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NCRR Reporter

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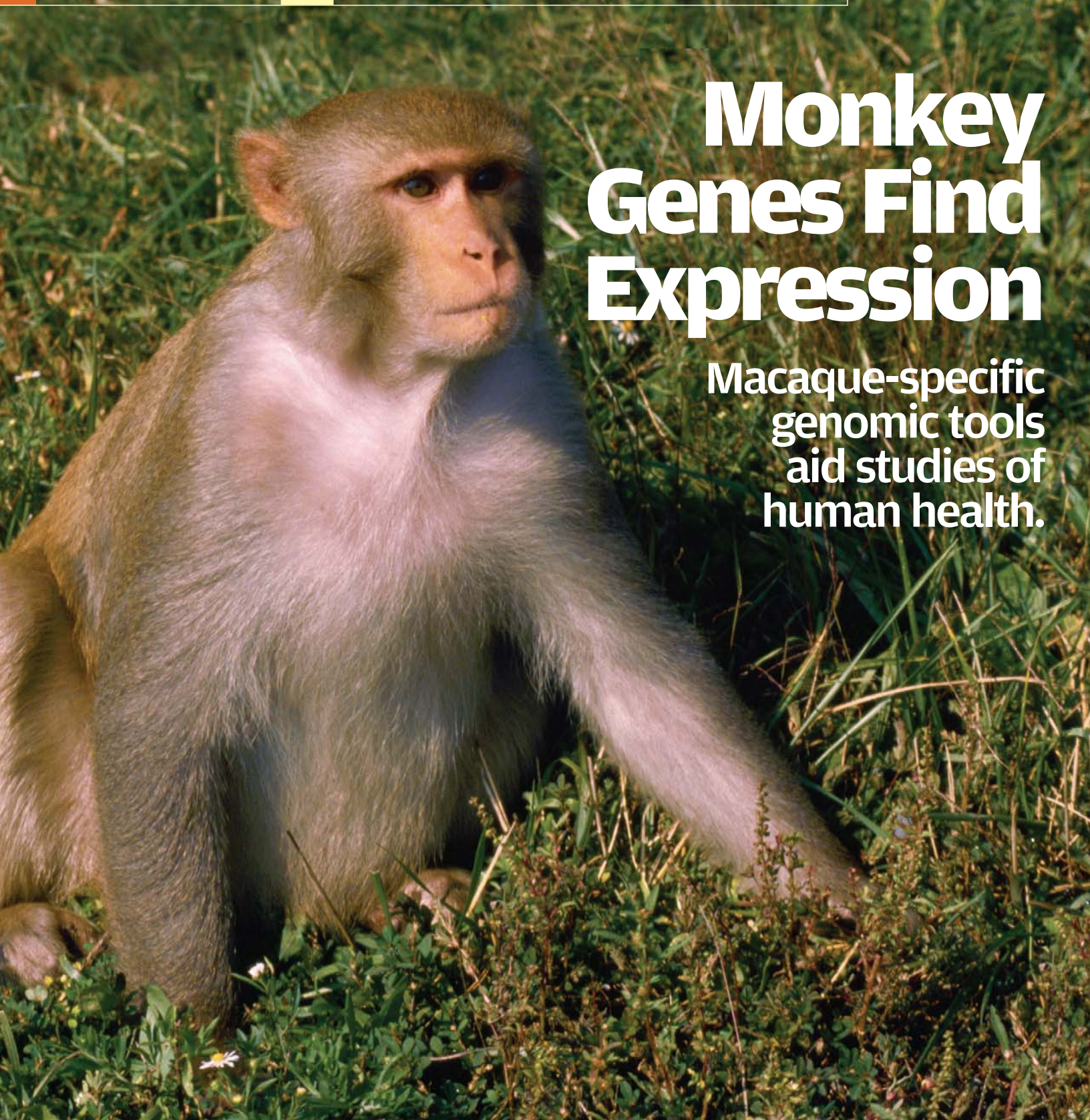
U.S. Department of Health and Human Services

Spring 2005

CRITICAL RESOURCES FOR YOUR RESEARCH



Monkey Genes Find Expression

Macaque-specific genomic tools aid studies of human health.

FROM THE DIRECTOR

A Transforming Vision for Clinical and Translational Research

As part of the NIH Roadmap for Re-engineering the Clinical Research Enterprise, NIH recently launched an initiative that is designed to revolutionize the way that clinical and translational research is conducted at academic health centers (AHCs) across the country. The goal of the initiative is to advance the academic standing of clinical and translational science as a distinct discipline, and to catalyze the development of an academic home for clinical and translational science. NCRR has been asked to implement this new and very exciting approach in coordination with the other NIH institutes and centers.

As many of you know from your own experiences, clinical and translational research involves specialized knowledge above that required for a medical, dental, or nursing degree, or specialty certification, and is a distinct discipline with a knowledge and skill base that must be learned. The increasing complexity of clinical and translational research requires a professional team that may include basic scientists, skilled clinical scientists, technology experts, and highly trained nurses, coordinators, and ancillary personnel. However, setting up these teams can be daunting for junior investigators and difficult even for more senior investigators unless the institution provides a conducive environment.

Dr. Zerhouni's vision under this initiative is to create a new home for clinical and translational research science that includes degree granting programs and opportunities for creative mentoring of the next generation of clinical and translational scientists. In supporting a range of academic, intellectual, and service activities, the new initiative will provide the financial resources and flexibility for institutions to establish an academic home that will likely encompass the following:

- Well-designed clinical studies and trials that include enhanced protocol development and regulatory oversight;
- Education, training, and career development, with an option of a clinical and translational science degree-granting program;
- Clinical research informatics and data management support with attention to leveraging efforts in healthcare informatics and facilitating inter-institutional collaborations;
- Clinical research resources, including space and personnel for inpatient, outpatient, and community studies and patient recruitment services;
- Core technologies and laboratories that provide clinical research services to investigators; and
- Pilot studies program for trainees, new investigators, and new innovative projects that need preliminary data before garnering independent support.

You will be hearing more about this new initiative when NCRR announces funding opportunities in the late summer or early fall. While NIH will make investments in this initiative, it also will require the support of the AHCs, industry, and foundations. As with all matters related to biomedical research, collaboration will be essential as we work toward our ultimate goal of finding ways to prevent, pre-empt, detect, treat, and cure more diseases.

Barbara Alving, M.D.
Acting Director, NCRR

(For more information on this initiative, see "News from NCRR," page 15.)

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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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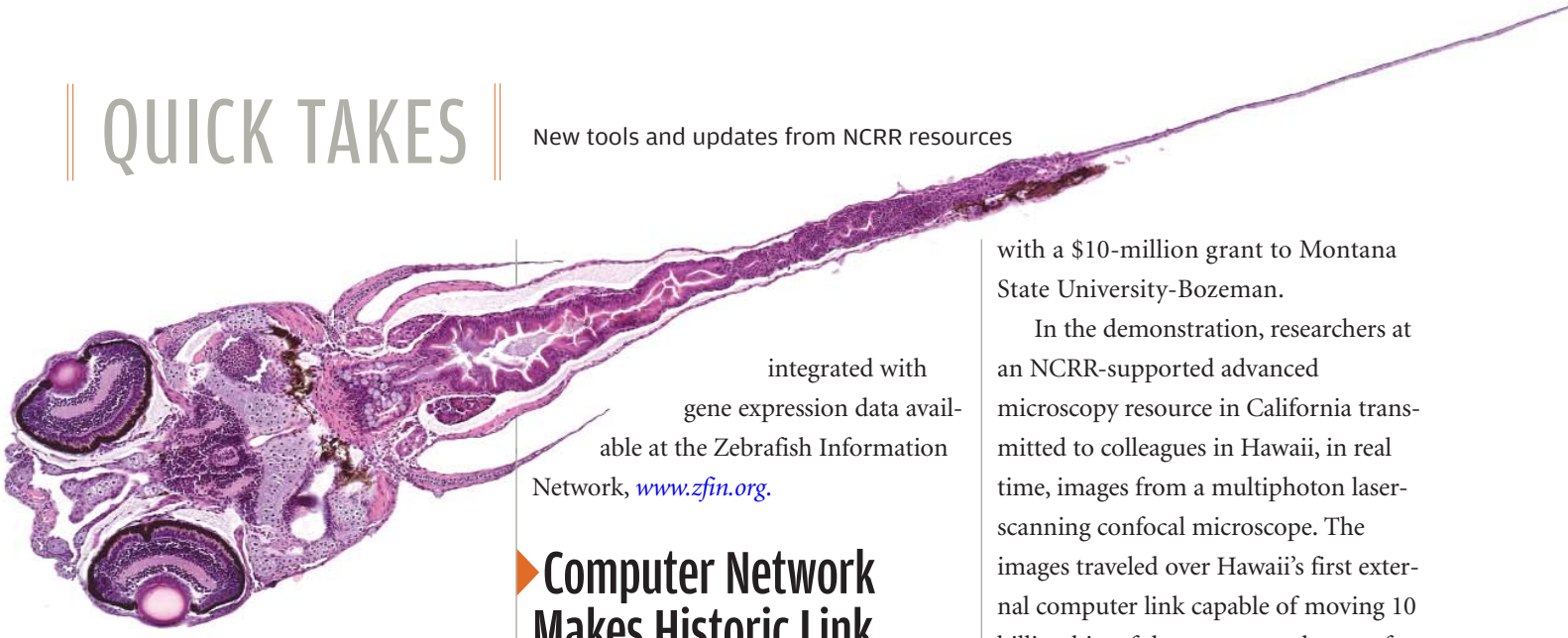
On the Cover: Studying gene expression in rhesus macaques opens a window into the genetic mechanisms underlying human health and disease. With two NCRR-funded projects now developing gene expression microarrays for these monkeys, rhesus genes can finally reveal their secrets.

PHOTO BY RICHARD T. NOWITZ/CORBIS

QUICK TAKES

New tools and updates from NCRR resources



The Zebrafish Virtual Atlas will show anatomical details like those in this cross-section of a zebrafish larva.

Zebrafish Anatomy Goes Online

A wealth of high-resolution anatomical images of zebrafish will soon be just a mouse-click away. NCRR has awarded $2.7 million over five years to Keith Cheng of the Pennsylvania State University College of Medicine to develop an online atlas of zebrafish anatomy. The freely available Zebrafish Virtual Atlas, which already has some images posted on its Web site, *www.zfatlas.psu.edu*, will aid the many scientists who rely on zebrafish models of human disease.

The atlas, which Cheng is developing with Stephen Moorman of the Robert Wood Johnson Foundation Medical School, will eventually contain annotated digital histology slides for every life stage and every part of the fish—the first such virtual atlas to be created for a vertebrate. Visitors can use their computers as virtual microscopes, changing their field of view and zooming in or out on the images. The atlas also will contain 3-D reconstructions of zebrafish organs and body structures, which will be viewed in rotation or movies, and be integrated with gene expression data available at the Zebrafish Information Network, *www.zfin.org*.

Computer Network Makes Historic Link

Earlier this year, scientists marked the first demonstration of a powerful computer link between Hawaii and the mainland, part of a high-speed computer network being built through an NCRR initiative called the Lariat Project (*www.lariat-west.org*). The project is creating high-speed connections for six Western states—Alaska, Hawaii, Idaho, Montana, Nevada, and Wyoming—that currently lack sufficient access to powerful computer networks. NCRR's Institutional Development Award (IDeA) Program launched the Lariat Project in 2003 with a $10-million grant to Montana State University-Bozeman.

A researcher in California transmits microscopic images through the Lariat Project's computer link to Hawaii.

In the demonstration, researchers at an NCRR-supported advanced microscopy resource in California transmitted to colleagues in Hawaii, in real time, images from a multiphoton laser-scanning confocal microscope. The images traveled over Hawaii's first external computer link capable of moving 10 billion bits of data per second, part of the Lariat Project's growing network of high-speed connections. Lariat is the first phase of a planned nationwide network called IDeANet, which will serve other states lacking adequate computer connectivity for conducting state-of-the-art biomedical research.

Islet Cell Program Now Serves Basic Science

NCRR's Islet Cell Resource (ICR) Program is now making human islet cells available to researchers engaged in basic scientific research. Until now, the program, which funds 10 ICR Centers nationwide, had provided islet cells only to clinical researchers, who then transplanted them into patients with severe type 1 diabetes.

Basic researchers whose projects are approved by the ICR Program's steering committee can receive islet cells at no cost. The application form to obtain the islets can be accessed at the ICR Web site at *http://icr.coh.org/info_invest.asp*. Applicants must describe why the islets are needed and how they will be used, outlining the specific research objectives, the methods to be employed, and the rationale for the number of islets requested. Once approved, applicants can arrange to receive islets from a specific ICR. ■

Monkey Genes Find Expression

Macaque-specific genomic tools aid studies of human health.

BY SCOTT J. BROWN

Microarrays being developed for rhesus macaques promise to revolutionize studies of human reproduction, development, and disease that depend on these animals.

MACAQUE MONKEY GENES have a lot to say, and researchers are finally getting a chance to listen. Genomic tools now being developed by NCRR-supported scientists will provide the research community with unprecedented opportunities to find out when and where genes are being turned on, or expressed, in rhesus monkeys, the macaque species most commonly used in biomedical research.

Although studies of macaques play a key role in our understanding of human reproduction, development, and disease, crucial genomic tools called microarrays have been lacking for these animals. A microarray is a small, flat plate spotted with thousands of molecular probes. Binding between these probes and fluorescently labeled DNA or RNA samples from various tissues reveals patterns of gene expression in the sampled tissues, shedding light on a variety of biological processes. Two NCCR-funded projects are now developing microarrays to study the expression of rhesus genes.

"Being able to use microarrays to do sophisticated genomics analyses is going to revolutionize the utilization of the macaque animal model," predicts Michael Katze, director of the Primate Genomics Division of the NCRR-supported Washington National Primate Research Center at the University of Washington. Katze, a professor of microbiology at the university, is spearheading one of the two rhesus microarray projects.

LOOKING AT THOUSANDS OF GENES AT A TIME

Microarrays allow scientists to determine how various portions of the genetic blueprint harbored in cells become activated, or expressed, as the body develops, carries out its normal functions, and responds to disease. An expressed gene is one that has been switched on by having its DNA sequence transcribed into messenger RNA (mRNA), allowing the gene's protein product to be produced within the cell. Unlike other techniques for studying gene expression, microarrays allow researchers to study the expression of thousands of genes simultaneously.

To produce microarrays, scientists collect—from various tissues—mRNA transcripts for expressed genes. They then create and clone DNA molecules that have sequences complementary to the mRNAs, called complementary DNAs, or cDNAs. These cDNA clones are then sequenced, and portions of those sequences are used to create short segments of synthetic, single-stranded DNA or RNA called oligonucleotides, which serve as the microarray probes. Labeled DNA or RNA samples derived from tissues will bind to probes that have sequences complementary to their own. If a sample binds to a probe, that binding reveals that the gene represented by the probe is expressed in the tissue that generated the sample.

Up to now, macaque researchers have been forced to examine the expression of one macaque gene at a time or have used human microarrays to study macaque gene expression. Although the human-microarray approach allows many genes to be studied at

PHOTO BY RANDALL C. KYES, UNIVERSITY OF WASHINGTON

once, some genes expressed in macaques will not be detected by human microarrays because of slight genetic differences between macaques and humans.

FROM GENOMICS TO PROTEOMICS

To provide researchers with macaque-specific genomic tools, Katze is working with Illumigen Biosciences, Inc., a biotechnology company in Seattle, to create libraries of cDNA clones from various macaque tissues. These clones are being sequenced to generate oligonucleotide probes and to create, in collaboration with Agilent Technologies, two gene-expression microarrays for the rhesus macaque. One of the microarrays, completed last summer, will soon be available for purchase from Agilent. The microarrays consist of glass plates the size of microscope slides containing thousands of rhesus sequences as well as some human sequences for comparative purposes.

The first microarray contains nearly 8,000 unique rhesus oligonucleotide sequences derived from cDNAs from a wide range of rhesus tissues. A second-generation microarray, slated for completion this summer, is expected to harbor more than 20,000 rhesus oligonucleotides. It will be based not only on Illumigen's ongoing cDNA sequencing, but also on sequences provided by the other NCRR-funded rhesus microarray initiative and by an ongoing rhesus genome project at Baylor College of Medicine. The sequences contained on the microarrays are being deposited in NIH's freely accessible GenBank database.

Many expression microarrays use oligonucleotide probes that contain sequences only from the noncoding regulatory end of a gene. Katze's probes, however, include the protein-coding regions of expressed genes, allowing scientists to see how gene expression correlates with protein production. Katze is among the many macaque researchers who will benefit from this type of microarray. His NCRR-funded investigations examine viral infection in macaques, including simian immunodeficiency virus (SIV) infection, a model for HIV infection and AIDS. Such studies depend on understanding how viruses alter the animals' gene expression and protein production.

"By using the microarrays at both the RNA and protein level, we can identify molecular signatures of virulence or pathogenesis, which could be used both diagnostically and prognostically," says Katze. The microarray analyses also shed light on host responses, help to reveal how viruses operate, and uncover the genes and cellular pathways that viruses impact. "That knowledge will allow us to become more adept at developing antiviral therapeutics and vaccines," he says.

MONKEY GENOME ON A CHIP

The other NCRR-funded microarray project is producing a more comprehensive microarray designed to represent all of the genes expressed in the rhesus macaque. Robert Norgren, associate professor of genetics, cell biology, and anatomy at the University of Nebraska Medical Center, and Eliot Spindel, senior scientist at the NCRR-supported Oregon National Primate Research Center at Oregon Health and Science University, are nearing completion of a microarray that will contain some 20,000 rhesus sequences. Affymetrix will manufacture the new rhesus microarray, which should be available for purchase by this summer. With technology borrowed from the computer industry, Affymetrix uses quartz chips to create thumbnail-sized microarrays called GeneChips.

"The rhesus GeneChip is going to have a huge impact. A number of prominent investigators have told me that it will greatly accelerate the speed of their research," says Norgren, who emphasizes the advantages of microarrays over single-gene approaches. "To try to guess which rhesus genes are important and then to study one gene at a time is just a painfully slow, incredibly inefficient, and frustrating way to do things," he says, "especially when you know this microarray technology now exists that allows you to look at all the genes at once and be done with it."

Scientists who have expressed interest in the rhesus GeneChip

are studying differentiation of rhesus embryonic stem cells, developing rhesus models of human reproductive biology, using SIV-infected macaques as a model for AIDS, and, like Norgren, investigating rhesus models of human neurological diseases.

"The biggest group of users will undoubtedly be the people who study AIDS," says Norgren. "Those people are desperate for a rhesus macaque GeneChip. I can imagine their frustration in not having the same tool for their animal model that they have for their human patients. They're storing their samples, getting ready to buy this GeneChip as soon as it's available."

MAKING USE OF THE HUMAN GENOME

Norgren came up with a novel way to produce the rhesus sequences necessary for the chip. His method relies on the close genetic similarity between humans and macaques and makes use of a preexisting Affymetrix GeneChip for the entire human genome. "The idea occurred to me that you could produce these sequences relatively fast and relatively cheaply by taking advantage of the human genomic resources already available," he says.

Michael Katze's microarrays cover the coding regions of macaque genes, helping researchers to understand how gene expression correlates with protein production.

Affymetrix created each of the probes on its human GeneChip from a selected region at the regulatory end of each gene, called the probe selection region (PSR). Norgren's team designs oligonucleotide primers whose sequences match sequences flanking the human PSRs. The researchers then use the human primers in a polymerase chain reaction (PCR) to amplify sequences in rhesus genomic DNA that match the human PSRs. The PCR amplifies rhesus sequences that are homologous to the human PSRs. These sequences, therefore, come from rhesus genes that are homologous to human genes. This strategy is thought to capture almost all rhesus genes, since the vast majority of rhesus genes have counterparts in the human genome.

The PCR products containing the rhesus sequences are cloned and sent to Eliot Spindel in Oregon. Spindel sequences the clones and annotates each of the sequences. He then places the annotated sequences, plus the primer sequences and PCR conditions used to generate them, in the GenBank database. "By making public the sequence information and how we got it, other people can use that information to make their own microarrays if they wish," notes Norgren. Affymetrix is using these rhesus sequences, plus sequences from the rhesus genome project and Katze's microarray project, to create the thousands of oligonucleotide probes that will make up the rhesus GeneChip.

After the GeneChip is out, Norgren says he will work to close any gaps in gene coverage that may exist on the microarray. He also foresees additional applications for the primers used to generate the rhesus gene sequences. These primers might help to generate sequences from other nonhuman primates, he says, noting that researchers who use African green monkeys have contacted him and expressed interest in a GeneChip for that species.

Norgren also would like to use the primers to discover mutations in rhesus genetic sequences known as single-nucleotide polymorphisms, or SNPs, which consist of substitutions of one base molecule for another in the DNA sequence. He notes that researchers could use rhesus SNPs to determine the genetic profiles, or genotypes, of individual monkeys, allowing them to choose the best animals for particular disease models. Rhesus SNPs also could serve as genetic markers to identify possible disease genes.

Norgren and Katze both view NCRR's support as vital to making rhesus expression microarrays a reality. "The support from NCRR has been absolutely fantastic," says Norgren. "Thanks to NCRR, many people funded by other NIH institutes will be able to do great things with these tools. It's going to pay off in a big way." ■

TO GAIN ACCESS: The Web site for the Affymetrix GeneChip Consortia Program (www.affymetrix.com/community/research/consortia.affx) will provide information on the availability of the rhesus GeneChip and how to order it. The consortia program designs and produces GeneChips for species identified as high-priority by the research community. Affymetrix's NetAffx Web site (www.affymetrix.com/analysis/index.affx) will provide the specific sequences contained in the rhesus GeneChip probes. Robert Norgren's Macaque GeneChip Web site (http://rhesusgenechip.unomaha.edu) includes information on some of the genes covered by the GeneChip, plus links to GenBank records for sequences from those genes.

The rhesus microarrays being produced by Michael Katze and Illumigen should be available this summer from Agilent Technologies (www.agilent.com). In the meantime, Illumigen has an NCRR-funded Web site (www.macaque.org) with links to GenBank records for sequences used in creating the microarrays. The site also gives details on Illumigen's cloning and sequencing efforts and provides information on ordering macaque cDNA clones.

PHOTO BY MARC STACY

Advances in Automated Microscopy

BIOMEDICAL RESEARCHERS have a new ally in their quest to understand the inner workings of cells. An NCRR-supported resource is automating the laborious process of cryo-electron microscopy (cryo-EM), currently the best technique for visualizing so-called molecular machines—the large macromolecular complexes of proteins that carry out most cellular activities.

The National Resource for Automated Molecular Microscopy (NRAMM) at The Scripps Research Institute (TSRI) in La Jolla, California, is striving to automate the entire cryo-EM process, from inserting the specimen into the microscope to creating a 3-D reconstruction of a molecular machine. The resource, launched in 2003 with NCRR support, hopes to free cryo-EM from dependence on long hours of microscopist labor, making the technique more attractive to researchers.

"By automating, we're hoping not only to enable different kinds of analyses to be done, but also to open up this technique more to the mainstream biologist," says Bridget Carragher, who codirects NRAMM with Clint Potter, both associate professors in TSRI's department of cell biology. NRAMM's advances in automation include a robotic system to load negatively stained specimens into the electron microscope for rapid screening, as well as automated tools to acquire and process cryo-EM images.

Because cryo-EM specimens are prepared by rapid freezing, complex structures like molecular machines are preserved intact. Many individual macromolecules, referred to as particles, are trapped in a layer of glass-like ice spread over a film of carbon mounted on a metal grid.

NRAMM has developed a software system called Leginon to acquire images of particles frozen on a grid. The Leginon system scans the grid, zeroes in on areas that contain the best particles, and then creates high-magnification images of particles in those areas and captures the images on micrographs. In a typical 24-hour session, the Leginon system acquires about 500 to 1,000 pairs of high-magnification images, which together contain anywhere from 10,000 to 300,000 particles, depending on the particle size and distribution.

As a result of Leginon training courses provided by NRAMM, 12 outside groups now have Leginon available at their own laboratories. Leginon has a Web site (http://leginon.scripps.edu) from which academic and nonprofit institutions can freely download the software.

Researchers set up and monitor their cryo-electron microscopy experiments from this state-of-the-art control room at NRAMM. The large screens display images acquired by the microscope as well as results of image analysis and processing.

Cryo-EM images acquired by the Leginon system are then analyzed by NRAMM-developed software, which automatically selects thousands of individual particles that best represent the structure of a macromolecule. Data from those particles are then averaged to create a composite 3-D reconstruction of the macromolecule, called a 3-D map. For this procedure, NRAMM uses reconstruction software developed by other NCRR-supported microscopy resources, (see *NCRR Reporter,* Summer 2004, pages 4-7).

Carragher, Potter, and colleagues now are working to link Leginon to the 3-D reconstruction software, thereby incorporating the construction of 3-D maps into the automated cryo-EM process. Linking Leginon to the reconstruction software should reduce the time needed to go from particle images to a 3-D map and further reduce the operator's burden in supervising the overall process.

NRAMM also has created a database to keep track of all images acquired with the Leginon system, as well as settings and parameters associated with those images. The database currently contains records on about 500,000 images from more than 50 projects. Researchers can view the images and query database records using Web-based tools that come with Leginon and can also view and download certain database images from the NRAMM Web site.

All of the automated tools housed at NRAMM are available to researchers for their cryo-EM projects. Outside researchers also can collaborate with NRAMM scientists on projects to enhance the resource's automated technologies. "People don't necessarily have to spend two to three years training a postdoctoral researcher to do cryo-EM," says Potter. "They can come to our resource instead."

—SCOTT J. BROWN

TO GAIN ACCESS: Researchers can apply to use the National Resource for Automated Molecular Microscopy (NRAMM) by filling out an online application on the resource's Web site (http://nramm.scripps.edu). NRAMM's Web site also provides access to the resource's cryo-EM database and software and gives information about NRAMM's automated cryo-EM technologies and training courses. For further information about NCRR-supported resources in biomedical technology, visit www.ncrr.nih.gov/biomedical_tech.asp.

PHOTO BY HEWITT/GARRISON ARCHITECTURAL PHOTOGRAPHY, SAN DIEGO

Bringing Veterinarians into Biomedical Research

Program cultivates expertise in whole-animal biology and microbiology.

As comparative medicine makes increasingly important contributions to biomedical science, the research community has a growing need for investigators with expertise that spans from molecular biology to the epidemiology of diseases in laboratory animal models. Yet biomedical researchers who are proficient in both veterinary science and biology are something of a rarity in the scientific community.

To encourage veterinarians to consider a career in biomedical research, NCRR offers Institutional Research Training Grants, called T32s. Awards are made directly to universities and other research institutions that are in a position to provide advanced training in comparative medicine or comparative pathology. T32 grants allow institutions to offer integrated courses of study drawn from a variety of academic disciplines. Trainees typically supplement their earlier clinical or pathology-related experience with formal instruction in ethics and experimental design, as well as basic science disciplines, such as biochemistry or molecular biology. And most important, they gain research experience in areas spanning the biomedical sciences.

PHOTO BY ALEXIS WENSKI-ROBERTS

The current shortage of veterinarians who know their way around a molecular biology laboratory is one reason for NCRR's vigorous support of the T32 program, says Franziska Grieder, associate director of comparative medicine at NCRR. "In biomedical research, it's essential to have well-trained veterinarians who understand not just a cell but the whole organism," she says.

Universities have flexibility in how they organize their T32 programs. The University of Washington School of Medicine, for example, requires participants to work toward a graduate degree, though not all T32 programs do so. First-year participants in the Washington program receive training in laboratory animal medicine and pathology and participate in animal-based research firsthand. Trainees also learn about research methods and ethics through courses and laboratory rotations, including a stint at the NCRR-supported Washington National Primate Research Center. In their second year, trainees begin to pursue advanced degrees in comparative medicine

■ ***Rachel Mo Peters is pursuing her Ph.D. in comparative biomedical sciences at Cornell University with support from an NCRR Institutional Research Training Grant. The grants provide essential training to prepare veterinarians for careers in biomedical research.***

or pathology or a basic science of interest to them.

"Our mission is broad: to take veterinarians and offer them the opportunity to get research training," says Denny Liggitt, who directs the university's T32 program. The program stresses what Liggitt describes as "hardcore research," preparing veterinarians to become collaborators with other medical researchers. Many graduates go on to work at academic institutions, including medical schools, while others enter private industry.

F. Claire Hankenson benefited from the T32 program as a graduate student. Today she teaches veterinarians who participate in the T32 program at the University of Michigan.

"The T32 program is extremely important for getting veterinarians into the medical research arena," Liggitt says. "Graduates of the program provide a perspective on research that a lot of veterinarians don't have."

The University of Washington program typically has six T32-funded participants at any given time and has no trouble attracting candidates, Liggitt says. "We create a positive environment for young people interested in research, and they prosper. We really have been fortunate to have this program," he says.

> "The T32 program is extremely important for getting veterinarians into the medical research arena."

One beneficiary of the Washington T32 program is veterinarian F. Claire Hankenson, who is today an assistant professor at the University of Michigan Medical School, where she teaches veterinarians, also funded by a T32 grant, and advises scientists who use animal models in their research. After graduating from Purdue University's School of Veterinary Medicine, Hankenson spent four years in the University of Washington's T32 program, earning a master's degree in microbiology in 2001. "I had an excellent experience there. It was a very stimulating environment," she says. The program enabled her to merge her interests in clinical work and basic research. Graduates of the program find that their unique combination of skills makes them attractive to potential employers, Hankenson says.

At Cornell University's College of Veterinary Medicine, the T32 program offers participants the opportunity to study biochemistry, cell and molecular biology, biomedical ethics, laboratory technology, and biostatistics, all under close faculty supervision. Trainees also participate in a seminar series on comparative medicine, designed specifically for program participants, says Douglas McGregor, director of training initiatives at the veterinary school.

Cornell requires participants to enroll in a doctoral program, and graduates finish with highly marketable skills, including a solid understanding of how to prepare a research proposal, McGregor says. "Industry is desperate for these people," he says. McGregor, an M.D., believes T32 graduates bring a much-needed, broad perspective to their research pursuits, a perspective that is sometimes lacking in classically trained medical researchers.

Some participants in the Cornell program have earned degrees in epidemiology, making them uniquely qualified to tackle research issues related to infectious diseases, from bird flu to bioterrorist attacks. "These trainees will be critical to the nation's biodefense structure," says McGregor.

In recent years, the number of students in NCRR's T32 program has doubled, with about 80 trainees now participating. "The program is geared toward research," says NCRR's Franziska Grieder, "but the broad education that trainees receive also will help equip them to take on leadership positions within the biomedical research community."

–PHILIP BULMAN

APPLY FOR FUNDING: NCRR supports research training and career development in comparative medicine through Institutional Training Awards, including three types of T awards. The two universities featured in this article received T32 awards as part of the Postdoctoral Program for Veterinarians. All applicants to the postdoctoral program must have completed their veterinary medical training. The other T32 options, which also go directly to the university or research institute, focus on predoctoral students. These programs—the Predoctoral Program for Veterinary Students and the Summer Program for Predoctoral Veterinary Students—allow veterinary students interested in biomedical research to participate in a one-year or summer program that provides a mentored, animal-oriented research experience. Trainees must have undergraduate degrees, be enrolled in a veterinary degree program, and be willing to devote 40 hours a week to the program. The third program is funded under the T35 mechanism and provides, directly to the university or institution, funds for short-term summer research training for veterinary students.

For more detailed information about NCRR's T32 awards, visit www.ncrr.nih.gov/compmed/cm_rcdtf.asp.

PHOTO BY KDH

Discovering the Heart's Repair Cells

"Discarded" cells may be key to mending damaged hearts.

BY TINA ADLER

TUCKED DEEP IN the newborn's heart are malleable progenitor cells capable of growing into mature heart cells, researchers recently discovered. The finding represents the first strong evidence that progenitor cells survive, at least for a few days, after birth.

The tissue containing these valuable cells is normally discarded when newborns undergo cardiac surgery. But researchers now hope that someday the cells may be harvested to help restore tissue in heart attack patients or to function as biological pacemakers.

Molecular cardiologist Kenneth Chien and his colleagues first found the progenitor cells, called isl1+ cardioblasts, in developing mouse and rat embryos. The researchers then identified the cells in five out of six human infants undergoing heart surgery. The cells' identities were verified by using the high-speed, multiphoton, laser-scanning microscope at the NCRR-funded National Center for Microscopy and Imaging Research, directed by Mark Ellisman at the University of California, San Diego.

"Ellisman's technology was particularly important to distinguish progenitors from mature cells—to identify the cells and compare their properties," says Chien, director of the Cardiovascular Research Center at Massachusetts General Hospital. With the high-resolution microscope at the NCRR-supported resource, says Chien, "we were able to monitor, in real time, the flux of calcium between progenitor cells and the cardiac muscle cells to show they were working compatibly."

Three studies since 2003 by other research groups have identified stem cells in the adult human heart, but these unspecialized cells can divide to produce any type of cell. Progenitor cells, in contrast, give rise to a distinct cell lineage, says Chien. "Our story is unique, because the cells we found can become—and spontaneously do become—fully functional cardiac muscle cells," says Chien. Other researchers had identified single cells that had muscle-cell characteristics but did not become functioning muscle cells.

Chien's team found not just one cell but a few hundred of the progenitor cells in the hearts of newborn humans, mice, and rats. In all of these species, the progenitor cells were in the heart's myocardium, which is the middle and thickest layer of cardiac muscle in the heart wall. By placing the cells on a layer of connective-tissue cells, called fibroblasts, Chien's group was able to produce millions of offspring of the progenitor cells. About 30 percent of the

Researchers were surprised to discover progenitor cells (green) in the cardiac muscle (red) of a newborn mouse, as those cells were not thought to survive after birth. Cellular nuclei are shown in blue.

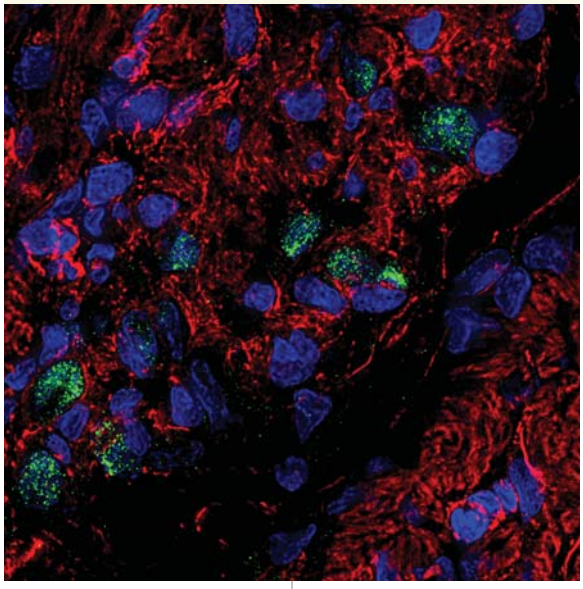


IMAGE BY KARL-LUDWIG LAUGWITZ, UNIVERSITY OF CALIFORNIA, SAN DIEGO

Sending in the Repair Cells

Patients who survive a heart attack are not necessarily in the clear. The episode creates scar tissue, which increases a patient's risk of developing complications. To help heal damaged hearts, researchers are experimenting with injecting the building blocks of heart muscle into the patient's damaged organ.

One such study has recently begun at the Johns Hopkins University School of Medicine, with assistance from the NCRR-supported General Clinical Research Center (GCRC). Researchers are injecting adult mesenchymal stem cells, which come from the bone marrow of adult donors, into recent heart attack victims to test the safety of the treatment. The Baltimore-based company Osiris Therapeutics developed the stem cell product and is supporting the study.

As of April 2005, the team had treated its first patient and had plans to treat an additional 47 patients, says cardiologist and senior investigator Joshua Hare, professor of medicine at the university. The GCRC is invaluable to this kind of research, he says. "We couldn't do the study without the GCRC, because it's a four-day in-patient study, and patients must be very carefully monitored." Staff from the Johns Hopkins stem cell laboratory are working with GCRC nurses to administer the infusions. "It's a big team effort," Hare notes.

In one recent study, reported in November 2004 at the American Heart Association Scientific Sessions conference in New Orleans, the team administered the stem cells to pigs with damaged hearts. Up to 75 percent of the dead scar tissue in the animals' hearts disappeared after the treatment. "When we looked at the function and structure of the animals' hearts, we found growth of new, normal cardiac tissue and really dramatic cardiac repair," says Hare.

In the human trial, candidates first undergo catheterization and echocardiography to ensure their main coronary vessels are not blocked. The participants then receive one of three possible doses of stem cells or a placebo. The stem cells, which do not trigger the body's immune system, are expected to migrate to the damaged areas of the heart muscle.

How mesenchymal stem cells may work to repair heart tissue is unclear. Mesenchymal cells normally give rise to a variety of cell types, including bone, cartilage, fat, and other kinds of connective-tissue cells. "There's some evidence that the cells differentiate into cardiac muscle cells and some evidence that they do not, but the larger area of controversy is whether differentiation is necessary for cardiac repair," says Hare. In the pig study, for example, the team saw no evidence that the cells had differentiated. Hare suspects that the mesenchymal cells do not become muscle cells but rather release growth factors and other molecular signals that elicit repair.

Hare is eager to bring the efforts of basic research to the clinic. "The ultimate goal of our research is the rapid translation of basic science into treatment," he says.

ADDITIONAL READING

■ Bhatia, R., Hare, J. M., Mesenchymal stem cells: Future source for reparative medicine. *Congestive Heart Failure* 11:87-91, 2005.

offspring cells differentiated into complete cardiac muscle cells.

Chien and his colleagues came upon the progenitor cells by taking a closer look at material that they once considered disposable. During their many years of studying heart tissue, the researchers typically separated out and discarded the nonmuscle cells from their samples. But when they finally examined some nonmuscle cells that had been left in a culture medium for two weeks, the cells appeared to be differentiating into cardiac muscle. The activity was occurring in tiny islands where there were cardiac fibroblasts, which the team now knows serve as a growth medium for the progenitor cells.

After seeing the cells in action, the team needed a way to identify them. Fortunately, Chien's colleagues in a nearby laboratory had recently identified a marker, called islet-1, that distinguished progenitor cells in the embryonic heart. By searching for the marker, Chien's group discovered that the progenitors in the newborn rats, mice, and humans were all expressing islet-1.

In hypothesizing why the cells remain in the heart after birth, Chien says that the progenitor cells may be important in remodeling the newborn's heart. Congenital heart defects may arise if the lingering cells are defective. Other researchers have experimented with injecting adult stem cells into patients' hearts to repair damage from heart attacks (see sidebar), but the results of these efforts have been mixed. Chien asserts that successful use of progenitor cells to heal heart attack patients is years away.

Whether progenitor or stem cells prove better at restoring healthy muscle in heart attack patients will depend on many factors, including which type of cell can best be grown in large numbers. The cells must take on the complex role of making electrical connections with other cells. Without those connections, Chien says, fatal arrhythmias may result. ■

The research described in this article depended on technologies developed at the NCRR-supported National Center for Microscopy and Imaging Research at the University of California, San Diego. For information about other NCRR-funded biomedical technology resources, visit www.ncrr.nih.gov/biomedical_tech.asp.

ADDITIONAL READING

■ Laugwitz, K. L., Moretti, A., Lam, J., et al., Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 433:647-653, 2005.

Virus Implicated In Lupus

NEW RESEARCH SUPPORTS the view that systemic lupus erythematosus, commonly known as lupus, can result from a viral infection. In what seems to be a case of mistaken identity, the body apparently thinks it is attacking the common Epstein-Barr virus (EBV) when in fact it is targeting a protein found in the body's own cells. Lupus is an autoimmune disease, in which misdirected antibodies, or autoantibodies, attack and injure a variety of tissues in the body.

"There's mounting evidence that EBV may be important in triggering these abnormal autoimmune responses," says Judith James, principal investigator of the new research and head of the NCRR-supported peptide synthesis core facility at the Oklahoma Medical Research Foundation (OMRF). Funded as part of a Centers of Biomedical Research Excellence (COBRE) award to the OMRF, the core facility provided more than a thousand protein fragments, or peptides, to study immune responses associated with lupus. In addition, NCRR's General Clinical Research Center (GCRC) at the University of Oklahoma Health Sciences Center helped with the study's statistical analysis.

Judith James has found that antibodies in lupus patients may confuse a common cellular protein with an Epstein-Barr viral antigen.

To investigate how the immune system might mistake the body's own proteins for EBV proteins, James and her colleagues used synthetic peptides representing portions of a common cellular protein called Ro, known to be an early target for autoantibodies in lupus, and of an EBV antigen called Epstein-Barr virus nuclear antigen-1 (EBNA-1). Reactions between these peptides and antibodies in serum previously collected from lupus patients revealed that EBNA-1 and Ro are both targeted by the same anti-Ro antibodies. These reactions also pinpointed the specific parts of these proteins that are attacked by the antibodies. When these targeted sections, or epitopes, were injected into rabbits, they triggered autoimmune reactions, and the rabbits developed lupus-like symptoms. This supports the idea that antibodies directed against EBV can lead to lupus by also targeting Ro.

These findings may lead to better diagnosis and treatment of lupus and may also aid a project at the OMRF, also supported by COBRE funding, to develop an EBV vaccine, says James.

The findings may lead to better diagnosis and treatment of lupus.

She and her colleagues next plan to explore genetic factors that might explain why most people infected with EBV produce antibodies to Ro only transiently or not at all, while individuals who go on to develop lupus make the antibodies for prolonged periods and progress to a more complex response. The researchers also want to enroll and follow patients through the University of Oklahoma's GCRC to determine how EBV-induced autoimmune responses to Ro might lead to clinical lupus symptoms. (*Nature Medicine* 11:85-89, 2005) **—SCOTT J. BROWN**

NCRR RESOURCES: The Center of Biomedical Research Excellence (COBRE) at the Oklahoma Medical Research Foundation is among more than 70 COBREs across the country (see *NCRR Reporter*, Winter 2004, pages 5-7). The COBRE Program funds researchers in states that historically have received fewer competitive research grants from NIH. COBRE awards provide five years of funding for multidisciplinary teams to enhance research expertise and competitiveness within their institutions. For more information, visit www.ncrr.nih.gov/resinfra/cobre.asp.

The General Clinical Research Center (GCRC) at the University of Oklahoma Health Sciences Center is one of 82 GCRCs nationwide. GCRCs provide optimal settings for inpatient and outpatient clinical studies. For more informtion, visit www.ncrr.nih.gov/clinical/cr_gcrc.asp.

Sleep Key to Speech Development, Songbirds Reveal

DESPITE WHAT their mothers may think, human infants and baby birds sound remarkably alike to the trained ear of a behavioral neuroscientist. "A few years ago, we discovered that the young bird is doing something very similar to babbling," says Ofer Tchernichovski of City College of the City University of New York (CUNY). The early vocalizations of both birds and humans are highly repetitive and become more complex over time. Because of these and other similarities, Tchernichovski and his colleagues study birds in an effort to understand speech development in humans.

Tchernichovski's research depends on support from one of

PHOTO BY MICHAEL DICKINSON

Because sleep is key to speech development, these young slumbering zebra finches may in fact be hard at work rehearsing the songs they heard during the day.

the NCRR-funded Research Centers in Minority Institutions (RCMIs), located at CUNY. RCMIs support health research at institutions that award doctorates in health-related sciences and also enroll at least 50 percent minorities that are underrepresented in the biomedical sciences. "The RCMI Program is a great initiative. It helped me in so many ways," says Tchernichovski, who received his salary and research funding for his first two years at CUNY through the RCMI. The support wasn't just financial; program staff provided the hands-on technical help he needed to set up his laboratory. "They worked night and day helping me," says Tchernichovski.

Researchers have known that sleep is key to learning and memory in both humans and animals. According to earlier studies, brain regions devoted to birdsong show spontaneous activity during sleep, suggesting that birds practice their songs while sleeping. But the effects of sleep on the development of speech had gone unexplored, says Tchernichovski. As it turns out, he and his colleagues have discovered that sleep has a curious effect on how young birds, and perhaps humans, learn to speak.

Birds learn to sing by hearing adult birds sing. Studies have suggested that baby birds readily commit the tunes to memory and then compare their own vocalizations to the tunes they remember. To uncover the role of sleep in this process, Tchernichovski's team recorded 50 zebra finches continuously, from when they first started learning to sing until adulthood. The finches stayed in individual cages where the scientists could control all aspects of the environment, including light and sounds.

To analyze the birdsongs, the team used its own software package, Sound Analysis Pro, developed earlier with RCMI funding. The software directs a computer to record continuously several birds at a time, differentiate the songs from each other, and separate the sounds into syllables. Each bird produces about 1 million syllables during about eight weeks of developmental learning, which the program analyzes for their acoustic features, such as frequency modulation and variance in pitch. Throughout the day, the computer can play the songs of mature zebra finches to the young birds, which copy the songs they hear.

The complexity of the young birds' songs changed from day to day, as the birds became master singers, but more changes occurred overnight than from one day to the next, the team discovered. However, the overnight changes were not what the team expected: the birds sang less well, meaning they made less structured sounds, in the morning than they did the night before. But after a few hours of morning singing, the birds' skills exceeded their previous day's performance.

"The effect of sleep is counterintuitive," says Tchernichovski. "In the short term, it looks like sleep does negative things, but in the long term it helps." Oddly enough, birds that declined the most overnight became the better singers. Additional experiments allowed the team to rule out sleepiness or lack of overnight practice as the cause of the poor morning performances. Also, the morning effect diminished as the birds got older, which points to a developmental cause.

Sleep has a curious effect on how young birds, and perhaps humans, learn to speak.

The researchers suspect that after rehearsing songs in their sleep, birds use the morning to explore their vocal abilities and improve their skills at imitating. The mechanism directing these overnight and early-morning language lessons is not clear, but structural changes at the cellular level are likely involved. (*Nature* 433:710-716, 2005)

—TINA ADLER

NCRR RESOURCES: City College of the City University of New York is one of 18 institutions that currently host Research Centers in Minority Institutions (RCMIs). Information about the RCMI at CUNY can be found at www.ccny.cuny.edu/rcmi/. To learn more about the RCMI Program, visit www.ncrr.nih.gov/resinfra/ri_rcmi.asp.

PHOTO BY SEBASTIEN DEREGNAUCOURT

Monoclonal Antibodies

From laboratory tool to effective therapies.

SINCE THE DISCOVERY of monoclonal antibodies (MAbs) was first published 30 years ago, these targeted biological tools have evolved from a laboratory mainstay to effective therapies for treating a variety of human disorders. Over the years, NCRR-supported resources have enabled the development and clinical evaluation of several therapeutic MAbs, including the radiolabeled drug ^{131}I-tositumomab, tradenamed Bexxar, which homes in on normal and malignant B cells in patients with non-Hodgkin's lymphoma.

Results of a phase 2 clinical trial in 76 patients, published in February 2005 in the *New England Journal of Medicine,* showed that Bexxar led to prolonged remission in more than 75 percent, and shrinkage of tumors in 95 percent, of patients with advanced and previously untreated follicular non-Hodgkin's lymphoma. Although researchers have yet to perform a direct comparison, a single week-long course of the less-toxic MAb-based therapy appears to produce similar efficacy and response rates in newly diagnosed patients as standard chemotherapy regimens, which typically last for several months. "I think the reduced treatment time is the most appealing thing about this new therapy," says Mark Kaminski, who created and developed ^{131}I-tositumomab at the University of Michigan with colleague Richard Wahl.

More than two decades ago, a team of scientists created an MAb that selectively attaches to a protein found on the surfaces of B cells, and Kaminski and Wahl saw an opportunity to devise a new therapy for B-cell-type non-Hodgkin's lymphoma. By attaching a potent radioactive payload—in this case, iodine-131—to the B-cell-targeted MAb, the Michigan researchers hoped to destroy rapidly dividing cancerous B cells in lymphoma patients. In theory, this radioimmunotherapy would kill B cells but leave other cells relatively unharmed.

In the early 1990s, radioimmunotherapy was in its infancy—consistent success in getting it to work in the clinic was still an elusive goal. But Kaminski and Wahl's pioneering clinical studies found that the experimental drug ^{131}I-tositumomab showed promise for treating non-Hodgkin's lymphoma patients who either failed to respond to chemotherapy or relapsed after the treatment. By 2003, the FDA had approved the radiolabeled drug for treating such patients. The most recent study, however, represents a first step toward obtaining approval for treating newly diagnosed patients, which is the ultimate goal of drug development projects.

■ *Mark Kaminski (far right) speaks with Richard Lowenthal, one of the first patients to receive Bexxar, as nuclear medicine technician Denise Regan administers the drug.*

Throughout 15 years of clinical evaluation and development, Kaminski and Wahl's team relied heavily on the resources of the NCRR-supported General Clinical Research Center (GCRC) at the University of Michigan, where GCRC staff performed patient scans and hourly blood draws. Because radioactive particles tend to escape from patients recently treated with radioimmunotherapy, the GCRC also built a special room to house Kaminski and Wahl's patients and protect the staff and other patients from the scattering radiation. "There was no way this project could have been done without access to the GCRC," says Kaminski.

"Wahl and Kaminski were a peas-in-the-pod team, with each researcher bringing his unique talents to the project," says John Wiley, who heads the Michigan GCRC. "Wahl had expertise in imaging and the radiological side, while Kaminski was the oncologist who wanted to use antibodies in combination with a high-intensity radioligand to treat the disease."

With approximately 15,000 new cases of follicular lymphoma diagnosed each year in the United States, finding a therapy that is effective and well-tolerated will have a profound impact. "From my perspective, having watched this experimental therapy evolve over the years, this project has been one of the wonderful bench-to-bedside stories that we love to see," says Wiley. "We just wish we could see more of them."

—RABIYA S. TUMA

PHOTO BY MARTIN VLOET, UNIVERSITY OF MICHIGAN PHOTO SERVICES

Meeting Seeks Input on Enhancing Clinical and Translational Science

In one of the first steps in a major effort to transform clinical and translational research, NCRR sponsored a meeting—called "Enhancing the Discipline of Clinical and Translational Sciences"—to discuss how the National Institutes of Health (NIH) and other research-oriented institutions might work together to create a new, integrated academic discipline of clinical and translational science. The meeting was held in Arlington, Virginia, on May 23, 2005.

With more than 300 biomedical scientists and administrators in attendance, the goal was to generate discussion on enhancing the translation of basic biomedical discoveries into more effective therapies and improved human health. NCRR convened the gathering on behalf of the NIH Roadmap initiative on Re-engineering the Clinical Research Enterprise. Discussions focused in part on the need for clinical research informatics; education, training, and career development; intra- and inter-institutional collaborations; and comprehensive integration and expansion of resources.

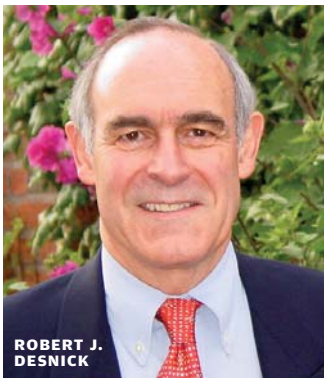
In his opening remarks, NIH Director Elias Zerhouni noted that NIH intends to take a more cohesive, systems-based approach to strengthening clinical and translational science by creating funding programs that are flexible enough to be tailored to the needs and strengths of individual institutions. He asked participants to think creatively about how NIH might help to bolster clinical and translational research at both the institutional and the national levels. "We realize that no one has all the answers, and we clearly believe that one size does not fit all," Zerhouni said. "The biggest challenge, in my view, is to be able to develop good science across disciplines. Collaboration is going to be the key to making progress."

At the close of the meeting, participants issued a series of observations and recommendations for NIH to consider in developing new initiatives and programs for clinical and translational science. NCRR Acting Director Barbara Alving noted that this input would greatly inform future NIH-wide activities in this arena. "The development of Dr. Zerhouni's vision for this initiative requires that the NIH work closely with the community of researchers and the academic health centers that choose to respond to this funding opportunity," she said. "By undertaking this change in how NIH supports clinical and translational science, we can work together to reach our ultimate goal—improving the health of the nation."

For more information about the new clinical and translational science initiative, part of the NIH Roadmap for Re-engineering the Clinical Research Enterprise, see "From the Director," page 2. For more information about the national meeting, including a videocast of plenary presentations, visit www.ncrr.nih.gov/clinicaldiscipline.asp. ■

Awards Honor Clinical Researchers

Robert J. Desnick, professor and chair of human genetics at Mount Sinai School of Medicine, received the 17th Annual Award for Excellence in Clinical Research at the General Clinical Research Center (GCRC) Program Directors Meeting, held April 29-30, 2005, in Washington, D.C. The GCRC Program Directors Association presented the award on behalf of the Jane and Charles Pak Foundation, which funds the award to recognize outstanding clinical investigators who have


ROBERT J. DESNICK

conducted studies at GCRCs within the previous decade.

Desnick received the $5,000 award in honor of his exceptional studies of genetic diseases, including the rare lysosomal storage disease known as Fabry's disease. He and his colleagues showed that lysosomal storage diseases can be effectively treated by using enzyme replacement, bone marrow transplant, or other novel techniques. Of particular note, Desnick's basic, translational, and clinical research over more than three decades ultimately led to a Food and Drug Administration-approved treatment (Fabrazyme) for patients with Fabry's disease.

Recently named a member of the National Academy of Sciences, Desnick is a past program director of the Mount Sinai GCRC (1991-1999) and a past member of the NCRR National Advisory Research Resources Council (2000-2004). He has authored more than 365 peer-reviewed articles.

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At the Clinical Research 2005 meeting, held in tandem with the GCRC Program Directors Meeting, **John Eisenach** of the Mayo Clinic received the $2,000 GCRC Outstanding Trainee Award. Eisenach was recognized for an abstract he presented on isometric exercise. His study demonstrates that a common genetic variation in the beta-2 receptor—a receptor in the heart, blood vessels, airway smooth muscle, and elsewhere—influences heart and blood vessel response to isometric exercise. "This finding provides further evidence that known gene variations play an important role in how our body regulates heart function and blood pressure," Eisenach explains.

Eisenach has an NCRR Patient-Oriented Research Career Development Award, or K23 grant. The K23 program supports physicians or dentists for three to five years as they train in advanced and experimental clinical research methods. Eisenach received his medical degree from the University of Colorado Health Sciences Center in Denver and completed his residency in anesthesiology at the Mayo Graduate School of Medicine. ■

JOHN EISENACH

Multimedia Product Wins Award

A multimedia educational tool created with NCRR support has won a coveted international award. The Pirelli Internetional Award competition this year gave one of its top prizes—15,000 euros, or about $18,000—to the makers of an interactive multimedia tutorial called the Microarrays MediaBook. Each year, the Pirelli Group, a multinational corporation headquartered in Milan, presents awards for the best Web-based multimedia products.

Produced by the Institute for Science Learning (ISL) at the University of North Carolina at Chapel Hill, the Microarrays MediaBook uses 3-D animations and dynamic, interactive learning tools—aimed at a variety of learning styles—to help undergraduate students understand the role of microarrays in genomics and bioinformatics. ISL's MediaBook project, funded primarily by a Small Business Technology Transfer Research grant through the National Human Genome Research Institute, receives supplemental support from NCRR and the National Institute of General Medical Sciences.

The Microarrays MediaBook is the first learning module of a larger MediaBook that will contain about 60 or 70 interconnected modules covering all major aspects of genomics and bioinformatics, says ISL Director Walter E. "Skip" Bollenbacher. The modules will be sold as interactive Web-based tutorials, although some, including the microarrays module, also may be released as CD-ROMs bundled to conventional textbooks. The Microarrays MediaBook could be available as early as this fall, says Bollenbacher, with the complete MediaBook slated for release in January 2007. ■

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