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NCRR Reporter

WINTER 2005

CRITICAL RESOURCES FOR YOUR RESEARCH

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U.S. Department of Health and Human Services

Hopping and Swimming Into the GENOMIC AGE

Building Partnerships Into the Future

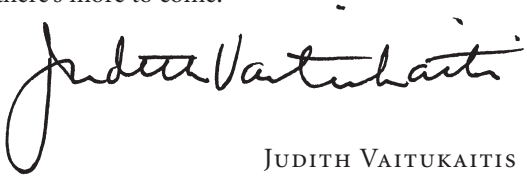
As a young investigator working in the field of reproductive endocrinology, I quickly learned that research is not a "solo" effort. I have watched as one person's discovery sparked hundreds of others. Throughout my many years of research and management, the importance of collaboration and partnerships was reinforced countless times over. Nowhere is this more true than at NCRR. In alliance with its NIH partners and the biomedical community, NCRR has worked to anticipate and provide the requisite resources to meet scientific needs.

I now have the opportunity to share this experience in another capacity. Dr. Elias Zerhouni, NIH Director, has asked me to serve as the Senior Advisor on Scientific Infrastructure and Resources. I hope to offer my insights on working in partnership with the research community to track emerging trends in biomedical science, so that essential research resources will be in place when they are needed.

During my time as director, I developed the greatest admiration for the work and dedication of the NCRR staff and the biomedical community that we serve. Each year more than 35,000 investigators—supported by competitive grants from other NIH components, other federal agencies, and the private sector—use NCRR-supported research resources. Because of the commitment of the NCRR staff and insights from researchers across the nation, we have been able to go beyond conventional approaches to find innovative resource solutions for scientists nationwide.

This support will continue. Dr. Zerhouni has asked Dr. Barbara Alving, who previously served as Deputy Director of the National Heart, Lung, and Blood Institute, to serve as the Acting Director of NCRR. I wish her well in this effort and know she will receive the same outstanding support that I received from all of you.

I look forward to building on the partnerships that I have established over the years and strengthening all lines of health-related scientific inquiry by ensuring that infrastructure and resources needs are addressed. I am thankful to have had the opportunity to work with NCRR's dedicated staff, and I look forward to the challenges of the future. As I frequently say—stay tuned; there's more to come.



Judith Vaitukaitis
Senior Advisor on Scientific Infrastructure and Resources

(For additional information about the appointment of Dr. Alving as Acting NCRR Director, see "News from NCRR," p. 15.)

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NCRR Reporter

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

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On the Cover: Genetic secrets locked inside the Mexican axolotl salamander may one day tell researchers how to regenerate human body parts. NCRR-funded studies of the salamander and other nonmammalian animals are shedding new light on human biological processes and disorders.
PHOTO BY JERAMIAH SMITH

NCRR Bids Farewell to Judith Vaitukaitis, M.D.

For the past 12 years, Judith Vaitukaitis has ably led NCRR in its mission to provide biomedical researchers with the tools they need to improve the nation's health. NCRR now bids a grateful farewell to a remarkable physician, scientist, and administrator. Dr. Vaitukaitis announced in March that she is stepping down as NCRR director to become Senior Advisor on Scientific Infrastructure and Resources to NIH Director Elias Zerhouni.

Dr. Vaitukaitis first came to NIH in 1970 as a postdoctoral researcher, initially for the National Cancer Institute, then for the National Institute of Child Health and Human Development. She worked on developing a more sensitive assay to detect elevated levels of human chorionic gonadotropin (hCG) in the body, as a way to diagnose certain malignant tumors that secrete this reproductive hormone. Dr. Vaitukaitis and her colleagues soon realized that such an assay, which they successfully developed by 1972, could also detect pregnancy at an early stage far better than pregnancy tests available at the time. Industry picked up on their new assay and turned it into the first home pregnancy test in 1978.

After contributing to this revolutionary breakthrough in women's health, Dr. Vaitukaitis left NIH in 1974 to become professor of medicine at Boston University School of Medicine. While there, she published numerous papers on glycoprotein hormones and reproductive endocrinology and also edited the book *Clinical Reproductive Neuroendocrinology.* For her clinical studies, she made use of the university's NCRR-supported General Clinical Research Center (GCRC) and became director of the GCRC in 1977.

Nine years later, in 1986, Dr. Vaitukaitis returned to NIH to direct NCRR's GCRC Program. She next served as NCRR's deputy director for extramural research resources before becoming acting director of NCRR in 1992 and then director in 1993. While director, she was elected to the prestigious Institute of Medicine of the National Academy of Sciences in recognition of her contributions to hormonal research.

During Dr. Vaitukaitis' directorship, NCRR's resources expanded significantly. NCRR's budget almost quadrupled, and program areas expanded to include a broad range of cutting-edge research resources, state-of-the-art technologies, and critical biological models of human disease. NCRR funded new grants for faculty mentoring and student training, and many new NCRR resources and programs were instituted.

In clinical research, for example, NCRR launched the Islet Cell Resource Centers to provide human pancreatic islets for basic research and for transplantation into patients with type 1 diabetes. In comparative medicine, Mutant Mouse Regional Resource Centers were created to accept and distribute mutant strains of mice for biomedical research. In biomedical technology, NCRR established the Biomedical Informatics Research Network, a system of shared neuroimaging databases designed to foster the development of data-sharing tools for biomedical and clinical investigations.

NCRR also boosted its efforts to strengthen the nation's research infrastructure. The Institutional Development Award Program, for example, enhanced research efforts in states where investigators historically have received less competitive research funding from NIH. As part of this program, NCRR recently began building IDeANet, a nationwide network of high-bandwidth Internet connections that will create vital links for scientists in regions that currently lack access to powerful computer networks.

These new programs and resources, and many others that came into existence during Dr. Vaitukaitis term as director, reflect the great determination and vision of a remarkable leader. Judith Vaitukaitis' record of extraordinary service will no doubt continue in her new role as Senior Advisor to the NIH Director. She will be greatly missed by everyone at NCRR.

Hopping and Swimming into the Genomic Age

Genetic technologies energize nonmammalian research models.

BY SCOTT J. BROWN

THE GENOMIC AGE is taking some rather lowly creatures to new heights. Invertebrates, fish, amphibians, and other nonmammalian species are becoming increasingly valuable to biomedical researchers as new genetic and genomic technologies emerge for these animals. Because they share a surprising number of physiological processes with humans, nonmammalian species provide critical clues to the biological mechanisms that underlie human health and disease. These animals are particularly valuable for studies of embryonic development, because the transparent eggs typical of these creatures allow researchers to easily observe and study embryos.

In recent years, NCRR's Division of Comparative Medicine has extended its long-standing support of research models by funding efforts to explore promising new nonmammalian models and to expand existing ones. That support is now paying off, as NCRR-funded studies spawn a host of new genetic and genomic tools and techniques for use with nonmammalian species, enhancing the research value and versatility of a number of these animals.

Xenopus tropicalis **(right) has a simpler genome and shorter generation times than its larger relative** ***X. laevis*** **(above), making it particularly useful for transgenic experiments.**

PHOTO BY ROBERT GRAINGER, UNIVERSITY OF VIRGINIA

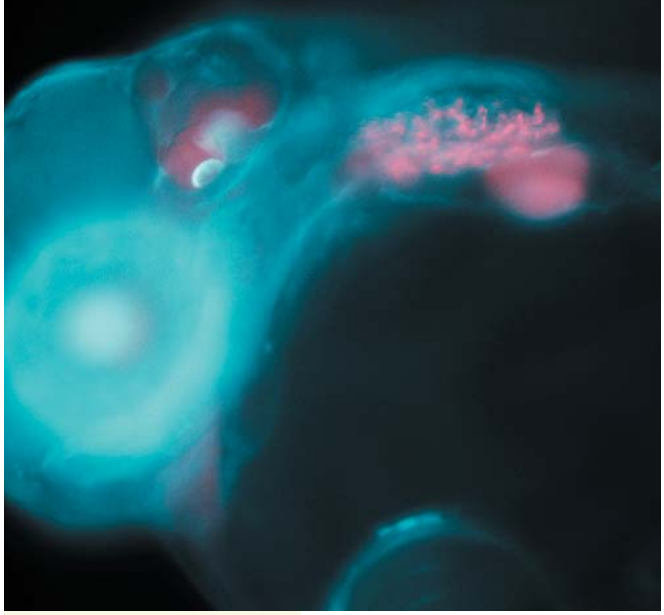
FLUORESCENT GREEN TADPOLES

Robert Grainger, W. L. Lyons Brown Professor of Biology at the University of Virginia, uses NCRR support to develop genomic

and genetic resources for *Xenopus tropicalis*, the western clawed frog of Africa. "Combining the new genomic and genetic tools with the power that frogs provide for studying early embryonic development should create a golden future for the *X. tropicalis* model," Grainger says. He views *X. tropicalis* as a much-needed complement to a related research frog, *X. laevis*, whose value is limited by its complex genome and long generation times.

Grainger and his colleagues have so far created about 100 different genetically altered lines of *X. tropicalis* for use in studies of development. They manipulate the genomes of these frogs with a technique called transgenesis, or gene transfer, using parts of genes known as control or regulatory elements. These elements control gene expression—the degree to which a gene's protein product gets produced within the cell—by either promoting or repressing the transcription of the gene's DNA sequence into messenger RNA (mRNA).

The researchers first take a regulatory element that promotes the expression of some particular gene of interest. They then hook up this promoter sequence with a reporter gene—a gene that produces an easily detectable protein product such as green fluorescent protein. When this transgenic construct is introduced into frog embryos, the result is embryos whose tissues light up with the reporter-gene product whenever and wherever the original gene of interest is expressed in the developing frog. Simultaneous development of different tissues can be visualized by combining specific promoters with reporter genes that produce differently colored proteins.



Red fluorescent probes in the liver and gall bladder of a see-through medaka fish embryo reveal enzyme activity associated with exposure to toxic substances.

Grainger also uses transgenes to interfere with the normal expression of a gene. One experiment introduced mutations to block expression of the gene *Otx2*, important for brain formation. This led to a form of blindness similar to an inherited human form of blindness known as coloboma. Grainger also is randomly introducing mutations in genes to see which ones produce developmental abnormalities and is then mapping and identifying the genes responsible.

THE WHITE MOUSE WITH FINS

Fish also serve as an important nonmammalian model for studies of development. Recently, Japanese researchers created a strain of medaka fish (*Oryzias latipes*) that has a transparent body throughout life. This "see-through" medaka lets researchers track how embryonic exposure to toxicants can damage DNA and lead to disease later in life.

> Nonmammalian models are useful for studying the embryonic development of body parts and may even shed light on the regeneration of adult tissues.

"The see-through medaka is truly unique among models," says David Hinton, Nicholas Professor of Environmental Quality at Duke University. "We can make verifiable changes in the fish, follow the animals during life, and then, at the end, have the opportunity to relate our histopathologic findings to the earlier life-stage exposure events." Hinton's NCRR-funded work with see-through medaka focuses particularly on toxicant effects on medaka livers, which are clearly visible through the animals' transparent bodies.

Hinton envisions a variety of practical applications for see-

PHOTO BY RON HARDMAN, DUKE UNIVERSITY (TOP)

through medaka, including testing for toxic effects of drugs, detecting hazardous substances in the environment, and identifying carcinogens and their effects. To aid his carcinogen research, Hinton is developing medaka lines that carry transgenic promoters for genes likely to be expressed in precancerous cells. By exposing fish to toxicants and then watching for expression of the transgenes in liver cells, researchers can identify carcinogenic toxicants and track their effects on various cell types. These transgenes also could be used to test the efficacy of anticancer agents.

Richard Winn, director of the Aquatic Biotechnology and Environmental Laboratory at the University of Georgia, also works with transgenic medaka, but he studies nontransparent fish. With NCRR support, Winn has developed medaka that harbor a transgene, derived from a bacterial virus, that serves as a mutation target. When a fish carrying this transgene contacts a mutagenic substance, the transgene mutates, as do the fish's endogenous genes. But unlike the endogenous genes, the transgene can be easily excised from any tissue of the animal to determine the extent of mutational damage.

"One of my ambitions is to use the medaka fish as the white mouse with fins," says Winn. "A transgenic fish for mutation analysis can be used in some of the same ways as rodents, but the fish might also make some contributions where rodents simply cannot be used practically." Medaka can be more easily exposed, for example, to environmentally realistic levels of a toxicant by having them swim in water containing low doses of a chemical.

Winn and his colleagues also are using medaka to quantify the degree to which certain substances inhibit mutations induced by toxicants. They have found green tea to be particularly effective at blocking such mutations, a finding already reported for rodents. Winn believes transgenic medaka can help researchers discover substances that might prevent cancer in humans. He is working with cancer researchers to identify possible agents for testing in medaka.

Guppy-sized medaka can stay immersed in water containing environmentally realistic levels of a pollutant, making them ideal for transgenic studies of the effects of toxic exposures.

HIGH-THROUGHPUT SEA URCHINS

Transgenic techniques like those used with fish and frogs are not confined to vertebrate models. One particularly useful invertebrate for transgenic research is the sea urchin. Humans and sea urchins both belong to a very ancient lineage of animals whose members share certain features of early embryonic development.

With NCRR funding, Eric Davidson, Norman Chandler Professor of Cell Biology at the California Institute of Technology, studies a regulatory network of about 50 genes involved in embryonic development in the purple sea urchin (*Strongylocentrotus purpuratus*). Davidson's research group uses transgenic sea urchins to determine when and where specific regulatory elements exert their control on gene expression and to reveal the identities and functions of the many transcription factors involved in this process. Studying these complex regulatory interactions requires a lot of genetic material, and sea urchins have no trouble supplying it. A single female can release millions of eggs at a time, and individual eggs are easy to inject with transgenes.

"The people in our lab can inject up to 8,000 sea urchin eggs in a morning, and 90 percent or more of the eggs will develop properly," says Davidson, "We've turned the sea urchin model into probably the world's highest throughput gene transfer system. It's possible to get quantitative, reproducible, spatial, and temporal results from gene transfer on a scale that's almost impossible with most other animal systems."

Davidson is also principal investigator of the NCRR-supported Sea Urchin Genome Resource at the California Institute of Technology (see *NCRR Reporter*, Fall 2002, pages 12-13). This resource develops libraries of complementary DNA (cDNA) clones derived from the purple sea urchin and related species. More than 15 million clones have so far been placed in libraries. The DNA sequences of these clones correspond to sequences of mRNA transcripts for genes expressed in the tissues of the animal. A single

A single female purple sea urchin is able to release millions of eggs at a time, yielding an abundant supply of genetic material for studies of embryonic development.

PHOTO BY LAURA FRANCIS, NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

library contains all the cDNAs derived from a specific tissue or developmental stage. The resource has also created several libraries of bacterial artificial chromosomes (BACs), which are clones of longer stretches of DNA, extending up to 200,000 bases. A major function of the resource is to provide clones and arrayed libraries to the research community on request.

The cDNA libraries help researchers to determine where and when during development particular sea urchin genes are expressed. BAC libraries, with their longer stretches of DNA, help to map the genomic positions of specific genes. The materials generated by the resource have been critical to the effort to sequence the sea urchin genome, now in its final stages. Davidson says the current libraries cover early sea urchin development extremely well. Future libraries will focus on later stages, when the sea urchin larva metamorphoses into the adult form.

TO LEARN MORE

Additional information about the NCRR-supported nonmammalian models described in this article can be obtained from the Web sites listed below.

Western Clawed Frog (*Xenopus tropicalis*). Descriptions of genetic studies of *X. tropicalis*, with links to relevant publications and resources, can be found at Robert Grainger's Web site, http://faculty.virginia.edu/xtropicalis/.

Medaka Fish (*Oryzias latipes*). For information on research projects benefiting from the medaka model and a list of related publications, visit David Hinton's home page at www.nicholas.duke.edu/people/faculty/hinton.html.

Purple Sea Urchin (*Strongylocentrotus purpuratus*). Details on the offerings of NCRR's Sea Urchin Genome Resource, access to genetic databases and computer downloads, and an online order form for library clones are available at the resource's Sea Urchin Genome Project, http://sugp.caltech.edu.

Axolotl and Tiger Salamanders (*Ambystoma*). Learn about salamander genetics, query EST databases, view genetic maps, and access relevant publications at Randal Voss's Web site for the Salamander Genome Project, http://salamander.uky.edu.

For information on other NCRR-supported nonhuman models for biomedical research, visit NCRR's Division of Comparative Medicine at www.ncrr.nih.gov/comparative_med.asp.

SALAMANDER MAGIC

Although nonmammalian models are particularly useful for studying the initial development of body parts in the embryo, some of these models also shed light on a related process—the regeneration of damaged or destroyed tissues in the adult. By far the best animal for this research is the salamander, the only vertebrate able to regenerate complex body parts such as limbs.

"People are interested in understanding the molecular basis of regeneration and in seeing whether we'll be able to take insights from the salamander and use them to advantage in mammalian systems," says NCRR-supported researcher S. Randal Voss, associate professor of biology at the University of Kentucky. Voss is identifying bits of DNA sequence, called expressed sequence tags (ESTs), in various larval tissues of the Mexican axolotl salamander (*Ambystoma mexicanum*) and tiger salamander (*A. tigrinum tigrinum*), including tissues from limbs in the process of regenerating. These ESTs are lines of genetic code revealed by sequencing cDNA clones from various salamander tissues. Voss has so far compiled more than 40,000 high-quality ESTs and placed them in databases.

Scientists can use the information in these databases to develop molecular probes that have sequences matching the ESTs. Oligonucleotide probes, for example, can be synthesized to study gene expression in regenerating tissues by seeing how the oligonucleotides bind to genetic material from the tissues. Voss plans to use the ESTs for such analyses and also for mapping salamander genes.

Voss wants to determine which genes in mammals, including humans, may be homologous to salamander regeneration genes, and to see if those mammalian genes might have regenerative potential. The distant goal of developing techniques for regenerating human tissues drives him on. "I'm pushing the salamander as a model on all fronts," he says. "Sure, there are problems to overcome, but we've made a lot of progress in a short time. I'm excited about the future." ■

ADDITIONAL READING

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- Putta, S., Smith, J. J., Walker, J. A., et al. From biomedicine to natural history research: EST resources for ambystomatid salamanders. *BMC Genomics* 5:54, 2004.
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- Hinton, D. E., Wakamatsu, Y., Ozato, K., et al. Imaging liver development/remodeling in the see-through medaka fish. *Comparative Hepatology* 3:S30, 2004.
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PHOTO BY PETER FREY, UNIVERSITY OF GEORGIA

Centers Unite Researchers from Multiple Disciplines

SEARCHING FOR SOLUTIONS to difficult biomedical problems can be like jumping into quicksand—the more you struggle to understand, the further you sink into the depths of complexity. Scientists hoping to escape this quagmire need the insight that comes from interdisciplinary collaborations. Fortunately, NIH has just thrown out a lifeline by funding 21 Exploratory Centers for Interdisciplinary Research. Together, the awards total more than $36 million over three years. The centers, established last fall and administered by NCRR, are part of the NIH Roadmap for Medical Research, a series of initiatives that tackle key health issues of the 21st century. A second phase of the program will create large-scale Interdisciplinary Research Consortia beginning in 2007 (for more information, visit http://grants.nih.gov/grants/guide/notice-files/NOT-RM-05-006.html).

"We have moved from the days when a single investigator working in a room by him or herself could accomplish big breakthroughs," says Patricia Grady, co-chair of the Interdisciplinary Research Implementation Group of the NIH Roadmap and director of the National Institute of Nursing Research. "By bringing together individual scientists with a variety of expertise to deal with new issues and new scientific challenges, we can advance science faster and much more effectively."

Gregory Farber, who oversees the exploratory centers program for NCRR, says that the large number of applications shows that researchers have been waiting for funding that supports interdisciplinary work. "The research community was ahead of us on this and was ready to respond," he says. Awards went to researchers investigating complex issues that have not been well served by single-discipline approaches (see sidebar). "None of these centers is going after low-hanging fruit," says Farber, a health scientist administrator in NCRR's Division for Biomedical Technology Research and Research Resources. "They're all addressing really hard problems."

Barry Popkin, who heads one of three Exploratory Centers for Interdisciplinary Research focused on obesity, is bringing together diverse specialists to address the many aspects of this growing health problem.

The new centers move beyond simple *multi*disciplinary work, where collaborators remain in their own scientific frameworks. Rather, the *inter*disciplinary research at the centers requires specialists to work together in an intertwined fashion, acquiring new knowledge and techniques and perhaps even forging new disciplines. Each exploratory center will plan interdisciplinary projects and create an interdisciplinary infrastructure to solve significant and complex biomedical problems. The follow-on program will move beyond the "exploratory" phase to create Interdisciplinary Research Consortia. Each consortium will consist of teams of investigators from various institutions working together on interrelated projects funded by a variety of grant mechanisms. NIH expects to fund 8 to 10 consortia. Applicants need not have received prior funding as an exploratory center.

Three of the new centers focus on the much-publicized problem of obesity. Most obesity research efforts have focused on isolated factors and adopted a "one size fits all" approach, says Barry Popkin, who heads the Inter-Disciplinary Obesity Center (IDOC), an exploratory center at the University of North Carolina (UNC) at Chapel Hill. "We need to find a way to open the field up, to think and work across the scientific spectrum in ways that will push the field forward," he says. "It requires not only a very different way of thinking but also a different way of interacting and much greater understanding of the multiple approaches to addressing each dimension of the problem."

The IDOC will investigate wide-ranging research areas associated with

John Beier, who heads the InterVector Exploratory Center, is assembling interdisciplinary teams, both in the United States and abroad, to tackle the growing threat of mosquito-borne illnesses.

PHOTO COURTESY OF TABITHA JACKSON(TOP), PHOTO BY TAMARA S. MILLER (BOTTOM)

obesity. These include genetics, metabolism, treatment and prevention, chronic obesity-related diseases, psychosocial and family influences, and macroeconomic and community factors that affect levels of diet, activity, and obesity. Popkin, an economist and nutrition epidemiologist and professor of nutrition at UNC's School of Public Health, also hopes to boost the public policy arm of the fight against obesity, borrowing tactics from anti-smoking campaigns. Seminars, retreats, and an interdisciplinary training program will provide opportunities for experts from a variety of fields to assemble and discuss different aspects of obesity. One pilot project brings together geneticists, endocrinologists, nutritionists, and epidemiologists to investigate obesity in an ongoing 20-year longitudinal study of women and their children in the Philippines.

Another exploratory center, headed by John Beier at the University of Miami, is examining vector-borne diseases, a major cause of child mortality in the developing world. The goal of the new InterVector Exploratory Center is to develop interdisciplinary approaches to control malaria, dengue fever, West Nile virus, and Rift Valley fever in urban environments, where these mosquito-borne illnesses pose a growing threat. Urban sprawl around some rapidly expanding cities has begun to encroach on swampy, flood-prone areas where mosquitoes thrive, says Beier, a professor of epidemiology and public health at the University of Miami. The exploratory center will allow Beier and colleagues to refine their interdisciplinary approaches to fighting malaria and dengue fever in two Kenyan cities and then to extend these tactics to cities in the Middle East, Caribbean, and Central America. The lessons learned will benefit not only those regions, but also cities in the United States, where West Nile virus and some other insect-borne diseases occur.

"This center has been wonderful, because it has allowed me to make connections with the right types of people," Beier says. "We have groups in Egypt, Israel, Trinidad, and Costa Rica, but we also have a really good team of faculty members from the university." The center forges links between various biomedical experts and nonmedical specialists such as entomologists, mathematicians, computer scientists, engineers, geographers, and social scientists.

"We hope that in about 10 years, there will be new disciplines that have evolved because of these interdisciplinary programs," says NCRR's Farber. In the meantime, "the centers are getting people with very different backgrounds into the room and getting them focused on how to solve a particular problem. It's pretty impressive to see what they've been able to do so far."

—SCOTT J. BROWN

Diverse Centers for Diverse Problems

The 21 Exploratory Centers for Interdisciplinary Research tackle complex biomedical issues, ranging from genetics and structural biology to major public health problems such as AIDS and obesity. Center Web-site addresses available at press time are provided below. For abstracts of the original research proposals for each center, visit www.ncrr.nih.gov/ncrrprog/roadmap/ecirdirectory.asp.

AIDS and Other Sexually Transmitted Diseases Among Youth
Exploratory Center in Behavioral Economic Epidemiology, University of California, Berkeley, *http://iber.berkeley.edu/bee/*

Antimicrobial Resistance
Center for Interdisciplinary Research on Antimicrobial Resistance, Columbia University School of Nursing, *www.cumc.columbia.edu/dept/nursing/CIRAR/*

Blinding Eye Diseases
Planning Grant for Research on Blinding Eye Diseases, Schepens Eye Research Institute, *www.theschepens.org/nih_roadmap.htm*

Chronic Inflammatory Disorders
Non-Invasive Approaches to Assessing Inflammation, University of North Carolina at Chapel Hill, *http://uncgihep.med.unc.edu/Sartor/RR20764.html*

Complex Genetic Traits Underlying Disease
Carolina Center for Exploratory Genetic Analysis, University of North Carolina at Chapel Hill, *http://renci.org/nih/*

Diabetic Cardiovascular Disease
Planning Interdisciplinary Studies of the Diabetic Heart, Washington University in St. Louis, *http://wup20.wustl.edu*

Drug and Chemical Toxicity
Diagnosis and Therapy of Drug and Chemical Toxicity, University of Florida

Elder Self-Neglect
Consortium for Research in Elder Self-Neglect, Baylor College of Medicine, *www.bcm.edu/crest/*

Gene-Environment Interactions in Child Health
Duke Center for Geospatial Medicine, Duke University, *www.nicholas.duke.edu/cgm/*

Genetic Variability in Drug Response
Human Pharmacogenomic Epidemiology, University of Pennsylvania, *www.cceb.upenn.edu/kimmel/P20.html*

Mind-Body Interactions in Disease
Exploratory Integrative Biology, University of Texas Medical Branch, Galveston, *www.utmb.edu/mbbh/*

Neuropsychiatric Disorders
Center for Cognitive Phenomics, University of California, Los Angeles

Transdisciplinary Imaging Genetics Center, University of California, Irvine

Obesity
Exploratory Center for Obesity Research, University of Washington, *http://depts.washington.edu/uwcphn/P20/*

Inter-Disciplinary Obesity Center, University of North Carolina at Chapel Hill, *www.cpc.unc.edu/idoc*

Taskforce for Obesity Research at Southwestern, University of Texas Southwestern Medical Center at Dallas, *www8.utsouthwestern.edu/utsw/home/research/TORS/index.html*

Obstacles to Vaccine Development and Use
Exploratory Center for Interdisciplinary Research in Vaccinology, Emory University, *www.medicine.emory.edu/id/ecirve.cfm*

Racial Disparities in Pregnancy Outcomes
Health Disparities: Leaders, Providers, and Patients, University of Michigan, *www.med.umich.edu/obgyn/HealthServicesResearch/nihp20.htm*

Stroke Rehabilitation
New Directions in Stroke Neurorehabilitation, University of Southern California

Structural Biology
Computational Center for Biomolecular Complexes, Baylor College of Medicine, *http://ncmi.bcm.tmc.edu/ccbc*

Vector-Borne Diseases in Urban Environments
InterVector Exploratory Center, University of Miami, *www.intervector.org*

Encouraging Small-Business Innovation

Early support helps to turn a rough sketch into a manufactured product.

Some time ago, in the emergency room at Albert Einstein Memorial Center in Philadelphia, physician Geoffrey Hart got a new idea for solving an old problem: how to give needed anesthesia to frightened, suffering children without increasing their fear and discomfort. Hart's idea came to fruition as the PediSedate—a medical device that looks like a toy. A child-sized headset that comes with a snorkel and a video gameboard, the PediSedate delivers to young children sedative gas for medical procedures while helping them to remain calm and entertained as the sedative takes effect. The research and development for this project was supported by funding from NCRR's Small Business Innovation Research (SBIR) Program.

Since its establishment in 1982, the SBIR Program at the National Institutes of Health has fostered small-business participation in federally supported research and development in the biomedical sciences, and encouraged private-sector commercialization of technology developed through federal support. SBIR grants have figured in the realization of projects ranging from medical devices to the pharmacological study of a little-known marine mollusk.

The original idea for the PediSedate took shape as Hart saw many young patients, already traumatized by the accident or injury that brought them to the emergency room, reacting with sheer panic when full-sized anesthesiology masks threatened to cover their entire faces. Young patients often struggled frantically to avoid the needle that contained the very painkilling medicine they needed. Hart observed, too, that the conventional means of holding a pediatric patient more or less immobile often added to the child's distress.

"It is very difficult for physicians who are not anesthesiologists to give children adequate painless medical care," Hart explains. He saw the need for an anesthesiology-delivery system that would make the experience easier for the child and also easier for most physicians and nurses to use. He left his emergency-room practice and went back to medical school, this time to become board-certified in anesthesiology.

Hart then enlisted the expertise of David Chastain, principal engineer at the consulting firm of Design Continuum, to help bring to reality the device he envisioned. The team at Design Continuum consulted, in turn, with a panel of experts ranging from anesthesiologists, nurses, and psychologists to the prospective users of the device—children—and their parents.

Hart and Chastain next applied for a Phase I SBIR grant, which they received from the National Heart, Lung, and Blood Institute. Phase I funding typically lasts for six months or longer for an amount of up to $100,000. This was followed by a Phase II SBIR grant, awarded by NCRR's Division for Biomedical Technology Research and Research Resources. "These grants made everything possible, all the product development," says Chastain. "They enabled the clinical trial work, as well as the industrial design, engineering, and production of headsets for clinical trials."

The care that went into the design is evident in the finished product, from PediSedate's colorful, semitransparent plastic to its swiveling yoke that keeps the snorkel out of the way until the child is comfortable in the headset. The snorkel itself monitors respiration, delivers mixed oxygen and nitrous oxide, and scavenges exhaled gas. The earpieces, which can connect either to a portable CD player or to a Nintendo Game Boy, give the child the choice of listening in on the medical procedure or blocking it out with music or a video game; at the same time, one earpiece monitors oxygenation of the blood.

The purple headset and snorkel look like something from a toy chest, but they're actually a medical device that delivers sedative gas to young, sometimes apprehensive, children.

PHOTO COURTESY OF DESIGN CONTINUUM

Researchers examine a laboratory colony of giant keyhole limpets, which produce a cancer-fighting protein known as KLH.

In clinical trials, PediSedate has shown good results for safety and has won approval from physicians, young patients, and parents. "We've been fortunate to get an extension on our second grant, to develop the product further," says Hart. This spring, the device will undergo its most extensive clinical trials yet, with hundreds of children receiving sedation either through PediSedate or by standard methods. The device already has received partial approval from the FDA.

Whatever comes next for the PediSedate, Hart has no doubt as to how the project became ready for commercialization. "The support I've gotten from NCRR is significant, providing the lion's share of the funding," he says.

In another example of SBIR support, a grant that has the ultimate goal of producing a promising new cancer therapy also may have the unexpected benefit of helping to restore the dwindling population of a marine animal known as the giant keyhole limpet. Termed "giant" because, at about 6 inches long and 4 inches high, it is larger than most other limpets, this mollusk can be found in the United States only off the California coast. Highly prized for its ability to make a cancer-fighting protein called hemocyanin, the animal was threatened by over-harvesting until Frank Oakes, chairman of Stellar Biotechnologies, took a scientific interest in it. Oakes was looking for a way to extract as much keyhole limpet hemocyanin (KLH) as possible from the animal without letting it bleed to death, as was then the common practice.

"We got recruited into this by a pharmaceutical company that was looking for a consistent source of KLH," he says. This was something that the hunting of limpets in the wild could not provide. With SBIR funding from NCRR's Division of Comparative Medicine, Oakes found a way to fine-tune the animals' diet and keep their water free of nitrogen and other contaminants, making his laboratory limpet colony the world's only reliable source of standardized, medically useful KLH. "We have demonstrated the feasibility of producing animals of a size and condition to generate a consistent supply of KLH that can't be distinguished from the KLH of animals in the wild," says Oakes. Equally important, Oakes developed a safe and repeatable way to extract the animals' blood—not an easy matter, since the animals have no blood pressure.

The SBIR seed money allowed Oakes to develop his project to the point that it attracted attention from another branch of the NIH. "After the SBIR grant from NCRR," says Oakes, "we were awarded a competing continuation grant by the National Cancer Institute to manufacture KLH into a fully purified medical product." The protein is now in clinical trials for the treatment of colorectal cancer and metastatic breast cancer. Like the PediSedate and numerous other projects now on their way to market, Oakes's limpet colony offers an example of the productive and far-reaching results of collaboration between the SBIR Program and an individual's creative scientific thinking. **—SANDRA J. ACKERMAN**

APPLY FOR FUNDING: NCRR, along with other NIH components, participates in two federal programs that provide funding to small businesses. For detailed information about these programs—the SBIR Program, as described in this article, and the Small Business Technology Transfer Program—visit the NIH Small Business Opportunities Web site at http://grants1.nih.gov/grants/funding/sbir.htm. For information about small-business funding opportunities from NCRR's four divisions—related to biomedical technology, clinical research, comparative medicine, and research infrastructure—visit NCRR's home page at www.ncrr.nih.gov and type "SBIR" into the search box.

PHOTO BY FRANK OAKES

Family Defect Sheds Light on Mitochondria

A rare gene mutation links common disorders.

BY TINA ADLER

MITOCHONDRIA ARE respectfully described as "the powerhouse of the cell." But when these vital fuel sources develop mutations, they become the root of a vast array of common disorders. High triglyceride levels, low levels of high-density lipoproteins, and insulin resistance have all been linked to mitochondrial dysfunction. Now, a study that began with a single patient, and ended up involving more than 140 of her relatives, has identified additional metabolic problems caused by genetically flawed mitochondria.

Richard Lifton, a professor of genetics and medicine at Yale University School of Medicine, learned about the family by chance, when the patient mentioned that other family members also had problems with high blood pressure, high cholesterol, and chronically low blood magnesium levels. The remark caught Lifton's attention, since magnesium deficiencies are rare. After learning more about the patient's family, Lifton and his colleagues recruited 142 of the blood relatives, spanning four generations, to undergo detailed clinical and genetic evaluations at Yale's NCRR-funded General Clinical Research Center (GCRC).

"What's vital for any clinical genetic study of this sort is the ability to accurately characterize phenotypes in patients. The support of the GCRC for this purpose was essential," says Lifton.

Of the group, 45 family members shared not only the same maternal lineage but also an unfortunate legacy: an increased risk of having hypertension, high cholesterol, or low magnesium levels. Of the 45 members, 38 had at least one of the three conditions, and 7 had all three conditions. Before the age of 50, "it's almost a roll of the dice which sign will occur," Lifton says. After age 50, 95 percent of family members on the same maternal lineage had high blood pressure.

Although diet plays a role in the conditions, the affected and unaffected family members had similar body mass index scores. Family members with low magnesium levels also had low levels of calcium in their urine, which suggests a renal defect in a particular cell type. Only family members on the same maternal lineage had low magnesium.

> This is the first time research has hinted at a genetic link between hypertension, high cholesterol, and low magnesium.

Whenever disorders are passed along the mother's side of a family, scientists take a good look at mitochondrial involvement, since children inherit only their mother's mitochondrial DNA. In such cases, affected mothers—but never affected fathers—transmit the trait to their offspring. The team assessed the study participants for disorders known to result from mitochondrial dysfunction—headaches, hearing loss resulting from problems in the inner ear or

With help from the Yale GCRC, Richard Lifton and colleagues assessed four generations of a family and uncovered a rare mitochondrial mutation.

brain, and hypertrophic cardiomyopathy—and found a high rate of these conditions among maternally related members.

Older family members were more likely than others to have high cholesterol, hypertension, and low magnesium, which was additional evidence that the conditions were mitochondria related, since mitochondria weaken with age.

MONITORING THE MITOCHONDRIA

Once they had their clinical evidence lined up, Lifton and colleagues examined the relatively small mitochondrial genome to search for mutations in the DNA sequence. They found one mutation of importance. It showed up in all the affected family members with common maternal lineage and in none of the healthy participants. The mutation markedly impaired the structure and function of one specific mitochondrial transfer RNA (tRNA), a key element in the manufacture of proteins.

Laboratory tests of muscle tissue taken from one family member revealed that the patient's muscle fibers had reduced mitochondrial function and other characteristics seen in patients with mitochondrial mutations. Nuclear magnetic resonance (NMR) spectroscopy of the same patient's skeletal muscle showed reduced production of adenosine triphosphate, the molecular fuel that cells use to synthesize molecules and contract muscles. By using magnetic fields and radio frequency pulses, NMR spectroscopy is able to measure metabolism at a cellular level, which is another indicator of the health of the mitochondria. "NMR spectroscopy gives us tissue-specific rates of metabolism," notes investigator Gerald Shulman, also of Yale University School of Medicine. "NMR can detect alterations in a specific tissue that other metabolism tests don't pick up."

GCRC nurses undergo special training to help perform the NMR spectroscopy experiments at Yale's spectroscopy laboratory, including all aspects of patient care. "It's critical to have nurses who are experienced in magnet safety," says Shulman, who directed the GCRC until 2003.

Lifton and colleagues, with help from the GCRC, began a preliminary study in January, comparing the metabolic rate of 10 family members who have the mutation with 10 who do not. This summer, additional family members will undergo muscle biopsies and NMR spectroscopy, says Lifton.

The goal of Lifton's group is to find the mechanism that links the genetic defect to the medical disorders. So far, they can only hypothesize. The genetic defect might disable the ability of mitochondria to produce energy. Or the defect might lead to an increase in the production of reactive oxygen species that cause wear and tear on blood vessels, which would contribute to high blood pressure and other conditions.

The team also hopes to determine the potential role of mitochondrial dysfunction in common cases of hypertension and high cholesterol, says Lifton. This is the first time research has hinted at a genetic link between hypertension, high cholesterol, and low magnesium. The finding raises the question of whether mitochondrial dysfunction is behind the forms of these conditions found in the general population.

Patients with high blood pressure are more likely than healthy individuals to have high cholesterol, high triglycerides, diabetes, insulin resistance, and obesity. Indeed, up to one-fourth of the U.S. adult population has some combination of these conditions, which, when they appear together, are called metabolic syndrome. Because these traits occur together in patients more often than chance would allow, scientists suspect they have common roots, other than diet and lifestyle. Those roots may go deep into the cell's mitochondria. ■

The research described in this article is supported in part by the NCRR Division for Clinical Research Resources; the Howard Hughes Medical Institute; the National Institute of Diabetes and Digestive and Kidney Diseases; and the National Heart, Lung, and Blood Institute.

ADDITIONAL READING

■ Lowell, B. B. and Shulman, G. I., Mitochondrial dysfunction and type 2 diabetes. *Science* 307:384-387, 2005.

■ Wilson, F. H., Hariri, A., Farhi, A., et al., A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science* 306:1190-1194, 2004.

PHOTO BY MICHAEL MARSLAND, YALE UNIVERSITY

Scrutinizing Nature's Flying Machines

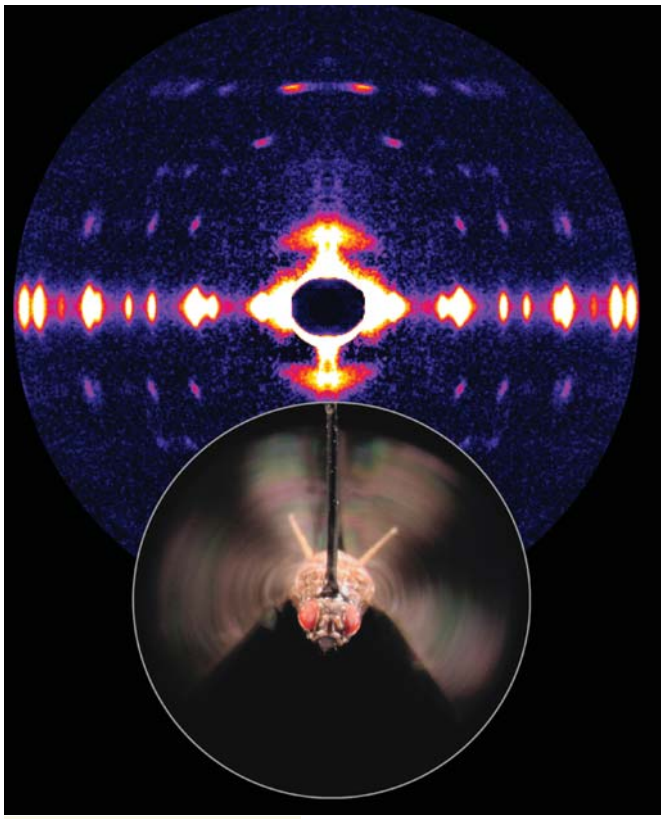
THE MOLECULAR maneuverings that power the fantastic flight of the fruit fly have been captured in an eight-frame movie. Not your average insect-in-flight flick, these flies are tethered in a flight simulator, and powerful X-rays at the Argonne National Laboratory's Advanced Photon Source (APS) function as the camera.

The movie reveals how the interactions between the muscle proteins actin and myosin enable the fly's muscles to contract, report Thomas Irving of the Illinois Institute of Technology and colleagues. Irving directs the NCRR-funded Biophysics Collaborative Access Team (BioCAT) facility at Argonne. BioCAT provides the scientific community with access to the brilliant synchrotron X-rays created by Argonne's vast particle accelerator. The movie also shows how protein fibers stretch and release as the insect flies, which will allow scientists to eventually calculate the amount of energy that the fibers store at any one time, says Irving.

BioCAT's narrow beam of focused X-rays was critical to the study's success. "Our work couldn't have even been conceived of without NCRR support of BioCAT," says Irving. With each wing beat taking a mere 5,000th of a second, synchrotron X-rays provide one of the few tools that can resolve the rapidly changing positions of muscle contraction molecules.

> "This research may shed light on human muscles, particularly the human heart."

To create the movie, Irving's team used small-angle X-ray diffraction, one of many X-ray techniques offered at the BioCAT facility. The X-rays scatter when they meet atoms, creating a diffraction pattern that yields sufficient information for Irving and colleagues to detect changes in the 3-D molecular structures. The X-ray exposures, enabled by the intense beam at BioCAT, total less than 1/20th of a second, the timespan of 10 wing beats. With laboratory sources, such patterns would take more than 24 hours to obtain, notes Irving. After shooting the individual X-rays, the scientists animate them, in much the same way that artists link still frames to create a cartoon, says Irving. Each frame of the movie is only 600 microseconds apart.



Powerful X-rays flashed at a tethered fly beating its wings (bottom circle) create a diffraction pattern (top circle). The positions and intensities of the spots reveal information about the molecular substructure of the insect's flight muscles.

Despite the current focus on flight, the new findings may someday prove useful to cardiologists. "All muscles are somewhat similar, so this research may shed light on human muscles, particularly the human heart," says Irving. The insect's flight muscles and the human heart both contract cyclically, among other shared traits. But before looking at clinical applications, he says, "we want to understand this system in the fruit fly a little better." His team is investigating, for example, the amount of force that the muscle is generating and how the energy is distributed and depleted. Another reason to study fruit flies among all of nature's flying machines: "You can mutate them easily, so you can test the action of any protein," notes Irving. Thus scientists will be able to determine if and how other proteins may be involved in the flight mechanism. (*Nature* 433:330-333, 2005)

—TINA ADLER

NCRR RESOURCES: The Biophysics Collaborative Access Team (BioCAT) resource is one of six NCRR-supported synchrotron resources available to the scientific community. BioCAT provides access to a variety of X-ray techniques for the study of cellular structures under conditions that replicate the cell's normal environment. More information about BioCAT is available at www.bio.aps.anl.gov. To learn about other NCRR-supported synchrotron resources, visit www.ncrr.nih.gov/ncrrprog/btdir/synchron.asp.

PHOTO BY MICHAEL DICKINSON

NCRR Welcomes New Acting Director

On March 25, 2005, NIH Director Elias A. Zerhouni, M.D., announced that **Barbara M. Alving, M.D.**, will serve as acting director of the National Center for Research Resources. Dr. Alving previously served—beginning in 2001—as deputy director of the National Heart, Lung, and Blood Institute (NHLBI) and director of the institute's Women's Health Initiative since 2002. She also served as acting director of NHLBI from September 2003 to January 2005. Dr. Alving joined NHLBI as the director of the extramural Division of Blood Diseases and Resources in 1999.

Prior to working at NIH, Dr. Alving served as director of the Department of Hematology and Vascular Biology at the Walter Reed Army Institute of Research and as director of the Hematology/ Medical Oncology Section at the Washington Hospital Center. She also was a research investigator in the Division of Blood and Blood Products at the Food and Drug Administration.

Dr. Alving earned her medical degree—cum laude—from Georgetown University School of Medicine, where she also completed an internship in internal medicine. She

BARBARA M. ALVING, M.D.

received her residency training in internal medicine at the Johns Hopkins University Hospital, followed by a fellowship in hematology.

A co-inventor on two patents, Dr. Alving has edited three books and published more than 100 papers in the areas of thrombosis and hemostasis. She is a professor of medicine at the Uniformed Services University of the Health Sciences in Bethesda, Maryland.

Advisor Appointed for Clinical and Translational Sciences

NCRR Acting Director Dr. Barbara M. Alving has appointed **Robert A. Star, M.D.**, to serve as her Senior Advisor on Clinical and Translational Sciences. Dr. Star will integrate initiatives from the "Re-engineering the Clinical Research Enterprise" theme of the NIH Roadmap into NCRR's clinical research programs. The Roadmap is a series of trans-NIH initiatives to transform the nation's medical research capabilities and speed translation of research discoveries from the bench to the bedside.

Dr. Star joined NIH in 1999 as a senior scientific advisor in the National Institute of Diabetes and Digestive and Kidney Diseases, where he is also a senior investigator and chief of the Renal Diagnostics and Therapeutics Unit. Since 2002, Dr. Star has served as a senior advisor for clinical research in the Office of the Director at NIH. He also cochairs the Roadmap Trans-NIH Clinical Research Workforce Committee.

Previously a professor of internal medicine at University of Texas Southwestern Medical Center, Dr. Star received his M.D. cum laude from Harvard Medical School and the Massachusetts Institute of Technology. He completed an internship and his residency in internal medicine at Michael Reese Hospital in Chicago, followed by a fellowship in renal physiology at the National Heart, Lung, and Blood Institute.

Dr. Star has published more than 80 papers on renal transport and acute renal failure. He also is a clinical professor of medicine at George Washington University in Washington, D.C.

NCRR Launches New Comparative Medicine Resources

NCRR's Division of Comparative Medicine is supporting the creation of two centers that will improve the study of disease progression in nonhuman models. With a five-year, $4.6 million grant, the University of Pittsburgh is setting up the first research center devoted to the use of viruses to trace the circuitry and architecture of the nervous system. The new Center for Neuroanatomy with Neurotropic Viruses, directed by neuroscientists **Peter Strick** and **J. Patrick Card**, will use modified herpesviruses that are naturally attracted to nerve tissue. When injected into laboratory animals, these so-called neurotropic viruses seek out and attach to nerve fibers, revealing intricate structural details that shed light on nervous system function. The new center will assemble experts from around the country to improve this virus-tracing technique, with an emphasis on collaborative and multidisciplinary studies.

At the University of North

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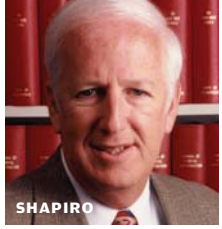
Carolina, Chapel Hill, researchers are launching the National Gnotobiotic Rodent Research Center. Funded by a five-year NCRR grant totaling $2.46 million, the new center will significantly expand the existing Mutant Mouse Regional Resource Center at the university. The center will provide scientists across the nation with access to gnotobiotic mice and rats, which will allow more precise explorations of how genes interact with the environment. Gnotobiotic organisms either are germ-free or have some contaminants that are known to the experimenter. Gnotobiotic techniques serve to produce germ-free and disease-free laboratories. ■

NCRR Welcomes New Advisory Council Members

Four new members have joined the National Advisory Research Resources Council, which advises NCRR on polices and programs and performs second-level peer review of grant applications. The new appointees are leaders in the scientific community.


KNOWLES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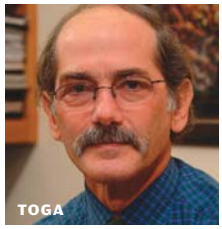
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Barbara B. Knowles, senior staff scientist and vice president for training, education, and external collaboration at The Jackson Laboratory in Bar Harbor, Maine. She is also codirector of the Institute of Molecular Biophysics at the University of Maine. Knowles studies the earliest stages of embryo formation.

Bettie S. Masters, co-director for research methodology in the department of biochemistry at the University of Texas Health Science Center in San Antonio. She has considerable expertise in biochemistry and pharmacology. Her research interests have included the characterization of nitric oxide synthesis.

Larry Shapiro, executive vice chancellor for medical affairs and dean of the school of medicine at Washington University in St. Louis. He previously directed and expanded the department of pediatrics at the University of California, San Francisco, and helped to establish the UCSF Children's Hospital.

Arthur Toga, professor of neurology and director of the NCRR-supported Laboratory of Neuro Imaging at the University of California, Los Angeles. His research focuses on neuroimaging, mapping brain structure and function, and creating a sophisticated atlas of the human brain. ■

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