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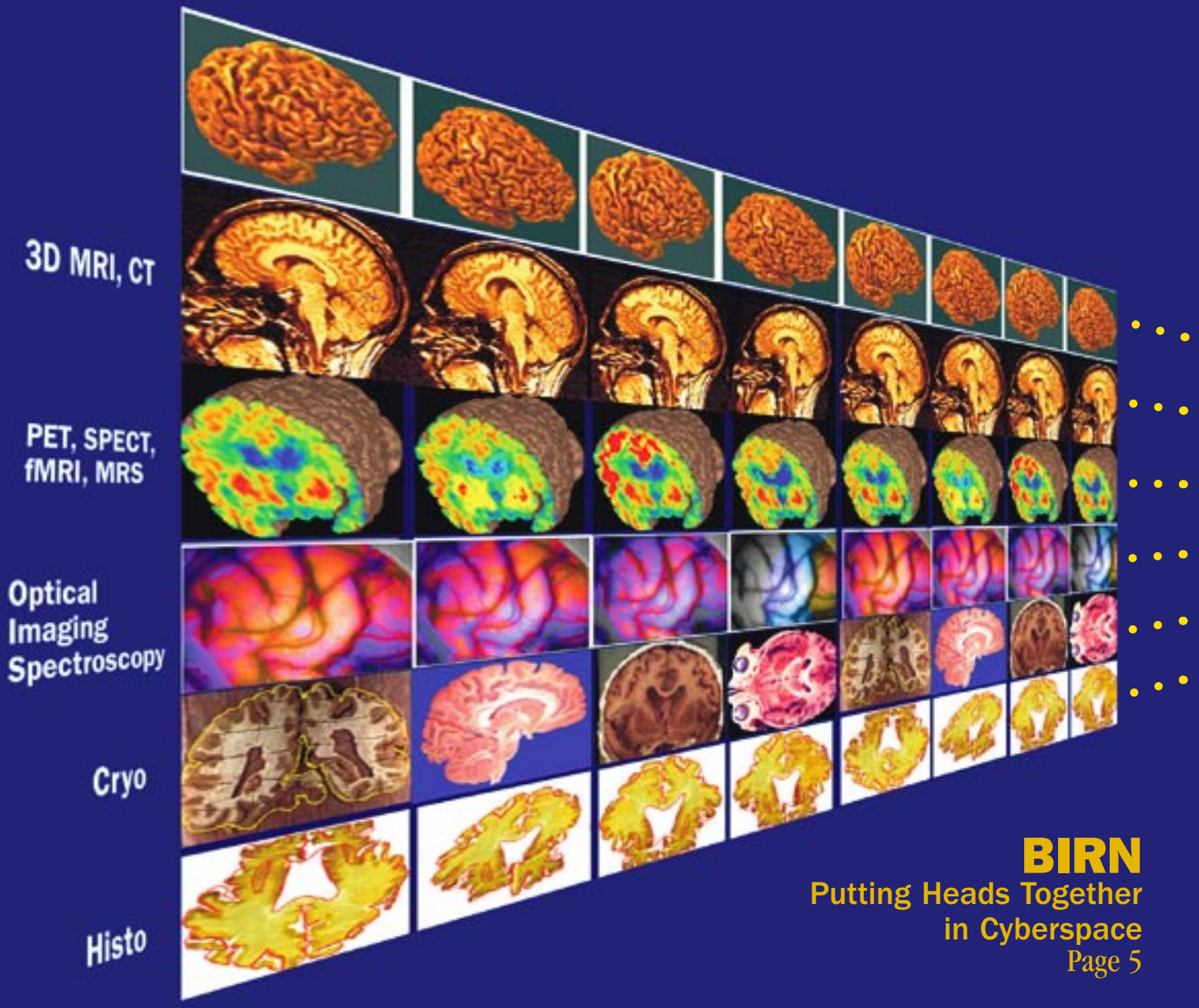


From the Director

Beyond the Conventional

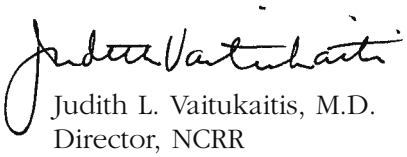
As biomedical research challenges have grown more complex, we've quickly learned that conventional methods may no longer be sufficient for unraveling the mysteries of disease and enhancing human health. Going beyond conventional approaches by sharing and comparing research knowledge, across disciplines and distances, has become paramount.

The Biomedical Informatics Research Network (BIRN), featured in this issue (page 5), illustrates the importance of forming partnerships that reach beyond the conventional boundaries of biomedical science to create research teams that draw on the expertise of computational scientists, engineers, and others. It also shows how effective these research teams can be when they break down barriers related to information sharing.

The infrastructure put into place for the BIRN testbeds allows researchers to address fundamentally different kinds of scientific questions by enabling integration of data from geographically diffuse patient populations and multiple biological scales—from the molecular level to the whole brain. From its onset, the BIRN was designed to be scalable—having the potential for future expansion and adaptation—so that new participants could join the network in a cost-effective manner. Its ultimate goal is to support the work of tens of thousands researchers. In essence, the BIRN is paving the road for the next generation of participants.

In October, Dr. Elias A. Zerhouni, NIH's Director, announced a series of far-reaching, cross-cutting initiatives, known collectively as the NIH Roadmap for Medical Research. The goal of the Roadmap is to transform the nation's medical research capabilities and speed the translation of research discoveries from the bench to the patient. One of the Roadmap initiatives—the National Centers for Biomedical Computing—takes information management to the next level. These new centers, in conjunction with individual investigator awards, will create a networked effort to build on the nation's infrastructure for biomedical computing. The goal is to establish a computer-based grid, wherein biologists, chemists, physicists, and computer scientists across the country will be able to share and analyze data using a common set of software tools. The envisioned system will resemble that of the integrated software packages for office tools installed on most home computers today, in which information can be traded seamlessly among software applications.

By going beyond conventional resources, we will be able to bring together the best minds from around the world to more effectively tackle unsolved biomedical mysteries. As the research paradigm evolves for biocomplexity and ever-expanding data sets, infrastructure must evolve as well. We must make the unconventional, very conventional.



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

NCRR Reporter

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Cover: Scientists today employ a wide range of technologies and methods to image the brain, with each technique providing rich and unique data on the structures or dynamic processes within this complex organ. A new NCRR initiative—the Biomedical Informatics Research Network (BIRN)—aims to create a collaborative research environment in cyberspace, allowing brain scientists to integrate data and knowledge across disciplinary, technological, and institutional boundaries. (Image courtesy of the Laboratory of Neuro Imaging Resource, University of California, Los Angeles)

NCRR Reports

Research findings with NCRR support

Switches for DNA Replication

Before a cell can divide, it must make one copy of its entire DNA so that each daughter cell will have a complete set of genes. The process is tightly regulated, because unrestrained DNA replication can have disastrous consequences for the survival of an organism. Previously, scientists identified one gene, called *cdt1*, that produces a protein required to trigger DNA replication. Now, using strains of *Caenorhabditis elegans* worms obtained from the NCRR-supported Caenorhabditis Genetics Center, researchers have identified another gene, *cul-4*, whose product helps degrade the product of the *cdt1* gene, thereby stopping DNA replication after one copy has been completed. Insight into the mechanisms that limit DNA replication could have important implications for the study of cancer, which is often triggered by aberrant amplification of genes. The Caenorhabditis Genetics Center currently houses more than 5,000 strains of nematodes (mostly *C. elegans*) that are available to the research community.

—*Nature* 423:885–889, 2003.

Copper Is Key to Artery Reclogging

When performing a balloon angioplasty procedure, surgeons insert a balloon-tipped catheter into an artery clogged with fatty deposits and then inflate the balloon to clear the blockage. In most cases, the narrowed arteries are opened. But in up to a third of patients, the arteries become blocked again, often within weeks. Now researchers have identified copper ions as the culprit in the reclogging and have shown in rats that a copper-binding compound can prevent the arterial thickening that contributes to renewed blockage. The study was conducted at the NCRR-supported Center of Biomedical Research Excellence in Angiogenesis, located at the Maine Medical Center Research Institute.

The scientists administered the copper-binding compound tetrathiomolybdate (TTM) to rats before and after inflating a balloon in the carotid artery. Control animals, which underwent the inflation procedure but received no TTM, showed thickening of the inner layer of the artery, a sign of vascular injury caused by balloon inflation. In contrast, TTM-treated animals exhibited less arterial thickening, and animals treated the longest had the least amount of thickening. These results suggest that TTM might eventually prove useful in preventing renewed arterial blockage in patients who have undergone balloon angioplasty.

—*Proceedings of the National Academy of Sciences USA* 100:6700–6705, 2003.

Diabetes Linked to Mitochondria

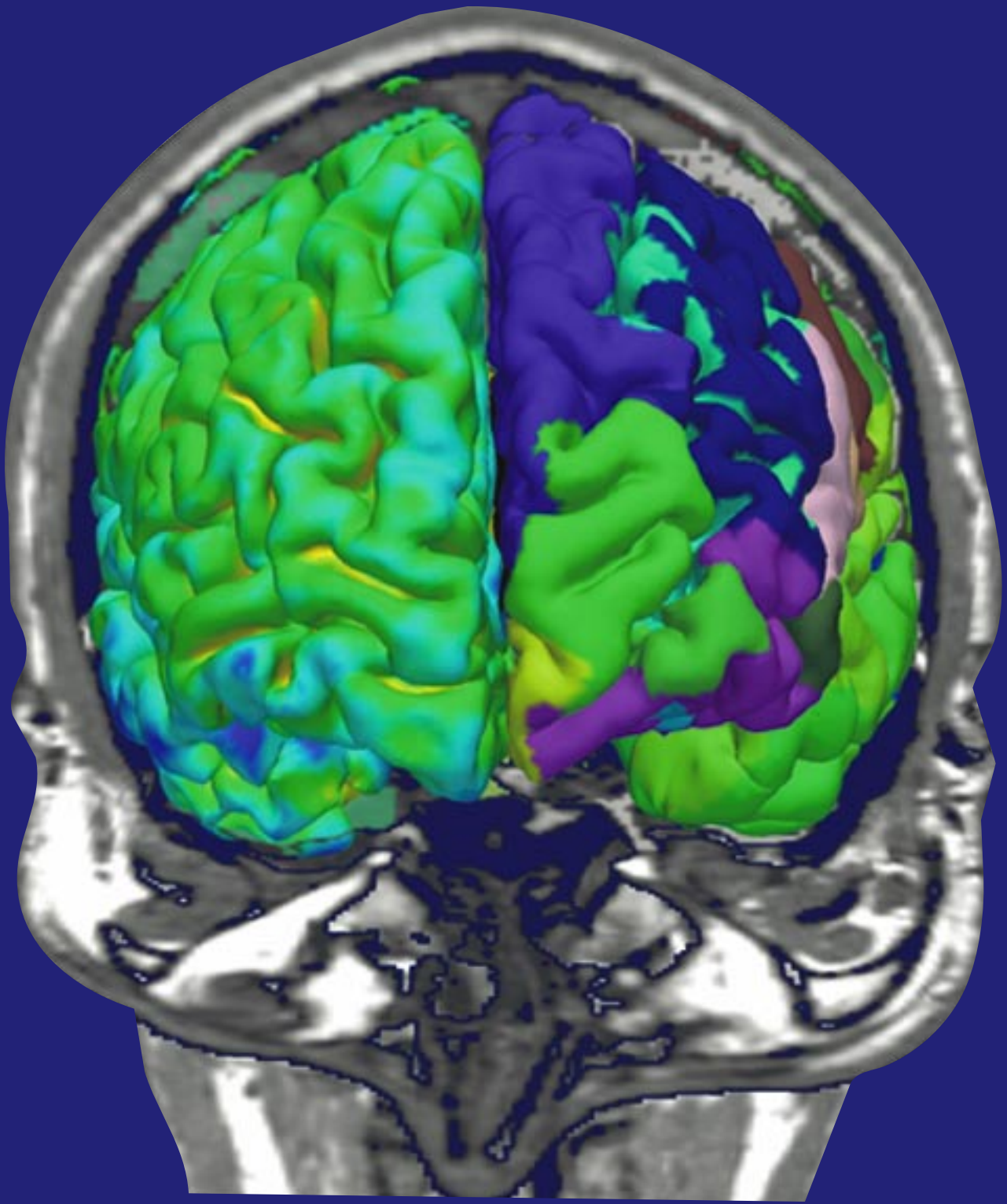
Scientists have uncovered evidence that the cause of type 2 diabetes may be in the mitochrondion, the microscopic body inside cells that converts food to usable energy.

With support from the Yale-New Haven Hospital General Clinical Research Center in Connecticut, investigators used nuclear magnetic resonance (NMR) spectroscopy to compare metabolic characteristics of 15 elderly and 13 young volunteers, all healthy, sedentary, and lean. NMR uses superconducting magnets to create field strengths high enough to study the metabolism of intact muscles in conscious subjects. Compared with their young counterparts, elderly participants were found to have 40 percent less activity in their skeletal muscle mitochondria, where lipids are metabolized. Reduced mitochondrial activity translated to elevated intracellular lipid levels. Previous studies by the investigators showed that elevated intracellular lipids interfere with insulin signaling within the muscle cells, reducing glucose uptake from the blood and thereby causing insulin resistance, a major factor in the pathogenesis of type 2 diabetes.

The researchers currently are investigating whether the reduced mitochondrial activity is due to a drop in mitochondrial content and whether physical activity in the elderly might counter the age-associated decline in mitochondrial function, thereby preventing the onset of type 2 diabetes.

—*Science* 300:1140–1142, 2003.

—Steven Stocker



BIRN

Putting Heads Together in Cyberspace

By Sandra J. Ackerman

It's an unprecedented meeting of minds, in both the literal and the figurative sense. The ambitious Biomedical Informatics Research Network (BIRN) links together some of the nation's top brain-imaging research centers, allowing scientists to share, compare, and morph together brain scans from healthy individuals, patients with brain disorders, and even nonhuman species, such as mice with neurological conditions. Launched by NCRR in 2001, the BIRN initiative represents an exciting new approach to collaborative research, an approach that will enable the type of large-scale team science needed for solving the complex biomedical problems that scientists grapple with today. Nearly 20 state-of-the-art NCRR-supported resources—including Biomedical Technology Resource Centers and General Clinical Research Centers (GCRCs) across the country—are now participating in the BIRN.

To create this brain image, scientists used noninvasive MRI techniques, automated software analysis, and visualization tools developed by BIRN researchers. Colors on the left side represent varying thicknesses of cortical gray matter, and colors on the right represent different anatomical brain regions. A standard imaging protocol is being performed on human subjects at each BIRN site to calibrate scanner variability and other factors that affect data sharing. *(Image courtesy of the Surgical Planning Laboratory, Brigham and Women's Hospital)*

"The promise of the BIRN is to provide a collaborative working environment that promotes the growth of interdisciplinary science, as well as an advanced biomedical cyberinfrastructure," says Dr. Mark H. Ellisman, professor of neurosciences and bioengineering at the University of Calilfornia, San Diego (UCSD), and head of the BIRN Coordinating Center. "In the context of test bed projects with practical goals, this environment will accelerate the pace at which we gain new understandings from increasingly shareable and comparable knowledge."

The BIRN is fulfilling its promise by forging unprecedented collaborations on multiple levels. "Biomedical researchers have agreed to stretch the boundaries of traditional research by sharing similar data across institutional lines," says Dr. Ellisman. "To facilitate the user-friendly exchange and mining of this data, biological and information technology experts are working together in a new and comprehensive manner to create infrastructure that solves computational, data analysis, and networking needs."

Although the initial emphasis is on neuroimaging, the sophisticated hardware and software developed for the BIRN ultimately will benefit the biomedical community at large, says Dr. Bret Peterson, health scientist administrator in NCRR's Division of Biomedical Technology. "The data-sharing tools and infrastructure developed for BIRN will be flexible and extensible, meaning they can be applied to collaborative research in many other scientific fields," Dr. Peterson says.

The creation of distributed databases, comprising compatible data collected from many different locations, has been a stubborn problem with no clear-cut solution. The challenge lies in translating data from one study into terms that permit direct comparison with the findings from other studies. For instance, brain structure and function often are assessed via magnetic resonance imaging (MRI), but the strength of the magnet varies from one MRI scanner to another, from 1.5 tesla to 3, 5,

or even 7 tesla. To address these inconsistencies, the BIRN scientists are developing mathematical algorithms to translate data from scanners of varying strengths into comparable data. Additional aspects of the scan are being standardized, such as the formats for storing all of the experimental conditions and variables, known as the metadata, in the database.

The BIRN is fulfilling its promise by forging unprecedented collaborations on multiple levels.

The BIRN will build not just one but three federated databases: Morphometry BIRN, which focuses on human brain structure; Function BIRN, which analyzes functioning of the human brain; and Mouse BIRN, which emphasizes studies of the mouse brain and cross-species comparisons. (See sidebar on page 7 for participating institutions.) A fourth and crucial component of BIRN is its coordinating center, headed by Dr. Ellisman. The BIRN Coordinating Center is responsible for developing the necessary software, setting up essential infrastructure, and managing the distribution and storage of the vast quantities of data that the BIRN already has begun to generate. To handle this enormous volume of data, the BIRN relies on Internet2, the "next-generation" Internet created through a consortium of government, industry, and more than 200 universities. Most remarkable about Internet2 is its speed: a file that would take 15 hours to transmit via a standard Internet service provider can cross the Internet2 backbone in 8 seconds.

The Morphometry BIRN project is building a database of structural brain images collected from multiple sites, which greatly increases the pool of patients who can be included in a given study. One subproject is examining thinning of the cerebral cortex, a process that occurs normally with age. Preliminary results suggest that cortical thinning is further advanced in patients with Alzheimer's disease than in age-matched, healthy subjects. Another research effort, headed by Dr. K. Ranga Rama Krishnan, chair of psychiatry at Duke University, uses the morphometry database to examine a brain region known as the orbital frontal cortex. "We are interested in people who have depression late in life," says Dr. Krishnan. "We find that they tend to have had numerous small, so-called 'silent' strokes—that is, strokes that are imperceptible to the patient—in the orbital frontal cortex." Because this same brain area also is damaged in some relatively young people who develop depression, Dr. Krishnan and his colleagues plan to explore whether damage to the orbital frontal cortex precipitates depression, or whether some aspect of depression leads to the atrophy in this brain region.

The Function BIRN project seeks to incorporate even more data into sophisticated, three-dimensional (3-D) images by adding information on brain activity. Dr. Steven Potkin, professor of psychiatry and director of the Brain Imaging Center at the University of California, Irvine, heads a Function BIRN study that is evaluating neuronal signaling—particularly the mental processing of sound and certain aspects of memory—in people with schizophrenia. The scientists will draw on the clinical resources of several GCRCs to conduct brain scans of diverse groups of patients not available at all sites—some with childhood-onset schizophrenia, some with a more common onset in early adulthood, and others with late-onset schizophrenia, as well as one group of patients before treatment and another whose treatment is under way. "Previously, the problem of understanding the development and progression of schizophrenia was not being able to study these important groups with the same methods, nor having the technology to meaningfully combine the data. BIRN now provides this methodology, which will allow us to better understand the development and progression of schizophrenia," says Dr. Potkin. "The problem with comparing the effects of particular treatments among such finely sorted groups was that there were just a handful of subjects at a few study sites. But a handful of subjects, multiplied by 11 or more sites, greatly strengthens the power of our studies."

Mouse BIRN is dedicated to developing and analyzing informative mouse models of human neurological disorders. Some initial efforts are correlating high-resolution, noninvasive MRI images with even higher resolution data gathered via advanced microscopy. Because the mouse strains are studied so exhaustively, they yield unique information about underlying mechanisms of a broad array of human disorders, from schizophrenia and multiple sclerosis to substance abuse. Ultimately, Mouse BIRN seeks to merge results from human and animal studies into a single, cohesive approach to understanding and treating particular diseases.

Taken together, the four components of the BIRN offer nearly limitless possibilities for enhancing scientific understanding of the brain and improving the treatment of brain dysfunction. One of the most promising aspects of this new effort, says Dr. Peterson, is the collegiality that the BIRN has begun to generate. "Science traditionally has been done by research teams working in their own labs, gathering their own data, and hoping that somehow their results might be tied together to a bigger picture. But with the BIRN, we're going at this collaboratively from the beginning," says Dr. Peterson. "We've got some of the nation's top scientists, from a variety of disciplines, working together in integrated teams. It's an exciting prospect." No one can predict where this unprecedented data sharing will lead, but the current consensus is that the BIRN is off to a good start.

For more information about the Biomedical Informatics Research Network, visit the web site at www.nbirn.net.

The BIRN is supported by the Divisions of Biomedical Technology and Clinical Research of the National Center for Research Resources. The computational and networking infrastructure that underlies the BIRN also is supported by the National Science Foundation.

A Nationwide Network in the Making

The four components of the BIRN each have distinct activities and goals, which are representative of the various participating institutions.

Brain Morphometry
With a focus on brain structure, this BIRN component aims to advance biomedical imaging for diagnosis and treatment of neuropsychiatric disease. The national imaging database is designed for future integration with databases in related fields, such as genomics.

Participants: *Duke University; Harvard University/Massachusetts General Hospital (MGH)/Brigham and Women's Hospital (BWH); Johns Hopkins University; University of California, San Diego (UCSD); University of California, Los Angeles (UCLA); University of Iowa.*

Brain Function
After assembling a centralized database of functional brain imaging studies, Function BIRN will monitor a multicenter clinical trial of potential treatments for schizophrenia. The trial will use fMRI to compare brain function in normal volunteers and patients with schizophrenia, both before and after medication. The study also will evaluate changes in the brain as the disease progresses.

Participants: *Duke University; Harvard Medical School/MGH/BWH; Stanford University; University of California, Irvine; UCSD; UCLA; University of Iowa; University of Minnesota; University of New Mexico; University of North Carolina.*

Mouse Brain
The brains of laboratory-bred mice offer a versatile resource for neuroscience research, since these animals can serve as models for human brain disease. Mouse BIRN assembles high-quality images at resolutions ranging from 100 microns (millionths of a meter) to less than 1 micron.

Participants: *California Institute of Technology, Duke University, UCLA, UCSD.*

Coordinating Center
This component unites all elements of the BIRN by deploying the network infrastructure, developing the web portal interface, converting data between various tools, and providing numerous support services.

Participant: *UCSD.*

Research Highlights

Escaping the Norwalk Virus

This past May, American soldiers encamped in northern Iraq fell victim to an invisible enemy: a norovirus, common cause of the so-called stomach flu. Within weeks as many as 2,500 soldiers, mostly from the 101st Airborne Division, developed symptoms—diarrhea, vomiting, nausea, headaches, and weakness—that ranged from mild to devastating. The virus probably spread on the spigots of jury-rigged faucets. But many soldiers who used those same faucets remained healthy, despite regular exposure to the highly contagious norovirus.

Such widely variable responses, typical of norovirus epidemics, have long puzzled scientists. Outbreaks tend to occur in close quarters, such as cruise ships, nursing homes, summer camps, or military encampments. As with the outbreak in northern Iraq, a sizable proportion of exposed individuals usually remains unaffected. Now, scientists have discovered possible clues to this viral resistance. For many individuals, the secret to protection appears to lie in a gene known as *FUT2*.

In a study conducted at the NCRR-supported General Clinical Research Center (GCRC) at the University of North Carolina at Chapel Hill, Dr. Christine L. Moe and her colleagues found that more than half of 77 volunteers exposed to Norwalk virus, a type of norovirus, were resistant to infection. About half of these protected individuals had *FUT2* mutations that blocked production of H type-1, a carbohydrate and blood group antigen found on many cell surfaces. All volunteers who had two copies of the nonfunctional *FUT2* gene remained healthy, even after receiving high doses of the virus. The findings suggest that H type-1 is the cellular receptor that binds Norwalk virus and allows it to enter cells. The study also jibes with earlier clinical studies that identified possible links between blood group antigens and norovirus susceptibility.

The North Carolina research also confirmed what had long been suspected based on anecdotal evidence: that the virus is infectious at incredibly low levels. "For those who were susceptible, the amount of virus necessary to develop infection was down to the limits of detection," says Dr. Moe, formerly at the University of North Carolina and now associate professor of international health at Emory University in Atlanta.

Knowledge of the mechanisms that reduce vulnerability to viruses could provide important clues for treating and preventing communicable diseases like norovirus infections, which cause an estimated 23 million cases of gastrointestinal illness in the United States each year. In developing countries, nearly 100 percent of children have contracted the viruses and developed antibodies to them by age five. Most often, noroviruses are spread through contaminated water and food.

Dr. Moe has been investigating noroviruses and other infectious agents for more than a decade. For the past five years, her clinical studies often have depended on the specialized research staff and infrastructure of the GCRC, which offers an ideal environment for analyzing the clinical aspects of viral exposure while ensuring the safety of volunteers. "It is incredibly important to be able to do these studies under safe and controlled conditions, like those available at the GCRCs," Dr. Moe says.

In preparing for the norovirus study, the GCRC staff recruited and carefully screened all volunteers to ensure that their exposure to the virus would not injure them, their families, or community members. When the study began, each of the 77 volunteers, men and women aged 18 to 50, was given a dose of virus mixed with water. Volunteers spent the next five days in the GCRC, receiving round-the-clock care. GCRC staff collected saliva, blood, and stool samples that were used for genetic screening and for tracking immune response and viral load. Volunteers returned at days 8, 14, and 21 for follow-up evaluations.

In clinical trials, Dr. Christine Moe (left) and her colleagues observed that some volunteers had a quick immune response when exposed to Norwalk virus and remained uninfected, perhaps because of past exposure to a similar virus. This immunoprotection may hold promise for vaccine development. *(Photo by Jack Kearse, Emory Health Sciences Photography)*

As expected, the researchers observed that a substantial number of volunteers—56 percent—did

not become infected. To determine possible causes, Dr. Moe's team collaborated with scientists at the Institute of Biology in Nantes, France. In earlier studies, the French scientists had evaluated norovirus infection in rabbit cells and shown that blood group antigens similar to H type-1 are required for viral docking, and possibly entry, to the cells. Previous clinical studies also have identified a link between red blood cell antigens and Norwalk virus infection.

The French scientists screened the volunteers' DNA for the *FUT2* gene. Normal versions of the gene produce the enzyme α-(1,2)fucosyltransferase, needed to generate the H type-1 molecule. The researchers found that all individuals with deactivated versions of *FUT2* remained healthy even after high-dose exposure to Norwalk virus.

Volunteers who had two copies of the nonfunctional gene remained healthy, even after receiving high doses of virus.

"H type-1 is the receptor that provides a door into the cell," comments lead author Lisa Lindesmith, a laboratory research specialist at the University of North Carolina's School of Public Health. "Without the receptor, the virus cannot gain access to the cell, regardless of how much virus gets into the gastrointestinal tract." The malfunctioning *FUT2* gene appears to provide genetic resistance to Norwalk virus, Lindesmith notes. "Because of this study, *FUT2* has become one of only a few human genes with a known link to viral resistance," she says.

But genes are only part of the story. Dr. Moe and her colleagues also discovered that some volunteers with normal, nonprotective versions of the *FUT2* gene remained healthy despite exposure to high doses of the virus. Studies led by Dr. Ralph Baric, professor of epidemiology at the University of North Carolina's School of Public Health, showed that these men and women mount an impressive mucosal immune response that peaks between the second and third days, perhaps because they had been exposed to a similar virus in the past. In contrast, volunteers who became sick did not produce mucosal antibodies to the virus until a week or more after viral challenge.

Such rapid development of immunoprotection is good news, says Dr. Moe, because it suggests that vaccination may one day protect against the virus. Since Norwalk virus accounts for only about five percent of norovirus outbreaks each year, finding signs of acquired immunity suggests either that people can develop long-term immunity to Norwalk virus, or that infection with a related norovirus confers protective immunity for more than one viral strain.

Many questions remain. For instance, not all of the norovirus variants enter a cell through the same molecular door. The H type-1 molecule, generated via the *FUT2* gene, has been implicated only in Norwalk virus infection, Dr. Moe notes. "We need to discover which receptors serve as cellular doorways for other noroviruses," she says. "We also must identify the factors that allow individuals to acquire and maintain immunity. Which viral strains must they be exposed to, and at what frequency, to gain and maintain protection?"

Dr. Moe, in collaboration with Lindesmith and Dr. Baric, plans to conduct additional norovirus studies at the Emory GCRC to further test the hypothesis that acquired immunity protects some people from infection. It is possible that a quick antibody response could signify reactivation of an earlier, so-called memory immune response. Additional evidence of acquired immunity would come if volunteers who got sick after a single viral challenge were later able to remain uninfected after a second challenge. If such studies ultimately lend support to the effectiveness of acquired immunity, Dr. Moe says, she and her colleagues are prepared to start designing a vaccine.

—Bernice Wuethrich

This research is supported by the Division of Clinical Research of the National Center for Research Resources and by the National Institute of Allergy and Infectious Diseases, the National Institute of General Medical Sciences, and the U.S. Environmental Protection Agency.

For more information about the NCRR Division of Clinical Research, see www.ncrr.nih.gov/clinical_rsrch.asp.

Additional Reading

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Transgenic Mouse Gets to the Heart of Human Disorder

It is well known that heart failure patients suffer from abnormal calcium cycling that disrupts the heart's ability to contract. But a "chicken-or-egg" question remained: Which strikes first—calcium dysregulation or heart failure? Finding the answer has proved problematic because many patients visit their physicians only in the late stages of heart failure, when it is difficult to identify root causes. But now, building on studies of transgenic mice, an international team of researchers has found an answer, which is moving science a step closer to enhanced therapies for cardiac conditions.

Dr. Christine Seidman, professor of medicine at Harvard Medical School in Boston, and her colleagues report that a genetic mutation known to disrupt calcium flow in the heart cells of mice also is responsible for a rare form of dilated cardiomyopathy in humans. Cardiomyopathy, which reduces the heart's pumping ability, causes heart failure and premature death in people in their teens and 20s. Approximately 30 percent of dilated cardiomyopathy cases are inherited. The mutation that Dr. Seidman analyzed affects a tiny protein, phospholamban (PLN), that also has caught the attention of drug companies developing treatments for heart failure.

Dr. Seidman and her research team sequenced the PLN gene in 20 unrelated patients with inherited cardiomyopathy and heart failure. Only one patient was found to have a mutant PLN gene. The scientists then sequenced the PLN genes of this individual's extended family. Only those family members with mutations in the PLN gene were found to have cardiomyopathy.

"We've known that many factors contribute to heart failure, but its molecular etiologies were poorly understood," Dr. Seidman says. "Now this study confirms that the gene defect causes this form of dilated cardiomyopathy." The condition the scientists studied is rare, but their findings may apply to other causes of heart failure.

Heart muscle cells contract when the cytoplasm becomes steeped in calcium, which is released from an internal cellular compartment known as the sarcoplasmic reticulum (SR). For cardiac muscles to then relax, allowing the heart to fill with blood, calcium must be pumped back into the SR with assistance from an enzyme known as sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA).

PLN contributes to these rhythmic contractions by inhibiting SERCA during muscle relaxation and then releasing SERCA during muscle contraction. During exercise or periods of stress, PLN becomes phosphorylated, which prevents its inhibition of SERCA and allows the heart to relax and refill faster. In patients with heart failure, PLN is too active, SERCA is chronically inhibited, and calcium levels in the cytoplasm of heart muscle cells are chronically high, explains coauthor Dr. Evangelia Kranias, professor of pharmacology and cell biophysics at the University of Cincinnati College of Medicine.

The mutant PLN gene discovered by the scientists was found to produce defective proteins that were not readily phosphorylated, thereby leading to chronic inhibition of SERCA and persistent contraction of heart muscle cells. By creating transgenic mice with the same mutation found in humans, the scientists could observe the events that triggered dilated cardiomyopathy, heart failure in early life, and premature death—all of which followed the same course as in their human counterparts. "The mice provided us with the means for exploring how cardiomyopathy evolves from the genetic defect," Dr. Seidman says.

By creating transgenic mice with the same mutation found in humans, the scientists could observe the events that triggered dilated cardiomyopathy.

The researchers now are investigating the cellular signals in young mice that may set them up for cardiomyopathy. Although the animals are born with the PLN mutation, the disease doesn't develop until later. "That delay suggests there might be a window of time when we can regulate calcium in the heart and prevent or slow the disease," says Dr. Seidman. Other gene mutations have been implicated in familial dilated cardiomyopathy in humans, but these genes primarily affect the heart's contractile proteins.

Dr. Kranias has worked for many years to develop the transgenic mouse models that made the current discoveries possible. Between 1997 and 2002, she was the principal investigator for the NCRR-supported Sarcoplasmic Reticulum Mutant Mouse Resource at the University of Cincinnati College of Medicine. She and her colleagues developed and studied transgenic mice that had altered calcium handling, including altered expression of PLN and its phosphorylation mutants in the heart muscle. (For more information about Dr. Kranias' mouse models, see the *NCRR Reporter*, Spring 2000, pages 12-13.) Dr. Kranias bred the first mice to produce either no PLN or excessive PLN. She and the NCRR-supported Mutant Mouse Regional Resource Centers now distribute the transgenic mice to other researchers.

The path to uncovering the role of PLN has not been simple or straightforward. Surprisingly, mice that lack the PLN gene actually fare quite well, whereas humans who lack functional PLN develop catastrophic heart conditions at an early age. In one study, Dr. Kranias and her colleagues found that inhibiting or even disabling the PLN gene in mice can normalize calcium cycling and prevent cardiac failure in experimental animals. But in a second study, the researchers evaluated 76 patients with dilated cardiomyopathy and 30 healthy subjects. Two families with hereditary heart failure were found to have a genetic mutation that led to a complete absence of PLN in the heart tissue of individuals who had inherited the mutation from both parents, and they developed heart failure before the age of 30. Two family members required heart transplants when they were only 16 and 27 years old. This was the first time that scientists had identified PLN null individuals. Unlike mice, "humans require a delicate balance of PLN activity," notes Dr. Kranias.

"Although mouse models give us an awful lot of information, we can't translate it directly to humans," Dr. Kranias adds. The mouse heart beats 600 times per minute, cycles calcium very rapidly, and PLN may not be essential for its proper function. But for now the mouse is the species best suited to genetic manipulation, says Dr. Kranias.

The mouse and human studies have opened the door to developing new medications for the treatment of heart failure. Dr. Kranias and her colleagues, as well as pharmaceutical companies, are experimenting with PLN inhibitors in human cells and in mice. "Administering a PLN inhibitor on a short-term basis to heart failure patients, who produce PLN, would probably be very beneficial," says Dr. Kranias.

Drs. Evangelia Kranias (left) and Kobra Haghighi examine a genetic mutation associated with enlargement of the heart's ventricles, which can lead to heart failure. *(Photo by Thomas P. Bevis, University of Cincinnati College of Medicine)*

PLN is a not an easy protein to tweak, since it is an intracellular protein and expressed in a sequestered part of the cell, says Dr. Seidman. But scientists will find a way to get at it, she adds. "I have a great belief in chemists who are engineering smart molecules. I suspect they will eventually create a molecule that can enter a cell and inhibit PLN."

—Tina Adler

This research is supported by the Division of Comparative Medicine of the National Center for Research Resources; the National Heart, Lung, and Blood Institute; and the Howard Hughes Medical Institute.

For more information about the NCRR Division of Comparative Medicine, see www.ncrr.nih.gov/comparative_med.asp.

For more information about the Mutant Mouse Regional Resource Centers, see www.ncrr.nih.gov/compmed/cm_mmrrc.asp.

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Critical Resources

Islet Cell Resources: Exploring New Therapies for Diabetes

As type 1 diabetes progresses, patients may fail to notice important warning signals, like shaky hands or confused thinking, that accompany a dangerous drop in blood sugar. Instead of automatically reaching for orange juice or warning their spouses to take the wheel, these patients may suddenly pass out. It is at this stage—when they have developed a condition known as "hypoglycemic unawareness"—that patients may become candidates for receiving an islet cell transplant, an experimental procedure that involves infusing insulin-producing islets derived from a donor pancreas into the patient's liver.

Islet cell transplants have been making headlines in recent years because of their unprecedented success in freeing some patients of the need for insulin injections. Since 1999, more than 200 patients with severe type 1 diabetes have undergone this procedure in the United States, the majority with positive results. Clinical-grade islet cells are now in great demand for ongoing clinical studies to assess the procedure's safety, effectiveness, and long-term effects.

To meet this increasing need, NCRR partnered with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to establish 10 Islet Cell Resource (ICR) centers across the country (see box). The mission of the ICRs is twofold: to investigate and improve methods for retrieving, preserving, and transporting islets, and to provide the maximum number of healthy islets to physicians who are performing these transplantations.

"Islet transplantation is an exciting area of research," says Dr. Richard Knazek, medical officer in the NCRR Division of Clinical Research. "NCRR wanted to move the field forward as quickly as possible, because the long-term complications of diabetes can be debilitating for so many people."

About 1.5 million Americans have type 1 diabetes, a condition with no known cure. Frequent insulin injections, a strict diet, and healthful lifestyle remain the mainstay of treatment. However, even faithful adherence to these necessary regimens may not eliminate the risk of complications such as vision impairment, nerve damage, kidney failure, amputations, and heart disease. "Islet transplantation may be considered for those patients whose disease continues to progress in spite of good, standard clinical treatment," says Dr. Knazek.

Dr. Camillo Ricordi examines islet cells in the operating room immediately prior to transplantation. *(Courtesy of Dr. Ricordi, University of Miami School of Medicine)*

Researchers have been seeking a cure for diabetes through islet transplantation for more than 30 years. A major advance came in 2000, when Dr. James Shapiro at the University of Alberta in Edmonton, Canada, and colleagues reported the outcome of a clinical research trial in which human islets were transplanted into seven patients with type 1 diabetes. All were able to stop taking their regular insulin injections. The ICR centers were launched in October 2001 to help scientists build on the success of this new cell transplantation approach, called the Edmonton Protocol.

Islets are retrieved from the pancreases of individuals who have donated their organs for transplantation. The involvement of nonprofit Organ Procurement Organizations and specialized surgical teams is critical, both at the time of donation and in all subsequent procedures, because minutes count. A delay of an hour or two can render an organ useless and deny the patient a chance at a healthy, productive life. These two groups, working closely with the ICRs, ship the pancreas in a special container, filled with both nutrient and oxygenating liquids, to an ICR facility.

The ICR researchers then incubate the donor pancreas for about 30 minutes in a solution of collagenase, a digesting enzyme, using a soccer ball-sized chamber invented by Dr. Camillo Ricordi, director of the ICR at the University of Miami School of Medicine. The pancreas then disperses into clumps of various types of cells. The islets are removed from this mixture of pancreatic cells during an hour-long process of continuous centrifugation, which is performed in a room held at refrigerator temperatures. The purified islets are then either shipped immediately to a clinical facility for transplantation or maintained in sterile flasks at body temperature for several days prior to transplantation.

This latter incubation approach is being studied by the ICRs to provide time for the islets to be shipped to a distant site or to allow transplant recipients to travel to the hospital. In the future, researchers may also take advantage of this two-day incubation period to transfer therapeutic genes into the cells, coat them with protective layers, or manipulate them in other ways to improve their longevity and function.

Key to success is production of high-quality islets, which depends on procedures being developed at the ICRs.

All ICR activities are coordinated by the Administrative and Bioinformatics Coordinating Center (ABCC) at the City of Hope National Medical Center and Beckman Research Institute in Duarte, California. The ABCC is developing a database that tracks procedures for harvesting, processing, and evaluating the quality of islet cell preparations. A parallel database, the Collaborative Islet Transfer Registry (CITR), funded by NIDDK, contains detailed information on procedures for cell transplantation, treatment sites, and clinical results. The two databases have been carefully integrated, so researchers can correlate transplant success with the many variables encountered during the harvest and isolation processes.

In June 2003, Dr. Shapiro announced preliminary results from the Immune Tolerance Network (ITN) clinical trial, to date the largest multicenter clinical investigation of islet transplantation. Overall, slightly more than half of the 36 transplant recipients participating in the study are managing without their insulin injections up to one year after receiving islet cell infusions. The study also suggests that experience counts when it comes to transplant success. The three institutions most experienced in islet cell transplantation—the University of Alberta and the ICR-affiliated sites in Miami and Minnesota—had a 90 percent success rate in freeing patients of the need for insulin injections. The differing success rates can be attributed in part to the complexities of the isolation and infusion procedures and immune suppression required to prevent subsequent rejection. Another key to success is the production of high-quality islets, which depends on the procedures now being developed or refined at the ICRs, says Dr. Ricordi, a co-principal investigator of the multicenter ITN study. "Islet preparation procedures are very complex and require a large investment of time and effort to do well," he says. "It is as much an art as it is a science."

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Islet Cell Resource Centers

NCRR currently supports Islet Cell Resource Centers at the 10 institutions listed below. Researchers interested in applying for access to islet cells for clinical studies should contact Dr. Rebecca Nelson, Project Administrator of the Administrative and Bioinformatics Coordinating Center at the City of Hope National Medical Center (e-mail: rnelson@coh.org, or visit www.infosci.coh.org/icr/).

City of Hope National Medical Center, Duarte, California. Director: Dr. Fouad Kandeel

Columbia University College of Physicians and Surgeons, New York City. Director: Dr. Mark A. Hardy

Joslin Diabetes Center, Boston. Director: Dr. Gordon Weir

Puget Sound Blood Center, Seattle. Director: Dr. Jo Anna Reems

University of Colorado Health Science Center, Denver. Director: Dr. Ronald G. Gill

University of Miami. Director: Dr. Camillo Ricordi

University of Minnesota, Minneapolis. Director: Dr. Bernhard Hering

University of Pennsylvania, Philadelphia. Director: Dr. Ali Naji

University of Tennessee, Memphis. Director: Dr. A. Osama Gaber

Washington University School of Medicine, St. Louis. Director: Dr. Thalachallour Mohanakumar

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Many hurdles still confront this field. Among the most important is the scarcity of viable donor organs. ICR research teams are trying to implement a variety of means to increase the number of donated organs that can be used in transplantation protocols. ICR researchers also are exploring innovative processes to maximize the number of functioning islets that can be harvested from these precious organs and are working closely with clinical research teams across the country to optimize their transplantation protocols.

Ultimately, the research and development conducted at the ICRs will have implications even beyond the treatment of diabetes, notes Dr. Daniel R. Salomon, chair of the ICR Steering Committee and associate professor of molecular and experimental medicine at The Scripps Research Institute in La Jolla, California. "This research is an investment not just in islet cell transplants but in cell transplants in general, which hold enormous potential for circumventing the problem of donor organ shortages," says Dr. Salomon. "Transplantation of genetically modified cells or even stem cells holds even greater promise for treating a host of human disorders, ranging from Alzheimer's and Parkinson's disease to cardiovascular disease and cancer."

—Tina Adler

The Islet Cell Resource Centers receive primary funding from the Division of Clinical Research of the National Center for Research Resources. Start-up funding for the centers also was provided by the Juvenile Diabetes Research Foundation International.

To learn more about the ICR centers, visit http://icr.coh.org/.

NCRR Launches Comparative Medicine Resources

NCRR's Division of Comparative Medicine has funded three new resources designed to research and develop critical nonhuman models for eventual dissemination to the scientific community.

The new Adult Mesenchymal Stem Cell Resource, located at Tulane University Health Sciences Center in New Orleans, will develop methodologies and technologies for producing and distributing adult mesenchymal stem cells (MSCs, also known as marrow stromal cells) for nonclinical research. MSCs normally give rise to bone, cartilage, and fat cells, but studies have shown that they also can differentiate into a wide variety of cell types, including neural cells. Recent studies have shown that MSCs may aid the treatment of spinal cord injuries, stroke, and cardiomyopathies by forming new tissue and stimulating the spinal cord. MSCs can be genetically engineered and are readily isolated from patients and grown in culture; however, preparing standardized quantities of MSCs has proved difficult. The new resource will prepare high-quality, standardized cells for distribution to other investigators, test the quality of MSCs prepared by investigators at other institutions, and develop new methods for isolating and characterizing human and rat MSCs.

At Texas A&M University in Kingsville, Texas, the new NCRR-supported Viper Resource Center (VRC) conducts research on medically important compounds in snake venom. The center houses more than 400 venomous snakes, representing 25 species and 33 subspecies. Snake venoms are a rich, stable source of biomedically important proteins such as disintegrins, metalloproteases, and fibrinolytic enzymes. Disintegrins are of particular interest because they can alter the shape, orientation, and movement of cells, and may play a role in the treatment of cancer, heart attacks, and strokes. The VRC plans to eventually house all venomous viper species from North America.

The third resource, the Drosophila Genomics Resource Center (DGRC), is housed at the Center for Genomics and Bioinformatics at Indiana University in Bloomington. The resource was created to assist researchers in applying genomics in the

model organism *Drosophila*, by assuring economical access to quality-controlled genomics materials. The DGRC will produce and distribute DNA microarray slides for gene expression analyses; test new and alternative genomics technologies; facilitate the collection and analysis of array expression data; and collect and distribute other reagents and materials essential for *Drosophila* genomics research, including large clone sets, common transformation vectors, and cell lines.

Chemistry Award Goes to Hochstrasser

Dr. Robin M. Hochstrasser, director of the NCRR-supported Ultrafast Optical Processes Laboratory at the University of Pennsylvania, received the 2003 Benjamin Franklin Medal in Chemistry from the Philadelphia-based Franklin Institute. The award recognizes Dr. Hochstrasser's pioneering role in developing ultrafast and multidimensional spectroscopies, and then applying these technologies to the study of molecular-level dynamics in complex systems. Dr. Hochstrasser also was honored for his effective mentoring of graduate and postdoctoral students throughout much of his career. He has been on the faculty at the University of Pennsylvania, Philadelphia, since 1962, and he has served as the Donner Professor of Physical Sciences since 1982.

As principal investigator of the Ultrafast Optical Processes Laboratory for more than two decades, Dr. Hochstrasser has led the development of innovative methods for investigating rapid structural changes and processes in proteins, enzymes, and nucleic acids. Researchers at the laboratory have invented and advanced a range of laser-based technologies that enable investigators to probe and measure biological reactions, including the binding of small molecules on hemoglobin and the dynamics of single proteins.

Evans Receives Cancer Prize

Dr. Ronald M. Evans, professor in the gene expression laboratory and Howard Hughes Medical Institute investigator at the Salk Institute for Biological Studies in La Jolla, California, was named corecipient of the 2003 Alfred P. Sloan, Jr. Prize, awarded annually by the General Motors Cancer Research Foundation. The prize recognizes outstanding recent contributions to basic science related to cancer research. Dr. Evans and prize corecipient Dr. Pierre Chambon, emeritus professor at the College of France in Paris, were both honored for elucidating the role of steroid and nuclear hormone receptors in cell division.

Because mutations to the estrogen receptor and other cellular receptors have been linked to cancer, understanding how these receptors operate is key to formulating cancer treatments. "Discovering the receptors opened the door to the wonderland of gene expression, the control of physiology, and the molecular basis of disease," says Dr. Evans. Several of his studies in recent years have depended on NCRR-supported biomedical technology resources, including the Laser Microbeam and Medical Program at the University of California, Irvine, and the National Center for Microscopy and Imaging Research at the University of California, San Diego.

Web Site Lays Out Human Metabolic Pathways

Humans are the latest species to have their metabolic pathways laid out for all to see on BioCyc, a web-based collection of databases with rich information on the metabolic pathways and genomes of 15 organisms. SRI International, a nonprofit research institute in Menlo Park, California, created HumanCyc, described as the first database that integrates the human genome with human metabolic pathways. BioCyc also is home to the NCRR-funded EcoCyc, which contains data on the genome and metabolic networks of *E. coli* and other disease-causing microorganisms, and to MetaCyc, which has metabolic pathway data on 160 species.

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The BioCyc databases enable computational analyses of metabolism, such as designing novel biochemical pathways for biotechnology and simulating or investigating the evolution of metabolic pathways. HumanCyc, called the "Encyclopedia of Human Genes and Metabolism," describes the function of each known human gene, along with the gene's products and predicted metabolic pathway. Still a "work-in-progress," HumanCyc can be accessed at http://HumanCyc.org.

Biomedical Technology Directory Updated

NCRR has published an updated directory of its Biomedical Technology Resource centers. The 89-page *Biomedical Technology Resources 2003 Directory* offers a complete listing of NCRR-supported technology resources, available for use by qualified biomedical investigators. The centers create, develop, and disseminate cutting-edge technologies, instrumentation, and research tools in such diverse areas as synchrotron

radiation, magnetic resonance imaging, optical and electron microscopy, and laser applications. The directory includes detailed descriptions of each center's research emphasis, resource capabilities, and contact information. The directory also provides an overview of technology-related funding opportunities from NCRR.

The *Biomedical Technology Resources 2003 Directory* and other NCRR publications, including directories for comparative medicine and clinical research resources, are available on NCRR's Web site at www.ncrr.nih.gov/publications.asp. Directories also can be obtained free-of-charge from NCRR/NIH, Office of Science Policy and Public Liaison, 6701 Democracy Boulevard, Room 984, MSC 4874, Bethesda, MD 20892-4874; telephone: 301-435-0888; fax: 301-480-3558; e-mail: info@ncrr.nih.gov.

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