preparing rhesus cDNA libraries and sequencing the resulting clones. A general description of the project plus microarray data that can be downloaded are available at http://primate.viromics.washington.edu. The cDNA sequences in FASTA format are available at www.illumigen.com/macaque.

In the second project, Dr. Robert Norgren, of the University of Nebraska Medical Center, and Dr. Eliot Spindel, of the Oregon National Primate Research Center, are designing rhesus-specific microarrays in Affymetrix format, with input from the research community. For a current list of sequences expected to be on the chips, plus information for providing input, see http://rhesusgenechip.unomaha.edu/index.html. All sequences determined by both projects also will be deposited in GenBank, which can be accessed through the National Center for Biotechnology Information Web site at www.ncbi.nlm.nih.gov.

Let There Be (More) Light

One of the world's first synchrotron facilities—the Stanford Synchrotron Radiation Laboratory (SSRL) at Stanford University in California—received a $58 million upgrade, thanks to NCRR, the National Institute of General Medical Sciences, and the U.S. Department of Energy (DOE). The upgrade project essentially rebuilt the existing storage ring—the machine in which electrons circulate at nearly the speed of light, producing visible and invisible forms of light called synchrotron radiation. This venture, which increased the brightness of the synchrotron radiation by one or two orders of magnitude, was begun in 1999 and completed in mid-December 2003, three months ahead of schedule and within budget. It involved removing 1 million pounds of old equipment, installing 1.25 million pounds of new equipment (including 290 magnets), and laying 68 miles of new cable. A dedication ceremony, held on January 29, 2004, featured presentations by Stanford's president, representatives from the funding agencies, the mayor of Palo Alto, and the SSRL director.

Synchrotron radiation at SSRL is used by NIH researchers to understand the structures of biological molecules through macromolecular crystallography, X-ray absorption scattering, and small-angle scattering. In anticipation of the storage ring upgrade, NCRR and the DOE/Office of Biological and Environmental Research have additionally invested in major improvements of the SSRL beamlines and experimental stations over the last 5 years to maximize utilization of the brighter X-rays. Each year, about 1,700 scientists from around the country use synchrotron X-rays at SSRL to solve biomolecular structures that have an impact on the design of new medicines, increase the understanding of how molecular systems work, and provide pictures and insights into molecular machines.

Funding Opportunities in Structural Genomics

NCRR and the National Institute of General Medical Sciences (NIGMS) are seeking grant applications to establish research centers for determining the 3-D structures of "challenging" proteins, including membrane proteins, small protein complexes, and proteins from humans and other higher organisms. The centers will focus on developing methods and technologies in structural genomics, a relatively new field that seeks to ascertain 3-D structures of most proteins from their corresponding DNA sequences in the genome. The new centers will form one component of the Protein Structure Initiative Research Network, which aims to greatly reduce the expense and time involved in determining 3-D protein structures.

The Protein Structure Initiative, launched by NIGMS in 1999, seeks to develop fast, automated methods for deciphering the structures of proteins that are characteristic of major protein families and then use computer models to identify the structures of remaining family members. Knowledge of protein structure facilitates drug development and allows researchers to compare proteins from normal and diseased tissues.

The Request for Applications (RFA) for specialized centers for the Protein Structure Initiative (RFA-GM-05-002) was released on April 1, 2004; the expiration date is October 16, 2004. The RFA can be accessed at http://grants.nih.gov/grants/guide/rfa-files/RFA-GM-05-002.html. For more information about NCRR funding for this RFA, contact Dr. Amy L. Swain, Division for Biomedical Technology Research and Research Resources, NCRR, phone: 301-435-0755; fax: 301-480-3659; e-mail: SwainA@mail.nih.gov.

NCRR Publishes Strategic Plan, Clinical Directory

NCRR has released two new publications, one of which will help the Center establish its future priorities, and the other listing research resources that are available to clinical investigators.

NCRR's *2004-2008 Strategic Plan: Challenges and Critical Choices* will guide NCRR's programmatic activities for the next five years. The strategic plan was developed from input provided through NCRR's Web site and from participants who attended a two-day Strategic Planning Forum in Arlington, Virginia, in September 2003. The *2004-2008 Strategic Plan* lists NCRR's guiding principles and provides goals and objectives related to clinical research resources and networks; informatics and computational biology; nonhuman models for biomedical research; emerging technologies and instrumentation; research capacity building; training and education; research partnerships; and communications.

The 2004 edition of NCRR's *Clinical Research Resources Directory* provides information about the resources supported by NCRR's Division for Clinical Research Resources. Included in the directory are listings for the more than 80 General Clinical Research Centers, which offer clinical investigators specialized research environments for conducting sophisticated patient-oriented research. The directory also contains listings for the Islet Cell Resource Consortium, which distributes human pancreatic islets for transplantation into patients with type 1 diabetes; the National Disease Research Interchange, which distributes normal and diseased human tissues for laboratory research studies; and the National Gene Vector Laboratories, which distribute clinical-grade vectors for human gene transfer protocols.

Both publications, as well as other NCRR publications, are available on NCRR's Web site at www.ncrr.nih.gov/publications.asp. The publications also can be obtained free-of-charge from the Office of Science Policy and Public Liaison, NCRR/NIH, One Democracy Plaza, Room 978, 6701 Democracy Boulevard, MSC 4874, Bethesda, MD 20892-4874; phone: 301-435-0888; fax: 301-480-3558; e-mail: info@ncrr.nih.gov.

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