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Research Festival

Getting to the Bottom Of the Beta Cell

by Fran Pollner

For some, the quest is to increase the progenitor pool of pancreatic beta cells, to derive stem cells that can be controlled in culture and serve as replacements for damaged or lost beta cells; for others, the quest is for new treatments.

Stem Cell Studies

Fran Pollner

Marvin Gershengorn

Typically, cultured human beta cells do not proliferate well or retain the mature phenotype, noted Marvin Gershengorn, chief of the Clinical Endocrinology Branch and scientific director, NIDDK, who has been exploring the optimization of hIPCs (human islet cell–derived precursor cells) for about five years.

Gershengorn's lab has established that hIPCs are a special type of mesenchymal stromal cells that can be induced to differentiate into adipocytes, chondrocytes, and osteocytes, as well as cells of the endocrine pancreas. "We can change the culture conditions that result in epithelial or endocrine-like cell clusters; we can upregulate the insulin transcript level, generating clusters of C peptide–expressing cells," he said, noting that his team has transplanted these cells into mice and demonstrated in vivo hIPC functionality.

The lab of Sushil Rane, of the Regenerative Biology Section in the NIDDK Diabetes Branch, has been examining the role of the cell-cycle regulator CDK4 in beta cell regeneration.

Hyperglycemic and hypoinsulinemic, CDK4-knockout mice have clearly lost beta cell mass, Rane said;

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Research Festival

Coming of Age: Tissue Engineering and Regenerative Medicine

by Julie Wallace

Julie Wallace

The Re-Generation: *(left to right): Pamela Robey, NIDCR; Cynthia Dunbar, NHLBI; Catherine Kuo, NIAMS; and panel chair Rocky Tuan, NIAMS*

Panel chair Rocky Tuan noted that this was the NIH Research Festival's first dedicated symposium on tissue engineering and regenerative medicine, a reflection that the field is steadily approaching the threshold of clinical application.

Indeed, applying biological and engineering principles to repairing and replacing damaged and destroyed tissues has attracted researchers across NIH; scientists from three institutes described their ongoing research involving adult stem cell–based approaches to tissue regeneration.

Tuan: Creating the Matrix

Adult stem cells and nanomaterials are Tuan's basic building blocks in his quest to regenerate skeletal tissues. Encouraging results thus far in repairing joint degeneration in rabbits foretell the application of his team's techniques to the treatment of patients with musculoskeletal diseases such as osteoarthritis.

With the right scaffold and physical and chemical environments, a cartilage micromass could be developed from human mesenchymal stem cells (MSCs) to replace the degraded tissue, said Tuan, chief of the Cartilage Biology and Orthopaedics Branch, NIAMS. The challenge is to generate a cartilage construct of sufficient size to transplant into a human joint, he said.

Tuan's lab evaluated whether differentiated MSCs could transdifferentiate—change from one differentiated state to another. The team found that osteoblasts, adipocytes, and chondrocytes derived from MSCs could indeed be made to switch identities. This capacity to de-differentiate generated the hypothesis that there might be "stemness" genes that regulate MSC self-renewal and multipotency.

Using microarray analysis, Tuan and his colleagues determined that differentiation genes were upregulated in differentiation and downregulated during

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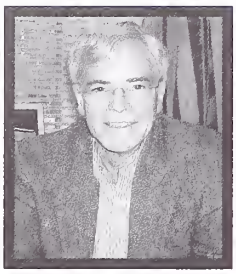
Trans-NIH Intramural Scientific Initiatives: Why? How? When?



Michael Gottesman

Last year, with the encouragement of NIH Director Elias Zerhouni, the NIH intramural research program (IRP) set out to identify important scientific initiatives that would be difficult for any one institute to support, that would exploit the special characteristics of the IRP, and would scientifically draw from and benefit multiple NIH institutes and centers.

After a series of meetings involving many of our principal investigators and scientific leadership, we settled on three major initiatives with cross-cutting impact: (1) Immunology, Autoimmunity, and Inflammation, (2) Molecular Imaging from Molecules to Cells, and (3) Systems Biology.

For nearly a year, groups have met to define the nature of each of these initiatives and how to bring them to fruition. The resulting consensus proposals on how to proceed were then presented to me and to Dr. Zerhouni—and, on October 22, 2007, to a larger group of NIH scientists and scientific leadership at a retreat held for this purpose.

Why?

Discussion at this retreat focused not only on the substance of the proposals, but also on the rationale for this new approach to science at NIH. In the past, virtually all of our scientific initiatives have been investigator-initiated or have sprung from programmatic imperatives of the institutes (for example, the Vaccine Research Center). This worked well for the IRP during the time of continuously rising budgets (real increases adjusted for inflation of approximately 2 percent per year from 1980 to 2000) when new funding was available to each institute or center to create a venture capital fund for high-risk, high-impact novel science, or even through the development of new intramural programs (such as the NHGRI IRP).

Even so, the IRP failed to be at the cutting edge of some new technologies and/or model systems—such as yeast genetics to study cell biology, and RNAi—until they were well established in academia or in industry. Now with real budgets dropping in the IRP for the past four years, it has become increasingly difficult to initiate large-scale new scientific initiatives, and the need for trans-NIH cooperation and planning has become obvious.

Some of the larger institutes have had the flexibility to provide core support for expensive new technologies (such as transgenic mice, microarray facilities, biostatistical support, and clinical research infrastructure), but the smaller institutes have definitely been disadvantaged.

All of these factors, plus the obvious observation that working collaboratively across NIH will increase creative input and reduce inefficiency and duplication, led to the trans-NIH initiatives concept.

How?

The original three proposals listed above were specifically chosen because they could provide infrastructure for the work of many different scientists throughout NIH and had the potential to revolutionize how we do science.

Neal Young, chief of the Hematology Branch, NHLBI, will lead the Immunology initiative. He proposed a Center for Human Immunology, which will occupy physically proximate space for common laboratories and core facilities to facilitate complete phenotyping of the human immune system and abnormalities in autoimmune diseases and in inflammatory processes that underlie or affect common diseases like cancer, asthma, and heart disease. Most of the studies will involve the NIH clinical center and human subjects.

Richard Leapman, scientific director of the new National Institute for Bioimaging and Bioengineering, will lead an effort to develop new imaging technologies for studying molecules and cells, beginning with a center in which senior fellows from physics, computational biology, and engineering can interact with NIH biologists to address the need for higher resolution, real-time molecular-imaging technologies.

The Systems Biology proposal is the least advanced of the three. The proposal involves recruitment into leadership positions as well as the creation of an incubator space for interaction of current and newly recruited NIH scientists. Most of us appreciate that systems approaches will begin to replace the more reductionist approaches we have taken in our laboratory and clinical studies, but how this necessary evolution will occur is still unclear.

When?

Both the scientific community and the NIH director expressed frustration at the October 22 retreat about why it has taken so long to get these initiatives off the ground—although we all realize that achieving true consensus among the working groups takes time.

With nearly every square foot of space on the NIH campus currently occupied with active laboratory and clinical science, finding appropriate space to initiate these programs has not been a trivial undertaking. But we have recently figured out how to reorganize existing space to some advantage. With budgets tight everywhere, funding was hard to find, especially before goals and programmatic needs were clearly defined. To jumpstart these three projects, the NIH director has provided $4 million from his Discretionary Fund for equipment needed in the instrument cores. Individual institutes and centers are expected to support the modest needs of these programs, at least during a period of evaluation, but most support will be in kind as the centers that are established reach out to the affiliate members in various institutes and centers. The NIH director will meet with the scientific directors to discuss fostering implementation of these centers. We fully expect the Center for Human Immunology and the Molecular Imaging Center to be functioning in calendar year 2008, and the Systems Biology initiative will soon begin to identify leadership.

Getting NIH scientists and scientific leadership together to discuss these ideas has been a new and rewarding experience in itself. We anticipate a continuing dialog between scientific leadership and scientists on how to ensure that the NIH IRP remains a vital, creative contributor to biomedical research. Committed to this objective, the NIH director has proposed a Grand Challenge program that provides one-time funding to launch innovative science that might otherwise not be initiated. Novel scientific ideas will be sought. As the existing trans-NIH initiatives transition to more stable footing, the transitioning of leadership is also underway. ■

From the Consortium

Biomarkers: A Call for Ideas

The Biomarkers Consortium, a public-private biomedical research partnership managed by the Foundation for the NIH (FNIH), provides an opportunity for intramural research staff to engage in joint projects with industry, FDA, and a variety of other partners to discover, develop, and qualify biomarkers across the spectrum of biomedical research.

The Consortium is soliciting project concepts from researchers and the general public worldwide. The Consortium's goal is to accelerate the delivery of successful new technologies, medicines, and therapies.

Tried-and-true biomarkers—such as blood pressure and cholesterol levels to assess heart disease risk, and CD4 T-cell counts and viral-load levels to assess HIV/AIDS—are windows into disease progression and regression. Biomarkers can help identify those at risk for developing disease, help stratify patients according to prognosis, demonstrate early signs of organ involvement or damage, and help assess response to treatment.

Such biomarkers can therefore help identify new drug targets or indicate which drugs under development can move to the next stage. They can be used to determine which patients will best benefit from a drug.

The Human Genome Project and proteomics research have unsealed a trove of potential biomarkers. Medical imaging, too, can identify a biomarker to trace visibly how cancer responds to treatment by tracking radioactively tagged glucose uptake by tumors.

The Biomarkers Consortium has raised more than $6 million in private funds for two NCI-led projects on lung cancer and lymphoma involving fluorodeoxyglucose positron emission tomography. Another NIH-led project, submitted by NIMH intramural scientists, focuses on PET radiopharmaceutical development for neurodegenerative and possibly atherosclerosis and brain cancer therapeutics.

Projects on imaging and biochemical markers for Alzheimer's disease progression and on adiponectin, a candidate marker for diabetes, are also being considered.

The Consortium has four subject-focused steering committees at this time: neuroscience, metabolic disorders, cancer, and inflammation and immunity. Future steering committees can be established as project concepts are proposed.

NIHers must submit proposed project concepts through their scientific director, using the form found at the website:

<**http://www.biomarkersconsortium.org**>.

If the concept is recommended for further development, a more detailed project plan is created by the PI and a project team created for this effort. The plan details such things as experimental methods, data collection, and intellectual property. Upon project approval, the FNIH begins the fundraising process.

The NIH Program on Public-Private Partnership (PPP) can help NIH staff, grantees, and contractors with Biomarker Consortium policies and procedures; the PPP program also can assist and advise in developing and submitting project concepts and plans and generally help keep the process on track. Barbara Mittleman is the PPP program director, and Shawnmarie Mayrand-Chung is the NIH program director for the Biomarker Consortium.

All the players have something to gain from this new research opportunity, says Consortium Director C. Anthony Altar of the FNIH. Researchers secure funding for important basic research; private companies can share the cost of research they otherwise would have had to fund independently; the FDA gets involved early in the drug- or technology-development process; measurements are standardized; and everyone is on the same page.

Participants in the Consortium are its founding members—FNIH, NIH, FDA, and the Pharmaceutical Research and Manufacturers of America—and more than 40 companies, trade associations, and advocacy groups. David Lee, deputy director for partnership development with FNIH, develops and manages these partnerships and is responsible for fundraising activities for both the projects and the contributing-membership program.

This precompetitive public-private partnership is designed to produce public resources rather then intellectual property. Having multiple discovery and regulatory parties involved in every project produces a synergy that quickens the pace of development and the translation of excellent science to benefits in public health. ■

From FELCOM

WALS: A Call for Speakers

The NIH Director's Wednesday Afternoon Lecture Series (WALS) features top biomedical researchers from around the globe. The Director's series has been bringing in outstanding seminar speakers since its inception in 1952, and in the current format since 1994. NIH Special Interest Groups are all soliciitng names of speakers for next year's WALS.

Many fellows, like me, have attended WALS lectures—held in Masur auditorium on Wednesdays at 3:00 p.m.—but many fellows may not realize that we have the opportunity to nominate speakers in this series.

If the prospect of attending a presentation in your field of study is not enough to persuade you to nominate a speaker, you, the nominator, may also participate in the hosting of the lecturer if he or she is chosen. As you can imagine, serving as a WALS host, and perhaps having breakfast with him or her, is an excellent way to become acquainted with an admired scientist—perhaps a future colleague.

FELCOM is collecting nominations from the NIH fellows community. So think about a speaker you have seen, perhaps at a recent conference, who gave an excellent seminar and nominate that person. FELCOM nominations for the 2008–2009 WALS series are now underway and will continue until **November 21**.

E-mail your nominations to Kristi Muldoon Jacobs

<**jacobskr@mail.nih.gov**>

with "WALS nomination" in the subject line. Include the nominee's name, institutional affiliation, professional title, and contact information—and a brief paragraph on his or her qualifications. FELCOM especially encourages the nomination of women and minority speakers. Priority will be given to nominees who have not recently presented a WALS lecture, so be sure to check

<**http://www1.od.nih.gov/WALS/**>

for the list of current and past speakers.

Your nominee might also be suited for one of the special NIH lectures listed at

<**http://www1.od.nih.gov/oir/sourcebook/ir-communictns/lecture-info.htm**>.

Finally, don't forget the Cultural Lecture slot—do you have a favorite writer or some other fascinating figure whose wit and wisdom you would love to bring to NIH? Think about it—and nominate.

—Lori Bibb

Regenerative Medicine

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de-differentiation; "stemness" genes, on the other hand, were found to support MSC self-renewal and proliferation—"differentiation readiness"—in the undifferentiated state.

A proper scaffold to support these stem cells, Tuan determined, would mimic the native extracellular matrix and be able to fit into a three-dimensional groove. Nanofibers of collagen and other macromolecules are the hallmark of the extracellular matrix of skeletal tissues, Tuan said, explaining how his group used electrospinning to prepare similar nanoscale fibers from a biodegradable polyester and then produced the desired tissue-engineering scaffold.

Their next step was to optimize nutrient supply to the developing cartilage construct to grow in size; the team succeeded in enhancing growth of the engineered cartilage in vitro from a diameter of 1.5 cm a few years ago to around 4 cm today.

Finally, the researchers are using minipig and rat models to test the ability of the engineered cartilage construct to repair cartilage defects and integrate into native cartilage. Initial observations have revealed promising signs of tissue repair in six months and four weeks in these respective models, Tuan said.

Robey: Skeletal Cells and Scaffolds

When bone-marrow skeletal stem cells are plated, they are able to form cartilage in vitro, and when they are transplanted, they form bone, marrow, fat, and the stroma that supports blood formation. This multipotentiality suggests broad therapeutic application for dental and craniofacial reconstruction, observed Pamela Robey, chief of the Craniofacial and Skeletal Diseases Branch, NIDCR.

Robey and her team have been characterizing these stem cells and evaluating different scaffold choices for tissue regeneration. The identification of markers for skeletal stem cells will aid the quality of purification of these cells from bone marrow. It will, however, also be necessary to generate large numbers of these cells via ex vivo expansion. The goal, explained Robey, is to trick the stem cells into dividing symmetrically to keep them as multipotent as possible.

In addition to researching ways to isolate and expand the skeletal stem cells, Robey's group has been testing potential scaffold materials. Currently, there are only a few FDA-approved, commercially available scaffolds. The nature of the scaffold is important, said Robey, observing that only one currently available scaffold (synthetic hydroxyapatite–tricalcium phosphate ceramic particles) supports both bone formation and the stem cell, so that marrow can be formed. The size and shape of the scaffold, which organizes and controls the structure of the bone formation, also matter, she added.

Among research questions requiring attention, Robey said, are determining the number of cells necessary for successful transplantation, identifying an appropriate transplantation method, stimulating incorporation of the transplant into the preexisting tissue, and developing a root structure in order to reconstruct a viable tooth from dental pulp cells.

Kuo: Mechanoactive Tenogenesis

Catherine K. Kuo, a postdoctoral fellow in the NIAMS Cartilage Biology and Orthopaedics Branch, brings her engineering perspective to investigating the potential of MSCs in tissue engineering and regenerative medicine. Kuo is particularly interested in the regeneration of tendons, which transmit forces from the muscle to the bone, and ligaments, which join bone to bone and thus stabilize joint structures.

Nearly half of all skeletal injuries involve tendons and ligaments. Poor healing ability of these tissues and imperfect repair strategies provide an opportunity for regenerative therapies.

There are no known growth factors to induce differentiation of MSCs into tendon/ligament fibroblasts (tenogenesis). These cells are distinct from other musculoskeletal lineages in that mechanical stimulation is the only known factor able to induce tenogenic differentiation of MSCs. Kuo created tendonlike constructs by seeding three-dimensional collagen type I gel scaffolds with MSCs; she cultured the constructs under uniaxial static or dynamic tension.

Kuo monitored tenogenesis via expression of scleraxis, a transcription factor that uniquely marks tendon progenitors during development. To determine whether dynamic mechanical stimulation (cyclic tensile loading) enhanced tenogenesis, Kuo developed engineering devices and tools to place the constructs under either static or dynamic tension.

She observed a similar increase in collagen mRNA in both static and dynamic conditions, but noticed more matrix deposition and persistent scleraxis expression over time with cyclic loading. In addition, expression of matrix metalloproteinases such as collagenase and gelatinase were differentially regulated by cyclic loading, implying that the increased matrix deposition and resulting tenogenic differentiation are regulated by changes in the expression of these genes.

Dunbar: Thwarting Mutagenesis

PI Cynthia Dunbar and her colleagues in the Hematology Branch, NHLBI, have been using gene-transfer techniques in their research on hematopoiesis.

Addressing the safety issues and complications associated with the use of viral transfer vectors, the group has investigated the risks of insertional mutagenesis after retrovirus and lentivirus gene transfer to hematopoietic stem cells (HSCs).

Viral gene-transfer vectors that are used to target HSCs must integrate into the host genome to be effective, but depending on the site of insertion, they can activate adjacent genes, including protooncogenes. These issues did not emerge in studies involving murine models and have come to light during clinical gene-therapy studies, Dunbar said.

In one study, patients with X-linked severe combined immunodeficiency (SCID) had reconstitution of their T-cell immunity and clinical improvement after HSC gene therapy. This optimistic outcome was interrupted three years later by the development of T-cell leukemias in four patients, due to activation of a growth-promoting gene by the inserted gene-therapy

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Engineering and Physical Sciences SIG Starting Up

The Working Group on Women in Biomedical Careers subcommittee on Integration of Women into Bioengineering Fields has created the Engineering and Physical Sciences Special Interest Group. The goals of this SIG will be to promote interaction between investigators and laboratories whose research interests involve integrating engineering or physical science with biology, and to educate the NIH community about these approaches.

Areas of research interests include tissue engineering and regenerative medicine, biomaterials, nanotechnology, physical regulation in biology, engineering-based enabling technologies, and quantitative approaches based on physical sciences.

This SIG will organize seminars by engineers and physical scientists from inside and outside NIH and identify mentors available to engineering and physical science students and fellows at NIH. Particular efforts will be made to identify outstanding women engineers and physical scientists. Contact Catherine Kuo (<**kuoc@mail.nih.gov**>, 301-451-4519) with questions or to join. ■

Getting to the Bottom of the Beta Cell

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conversely, a knocked-in point mutation yields "huge islets and increased beta cell mass."

The knock-in cohort demonstrates increased regeneration potential via differentiation of stem cell–like progenitors in the pancreatic duct, increased beta cell proliferation, and accompanying functionality reflected in superior glucose tolerance, insulin secretion in response to glucose, and protection against streptozotocin-induced diabetes.

The team intends to explore the regenerative potential of other cell-cycle proteins as well, Rane said.

Rough Measures

Accurate measurement of pancreatic beta cell mass, a key indicator of beta cell function that could elucidate diabetes progression and assess response to treatment, is in a "sorry state," said Dave Harlan, chief of the Diabetes Branch, NIDDK. He apologized for a talk that would highlight questions eluding answers and somewhat disappointing research results.

PET imaging using dihydrotetrabenzene (DTBZ), the radioactive ligand for the vesicular monoamine transporter-2 found in pancreatic beta cells, has been getting a lot of attention as a potential noninvasive method to measure beta cell mass, Harlan said.

He noted that PET images obtained from pancreas-transplant recipients reveal a bright signal from the transplanted organ, while the individual's native pancreas sends out a low signal; even so, results from individuals with long-standing type 1 diabetes have been inconsistent—as have results in monkey studies. For instance, autopsy findings show that the ligand binding is not specific in monkey pancreatic beta cells.

"We conclude that DTBZ PET scan signals do not accurately measure pancreatic beta mass," Harlan said, adding that though beta cell regeneration may occur in vivo—at least in mice—improved function has not yet been shown in humans.

Fran Pollner

Beta Cell Mates: *(left to right) symposium chair Sushil Rane, NIDDK; Jurgen Wess, NIDDK; Marvin Gershengorn, NIDDK; Josephine Egan, NIA; and David Harlan, NIDDK*

Pathways to Treatment

Introducing her research into islet biology to discern new approaches to treat type 2 diabetes, Josephine Egan observed that the workings of the pancreatic islet cells are only inferred from changes in hormone levels in response to stimulants.

The disordered responses that trumpet diabetes onset provide clues to treatment strategies, said Egan, senior investigator and chief of the Diabetes Section of the Laboratory of Clinical Investigation, NIA.

These derangements include decreased insulin secretion; the loss of the pulsatility of insulin release, a reflection of beta cell dysfunction; blunted response of the gut protein glucose-dependent insulinotropic polypeptide (GIP); increased glucagon secretion; and increased pancreatic polypeptide secretion.

Among agents Egan has been exploring is GLP-1, another gut hormone, which, unlike GIP, releases insulin and normalizes blood sugar in patients with diabetes and appears to correct imbalances more effectively than exogenous insulin.

Jürgen Wess and his colleagues have generated a series of mutant mouse models to better understand the role of M_3 muscarinic acetylcholine receptors in beta cell function. The selective overexpression of M_3 receptors in beta cells leads to enhanced insulin release and improved glucose tolerance, suggesting that "therapies aimed at enhancing this pathway might be useful in the treatment of type 2 diabetes," said Wess, chief of the Molecular Signaling Section, Laboratory of Bioorganic Chemistry, NIDDK.

The team has also created a series of mutant M_3 muscarinic receptors that are unable to bind acetylcholine (the endogenous neurotransmitter) but can activate different classes of G proteins when treated with the pharmacologically inert compound clozapine-*N*-oxide (CNO). Wess discussed findings in transgenic mice that selectively express these receptors in their pancreatic beta cells.

CNO treatment of different transgenic mouse lines resulted in the selective activation of either G_q - or G_s -type G proteins in pancreatic beta cells. In both cass, acute CNO administration led to a significant increase in insuin release and improved glucose tolerance.

Key questions that remain to be addressed, Wess, said, are: What are the chronic effects of activating these different signaling pathways—or a mixture of both—on insulin synthesis and release and beta cell mass and survival, and how can these findings guide the development of novel therapeutic agents? ■

Regenerative Medicine

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vector.

The need for an experimental model more predictive and more closely related to humans prompted Dunbar's laboratory to turn to the rhesus macaque as a model organism for studies of gene therapy.

Dunbar's group took advantage of the rhesus macaque model to study the patterns of virus (either murine leukemia virus [MLV] or simian immunodeficiency virus [SIV]) integration sites. The integration sites were determined in circulating granulocytes and lymphocytes of the monkeys up to seven years post-transplantation.

The researchers observed that integration was nonrandom: MLV integrated around transcriptional start sites; SIV integration sites were evenly spaced along the length of a gene.

The researchers also noted that animals receiving MLV-transduced HSCs had marked overrepresentation of cells containing integration events in the Mds1/Evi1 gene complex, suggesting significant in vivo selection for these clones, a very worrisome pattern, Dunbar noted. One macaque developed leukemia due to insertional activation of the BclaA1 locus.

Dunbar discussed approaches to decrease the risk of insertional mutagenesis, including alternative vector systems and modification of current vectors to decrease the likelihood of activation of adjacent genes. ■

Chemistry: From the Beaker to the Bedside

by Fran Pollner

They stopped short of saying "Chemistry rocks!" but the Research Festival panelists presenting their work "From the Beaker to the Bedside" did not blunt their reverence for chemistry in the quest for diagnostic probes, therapeutic agents, and innovative paths to elucidating the nature of pathogenic processes.

Designer Drugs

Nigel Greig and Amy Hauck Newman elaborated on some of their efforts to synthesize drugs aimed at treating Alzheimer's disease and addiction, respectively. Their ultimate objective is tech transfer.

Newman's laboratory has synthesized dopamine D_3 receptor ligands and demonstrated their selective localization and binding in the nucleus accumbens in rat brains, as well as their ability to thwart the compulsion to cocaine relapse in squirrel monkeys.

The D_3 receptor antagonists are not a "golden bullet" against all aspects of addiction, observed Newman, senior investigator and chief of the Medicinal Chemistry Section, Medications Discovery Research Branch, NIDA. "They will not block self-administration of cocaine [in squirrel monkeys], but they are very effective in the relapse model."

The team currently has a "lead candidate" among the compounds under study and is pursuing ways to optimize its bioavailability and functionality.

Greig, chief of the Drug Design and Development Section and senior investigator, Laboratory of Neurosciences, NIA, traced the development of synthetic analogues of physostigmine—from the *Physostigma venenosum* plant—through their testing first in animals and then in clinical trials in patients with Alzheimer's disease.

The novel agents—phenserine, a selective acetylcholinesterase inhibitor, and its enantiomer, posiphen—were designed to inhibit the synthesis of the amyloid-β precursor protein (APP).

Both have been found to lower APP and amyloid-β in mouse brains, and both have moved into clinical trials. Results thus far with phenserine are promising, Greig said, showing a dose-related lowering of amyloid-β plaques compared with placebo.

Posiphen, now in phase 1 clinical trial, may prove more beneficial than phenserine, he added. Cholinergically inert and therefore not associated with the nausea and vomiting that may accompany phenserine, the drug is better tolerated in humans and can be administered in higher doses (120 mg compared with 15 mg), he said. He speculated on the possible value of combining the two.

Fran Pollner

***Chemists**: (left to right) Nigel Greig, NIA; Daniel Appella, NIDDK; Amy Hauck Newman, NIDA; Craig Thomas, NIH Chemical Genomics Center; and panel chair Matthew Hall, NCI*

Molecules and Probes

Providing the molecular tools to identify, interrupt, or otherwise manipulate pathogenic and genetic mishaps is the daily work of Craig Thomas and Daniel Appella, whose efforts serve the needs of investigators across the NIH institutes.

One such need was reflected in the question, "Could a misfolded protein refold with the aid of a chemical chaperone?" Thomas recalled.

The answer was critical to devising a new approach to Gaucher disease, a lysosomal storage disorder characterized by 200 point mutations in the glucocerebrosidase (GC) gene. NHGRI's Ellen Sidransky brought the question to the NIH Chemical Genomics Center (NCGC).

Quantitative high-throughput screening facilitated the identification of three novel structures with potent selective inhibition of GC, said Thomas, chemistry group leader at the NCGC.

"But optimizing was a challenge" that took four months and five rounds of synthesizing nearly 300 compounds before the team delivered four novel chemotypes across the potency range of GC inhibition with the potential of binding to the enzyme to restore shape and function, he related.

Thomas listed several other projects undertaken with both intramural and extramural labs that involved in silico docking, virtual screening, advancing cellular pathway assays, and uncovering unforeseen toxicity in a potential therapeutic agent.

A major focus of Appella's group in the Laboratory of Bioorganic Chemistry, NIDDK, is manipulating peptide nucleic acids (PNAs) to improve their ability to bind to DNA. What'Chemically modifying these backbone units to improve their DNA binding affinity and sequence specificity in a predictable manner is aimed "not so much at making a drug but at developing a tool—for instance, to identify a pathogen" and improve diagnostic assays, Appella said.

A project to stabilize PNA-DNA duplexes—and, more recently, quadruplexes—by incorporating cyclopentane into different PNA sequences "has been a synthetic challenge—now in its third iteration," Appella recounted, "but we have demonstrated, after a lot of work, that binding *is* improved."

The group has synthesized PNA capture and detection probes and is working on sidechain incorporation to develop improved light-up probes. The team developed a "PNA sandwich-hybridization" assay that can be used to distinguish protective antigen from anthrax. The lab's latest project involves tethering three PNA sequences together that simultaneously clamp onto DNA to form a highly stable complex. ■

Forestalling Blindness: Two Decades of Progress

by Fran Pollner

Three spotlights have converged on the eye over the past 20 years, illuminating the genetic underpinnings of eye disease, the immunological environment of the eye, and the environmental modifiers of inborn propensities to eye disorders.

Following these pathways leads to formidable strategies to combat retinal neurodegeneration and choroidal neovascularization, the major instruments of blindness, panelists agreed at a Research Festival symposium focused on recent advances in eye research. The panelists, all NEI investigators, were themselves instruments of many of these advances.

Fran Pollner

The eyes have it: *(NEI colleagues, left to right) Paul Sieving, symposium co-chair Michael Redmond, Emily Chew, co-chair Patricia Becerra, Juan Amaral, and Robert Nussenblatt*

RPE65

In the early 1990s, the lab of Michael Redmond identified and cloned the *RPE65* gene. Since that time, researchers have found more than 60 mutations in its 14 exons, all causing eye disease ranging from early-onset severe disease to milder late-onset phenotypes. Half were missense mutations. The team looked for mutations in patients with Leber congenital amaurosis (LCA) and childhood-onset severe retinal dystrophy—two of the earliest blinding conditions—and found them, recalled Redmond, chief of the Molecular Mechanisms Section in the Laboratory of Retinal Cell and Molecular Biology (LRCMB).

The lab developed an expression system and proved that RPE65 is required for the production of 11-*cis* retinal, essential for vision. They generated *Rpe65*-knockout mice that could not produce 11-*cis* retinal and lacked rhodopsin.

The lab has also been involved in gene therapy studies involving dog LCA models, in which functional recovery of the treated eye has been demonstrated on electroretinogram. Four early-phase clinical trials are currently underway in the United States and abroad, Redmond noted.

CNTF

As of a year ago, said NEI Director Paul Sieving, 117 genes contributing to the death of photoreceptor cells had been cloned. Animal work with neurotrophic protective factors has shown that such lost vision is salvageable.

A neurotrophic protective factor with demonstrated photoreceptor-rescue ability in 13 different rat and mouse mutants with retinal degeneration was put to the test in 10 retinitis pigmentosa patients in a Phase I NEI study. Not only was safety shown, but potential efficacy was promising enough to warrant a move to Phase II, said Sieving, the study PI.

The agent—ciliary neurotrophic factor (CNTF)—was encapsulated within a semipermeable intravitreal implant. The implant was removed after six months, but retinal cells continued to secrete CNTF, possibly as a result of persisting vitreous nourishment, Sieving remarked.

The CNTF-implanted eye showed a trend to higher acuity, with several of the patients demonstrating a three- to four-line improvement in reading the eye chart in the treated eye. "The patients' enthusiasm was gratifying; they wanted implants in their other eye as well," Sieving said.

PEDF

Pigment epithelium–derived factor (PEDF) is another agent that has passed Phase I human safety tests. "It is neurotrophic, antitumorigenic, antiangiogenic, and anti-inflammatory—it is a very versatile protein," said Juan Amaral, a staff scientist in Patricia Becerra's Section of Protein Structure and Function, LRCMB. PEDF was also cloned in the early 1990s in the LRCMB and has been studied extensively in this lab

Using rat models, Amaral and Becerra have been exploring the potential for therapeutic delivery of this protein. "We propose subconjunctival delivery, either by injection or, simpler and safer, a sustained delivery device," he said, noting that the team has documented rapid diffusion to relevant areas, with intense scleral, choroidal, and retinal signals.

Subconjunctival injections of PEDF proteins can inhibit progression and induce regression of laser-induced neovessels in the choroid of rats, Amaral said.

Immune Mediation

The mechanisms underlying atherosclerosis also underlie choroidal neovascularization and age-related macular degeneration (AMD)—they are all inflammatory, immune-mediated conditions, suggested Robert Nussenblatt, head of the Laboratory of Immunology.

The therapeutic implications of this perspective are now being investigated in an NEI clinical trial of the anti-inflammatory agents inflixamab, sirolimus, and dacluzimab to treat choroidal subretinal neovascularization in patients with AMD, said Nussenblatt, the trial PI. He noted that restoration of the downregulatory immune environment of the eye is the hoped-for therapeutic mechanism.

"But while this is a great idea," he added, "we don't know that these medications will achieve that goal. Rather, we are treating the result of the immune dysregulation."

AREDS and AREDS2

Whatever the genetic and immune components of AMD, the ability of a regimen of antioxidants, copper, and zinc to stem progression in patients with moderate AMD speaks loudly to the role of nutrition in warding off an otherwise relentless disease course, observed Emily Chew, deputy director of the Division of Epidemiology and Clinical Research and chief of the Clinical Trials Branch.

The trial regimen in the NEI-sponsored Age-Related Eye Disease Study (AREDS), which involved nearly 5,000 patients, yielded a 25 percent reduction in progression to advanced AMD. The regimen has become a standard nutritional supplement for patients with AMD and, if taken by all at-risk individuals, could be expected to prevent progression in about 300,000.

AREDS2 is currently recruiting 4,000 people into a five-year study of the effects on AMD of ω-3 long-chain polyunsaturated fatty acids and the antioxidant carotenoids lutein and zeaxanthin. Chew noted that lutein is now viewed as a substitute for β-carotene, shown in two NCI-supported lung cancer trials to increase lung cancer and mortality risk among smokers. ■

Chromosomes in Modern Biology and Medicine: A Looking Glass into Common Diseases

by Julie Wallace

Julie Wallace

Understanding chromosomes: *(left to right) Shiv Grewal, NCI; Thomas Ried, NCI; session chair John Niederhuber, NCI director; Gary Felsenfeld, NIDDK; and Francis Collins, NHGRI*

Francis Collins—The Genetics of Common Disease

Efforts to uncover the genetics of common disease are "rocketing forward" thanks to a revolution in genomic tools and technologies and the emergence of new, multidisciplinary collaborations, said NHGRI Director Francis Collins.

A huge leap forward was the recent creation of a map of common human genetic variation, called the HapMap. The HapMap confirmed that variations in DNA sequence—single nucleotide polymorphisms (SNPs)—travel in neighborhoods, called haplotypes. More important, the map paved the way for genome-wide association studies (GWAS), a new approach that involves rapidly scanning SNPs across the genomes of many affected and nonaffected people to find genetic variations associated with a particular disease.

An impressive array of NIH-led initiatives, including the Genetic Association Information Network and the Genes, Environment and Health Initiative, have been launched to tap into this powerful resource. Data generated by these and other NIH-supported GWAS are already being deposited in the Database of Genotype & Phenotype (dbGAP), a powerful database developed by NCBI for use by the scientific community, Collins said. To access dbGAP, go to **<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap>**.

Collins has applied the new tools to his own research endeavors. Working in close collaboration with two other international teams of epidemiologists, geneticists, and bioinformaticians, Collins' group in April 2007 published results of a genome-wide survey of genetic risk factors involved in type 2 diabetes—a disease long considered "the geneticist's nightmare."

The studies identified at least four new variants associated with increased risk of diabetes. The variants lie in an intergenic region of chromosome 11 and near *IGF2BP2*, *CDKAL1*, and *CDKN2A/B*, a cell-cycle gene also implicated in myocardial infarction. The research also confirmed several variants previously associated with type 2 diabetes.

Thomas Ried—Cancer: A Disease of the Chromosome

It was just in 1956 that the correct number of human chromosomes was established. Since that time, the role of chromosomal abnormalities in blood cancers has been definitively recognized with the identification of the Philadelphia chromosome in chronic myeloid leukemia and other translocations in lymphomas, Thomas Ried observed. He then addressed the complexities of chromosomes and solid tumors.

Chromosomes in epithelial cancers are defined by catastrophic mitoses and centrosome amplifications that lead to cells with abnormal chromosome numbers, complex karyotypes, and ongoing chromosome instability, noted Ried, head of the Cancer Genomics Section, Genetics Branch, NCI.

Studies to determine the cytogenetic makeup of solid tumors, however, have been slower in coming, requiring advances in visualization techniques, such as comparative genomic hybridization and spectral karyotyping.

These are the tools that Ried's group has used to study cervical cancer, identifying chromosomes that are altered in invasive tumors and following progression from early- to late-stage cancer.

Studies of invasive cervical carcinomas have revealed that the long arm of chromosome 3 is frequently amplified, suggesting a "fundamental genomic insult" and a driving event for disease progression, Ried said. A Pap test can identify morphologically altered cells indicative of cervical cancer, but, he noted, it doesn't predict invasive propensity. Recognition of the role that amplification of chromosome 3 plays in progression from low- to high-grade lesions may lead to using cytogenetics in the staging and prognosis of cervical cancer.

Aneuploidies, in general, are a defining feature of carcinomas, Ried said, and there is a continuous selection for and maintenance of aneuplodies that are not fatal to cells, but rather confer "stability on a different plateau."

These aneuplodies are tumor-specific and acquired early in tumorigenesis, suggesting they may be useful in diagnosis. Ried's laboratory is currently investigating mouse models of hematological malignancies such as Burkitt's lymphoma and of mammary gland adenocarcinomas.

In addition to studying mouse models, Ried is particularly interested in looking at the entire transcriptional landscape of cells with aneuploidies, addressing what happens to genes on an extra chromosome. By generating artificial trisomies in human cell lines using microcell-mediated chromosome transfer, Ried's group found that, in general, there is an increase in the average message levels of genes on the affected chromosome. The researchers also noted that the expression of genes on other chromosomes was affected as well, indicating that aneuploidies not only target a few specific genes for increases in transcription, but also lead to a "massive and complex deregulation of [the] cancer transcriptome."

Gary Felsenfeld—Chromatin Boundaries

His lab's work on DNA insulators, observed Gary Felsenfeld, has shed light on the nature of chromosomes and the role of chromatin boundaries in gene expression.

To maintain specific patterns of gene expression, organisms have evolved a variety of ways to "establish boundaries between regions with different properties," observed Felsenfeld, chief of the Physical Chemistry Section, Laboratory of Molecu-

lar Biology, NIDDK. As a result of thoroughly characterizing the chicken β-globin locus, Felsenfeld's lab identified a specific DNA sequence that is capable of blocking enhancer-promoter interactions. DNA insulators, as these sequences are termed, are found in different places in the genome and help form boundaries by blocking inappropriate interactions within the nucleus.

Felsenfeld's group identified a single protein in vertebrates, CTCF, for its ability to bind the chicken β-globin insulator and confer insulation. Recent studies of CTCF have begun to elucidate its genome-wide locations as well as the role of CTCF sites in mice that mediate the formation of DNA loops and higher-order structures.

In addition to their enhancer-blocking role, DNA insulators can act as barriers to block the spread of condensed chromatin. By systematically analyzing the presence or absence of various modified histones throughout the chicken β-globin locus, Felsenfeld's laboratory discovered that nucleosomes at the insulator are highly marked with modifications frequently associated with open chromatin.

Further studies established that a heterodimer—USF1 and USF2—binds the insulator and recruits the histone H4R3-specific methyltransferase PRMT1 and, subsequently, a barrage of "positive" histone modifications to maintain a local environment of open chromatin.

Nucleosome stability can also play a role in regulation of chromatin-coupled mechanisms, Felsenfeld's group has recently discovered. In addition to regulation of histones by modification, histone variants such as H3.3 and H2A.Z are incorporated into nucleosomes and can mark specific sites in the genome.

In particular, H3.3 is concentrated at regulatory sites and is displaced through nucleosome-disrupting activities such as transcription.

By studying the salt-dissociation properties of nucleosomes containing different combinations of these variants, Felsenfeld's group has been able to determine a hierarchy of nucleosome stability. Nucleosomes with both H3.3 and H2A.Z are highly unstable, Felsenfeld said. These nucleosomes are predominantly found on the promoters of transcriptionally active genes and over the coding regions of genes transcribed at high levels, suggesting that these histone variants can serve as an epigenetic signal in the genome.

Shiv Grewal—Heterochromatin: A Versatile Platform of the Genome

Continuing Felsenfeld's theme of context within chromosomes, Shiv Grewal

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Genome-wide SNP Assays and the Genetics of Normal and Abnormal Variation

by Julie Wallace

Stephen Chanock, head of the Genomic Variation Section, Pediatric Oncology Branch, NCI, is using genome-wide SNP assays to study solid tumors.

He and his colleagues are aiming to identify genetic regions associated with risk for specific cancers, such as breast and prostate cancer. The results of their initial screening of more than 540,000 SNPs prompted further exploration of a specific chromosomal region (8q24) commonly amplified in prostate tumors. Moreover, regions in a 1.5-Mb section of this locus were also implicated in breast and colon cancers—leading to the tantalizing suggestion, Chanock said, that this may be one of several master-regulator regions important in different cancers.

Chanock's group is now extending its focus to pancreatic, lung, and bladder cancers.

Julie Wallace

Traveling the genome: *(left to right). Stephen Chanock, NCI; Francis McMahon, NIMH; session chair Andrew Singleton, NIA; and Anjene Addington, NIMH*

Bipolar Disorder

In the realm of psychiatric diseases, where patients are diagnosed primarily by behavior, "reliability in the diagnosis does not equal validity [in molecular genetics]," and the underlying biology for each patient may not necessarily relate to their psychiatric label, observed Francis McMahon, chief of the Genetic Basis Unit, Mood and Anxiety Disorders Program, NIMH.

To limit this variable, McMahon's lab focuses on bipolar disorder, which appears to have a strong genetic component. Using samples from the NIMH Genetics Initiative Bipolar Disorder Consortium, McMahon's lab identified around 80 SNPs in or near genes.

In this "first look" at bipolar disorder, a significant SNP was found near the *DGKH* gene, a good candidate gene for mental illness that is related to lithium signaling. The study also suggested the involvement of several risk alleles in bipolar disorder, which may be the "result of many genes of small effect acting together," McMahon said.

Childhood-Onset Schizophrenia

Anjene Addington, also of NIMH, is charting the chromosomal aberrations and copy number variations in patients with childhood-onset schizophrenia (COS). She hopes to identify a homogenous group of patients less likely to have been exposed to environmental factors. Patients with early-onset disease are also thought to have a greater genetic risk and a more severe disease course. In a study of 96 children diagnosed with schizophrenia by age 12, Addington's group identified multiple chromosomal aberrations, including a novel balanced translocation between chromosomes 1 and 7, three cases of X chromosome abnormalities, and four cases of a 3-Mb deletion on chromosome 22q11, associated with velocardiofacial syndrome.

Copy number variants—DNA segments larger than 1 kb that are present in abnormal numbers—were also higher in children with COS than their unaffected siblings (an average of 32 compared to 24). These variations suggest "general genomic instability," Addington said, but noted that it is not easy to distinguish disease-related from garden-variety copy number variations.

Homozygosity and Complex Diseases

While genotyping large numbers of Caucasian males, Andrew Singleton, an investigator in the Molecular Genetics Unit, Laboratory of Neurogenetics, NIA, and his colleagues observed large tracts of allele homozygosity in 7 percent—a finding that suggested a single origin for maternal and paternal chromosomes. Of these 7 percent, 59 percent possessed at least one homozygous region greater than 5 Mb in size. Identifying homozygous populations, Singleton said, should enhance the power of genome-wide association studies of complex traits and diseases such as Parkinson's. ■

Uncovering Secrets in Sweat: Stress and Immune-related Biomarkers

by Evan Galloway

At the NIH Research Festival symposium on a new noninvasive method for detecting stress and immune-related biomarkers in sweat, immunochemist Terry Phillips described his work as "pulling nothing out of nothing." However, the "nothing" he wrings from a tiny sponge soaked in sweat can reveal the emotional state of the sweat's producer.

The motivation for this project, neuroendocrinologist Esther Sternberg recounted, was the request from some architects for a noninvasive way to measure the stress levels of employees in traditional and modern workplace environments.

The architects wanted to design better offices. Sternberg, director of the Integrative Neural Immune Program and chief of the Section on Neuroendocrine Immunology and Behavior, NIMH, envisioned a case study of the interdisciplinary development of an assay.

The first step was to develop a collection method. A small absorbent skin patch was already commercially available. The trick was in squeezing it dry.

Phillips, chief of the Nanoscale Immunodiagnostic Group at NIBIB, tried different centrifuge-and-squeeze approaches before settling on a solid-phase extractor that was gathering dust in his lab. After developing a protocol for cleaning the sweat sample of salt and recovering the integral proteins, he then created a microfluidic chamber for high-sensitivity recycling immunoaffinity chromatography.

Evan Galloway

Sweat equity?: *(left to right) Julian Thayer, Ohio State University; Esther Sternberg, NIMH; Andrea Marques, NIMH; and Terry Phillips, NIBIB*

The next step was to test this assay in a clinical setting, continued Andrea Marques, a postdoc in Sternberg's section. A comparison of the levels of neuroimmune biomarkers in the sweat and plasma of depressed women and healthy volunteers enrolled in an ongoing study of osteoporosis in women with depression led by Giovanni Cizza, NIDDK, revealed an abundance of proinflammatory cytokines and stress-related neuropeptides in both the sweat and plasma of women with depression, even though they were medicated and in remission at the time of the study.

Psychophysiologist Julian Thayer, of Ohio State University in Columbus (and formerly of NIA), is now verifying these results against another measure of stress—heart-rate variability. A decrease in heart-rate variability, especially while sleeping, indicates an increased likelihood of heart disease. However, it may also signify an increase in stress.

In a previous study, when Thayer and his colleagues told healthy study subjects that they would have a test in the morning, they displayed decreased heart rate variability at night compared with other healthy subjects who were simply told to return to the lab in the morning. Interestingly—especially to the architects—when applying this method to office workers, the researchers found that working in a more modern workspace was associated with an increase in heart-rate variability.

Although Thayer's group has not finished comparing the results of the two approaches in the office workers, they anticipate that the use of both sweat-patch biomarkers and heart-rate variability will be helpful in establishing the status of the subjects' stress responses from two different perspectives. ■

Chromosomes and Common Diseases

continued from page 9

focused on the organization within chromosomes of so-called "junk" DNA—which, he observed, not only constitutes a significant fraction of eukaryotic genomes, but also contributes to regulating cellular processes and genomic evolution and stability.

Senior investigator and head of the Chromosome Biology Section, Laboratory of Biochemistry and Molecular Biology, NCI, Grewal has paid special attention to heterochromatin.

Within the fission yeast *Schizosaccharomyces pombe*, there are distinct peaks of heterochromatin, marked by the presence of a specific histone H3 modification on lysine 9 (H3K9), which recruits heterochromatin protein 1. These peaks are found at centromeres, telomeres, regions of ribosomal DNA, and the mating-type locus.

Grewal's studies of these regions has revealed a role for small RNAs in the formation of heterochromatin: In a self-reinforcing loop mechanism, repeats in the DNA sequence in these regions are transcribed and resulting RNAs are processed into small RNAs that lead to the recruitment of H3K9 methyltransferases and the spreading of heterochromatic marks.

More recent work has demonstrated that heterochromatin can recruit diverse regulatory proteins involved in histone modification, transcriptional silencing, RNAi, chromosomal architecture, and chromatid cohesion. Specifically at the mating-type region, heterochromatin is necessary for the spreading of the RNA-induced initiation of transcriptional gene-silencing complex and also inhibits RNA polymerase II from localization to these sites.

Grewal's group recently identified a multiprotein effector complex, called SHREC, that coats all major heterochromatin domains and contains the histone deacetylases CLR3. This complex is recruited to heterochromatin in both RNAi-dependent and -independent manners.

Grewal's work has contributed to challenging the dogma that heterochromatin is a static, rigid structure. Rather, Grewal and his team are discovering heterochromatin changes throughout the cell cycle. Their ongoing studies on the dynamic regulation of heterochromatin have provided new insights into the mechanisms of heterochromatin assembly, Grewal said.

The establishment and maintenance of these important heterochromatic sites, he said, are conducted through a carefully orchestrated dance of histone modifications and protein binding. ■

Job Fair: Exploring the World of Work Beyond the Research Festival

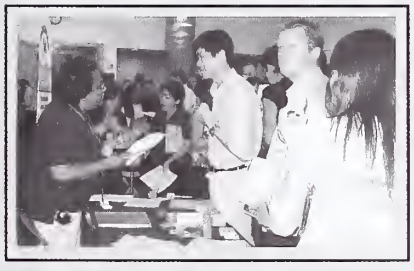
text and photos by Julie Wallace

More than 40 exhibitors—among them industry giants, local biotech companies, government agencies, professional societies, and foundations—were staffing tables and collecting résumés the third day at the Job Fair, the centerpiece of the third day of the NIH Research Festival.

Based on the comments of randomly selected fellows traversing those tables, one would conclude that preparation for the Job Fair had been on target and the array of potential employers and positions encouraging.

■ NIDDK postdoc Peter Choi was especially interested in a biotech and/or defense contractor research position and spoke to no fewer than seven different companies fitting those descriptions.

Sandeep Dayal, NIDDK, considers applying for an AAAS Science & Technology Policy Fellowship.



Activity around the FDA table

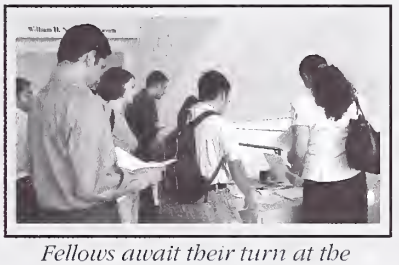
Peter Choi, NIDDK, inquires about programs at The Biotechnology Institute, Arlington, Va.

■ NCI postdoc Christine Horak arrived early, which gave her easier access to the much-visited Genentech table. Seeking a position involving oncological drug discovery and development in the pharmaceutical industry, Horak said the Genentech representatives were "extremely helpful, [taking] time with my CV and [telling] me exactly what their requirements are for hiring scientists."

■ NCI postdoc Jennifer Seiler checked out possible project-management positions and found several appealing prospective employers, including Discovery Logic Rockville, Md.), Lockheed Martin (headquartered in Bethesda, Md.), and the FDA.

In general, the fellows interviewed by the *Catalyst* said they were well-prepared for the Job Fair. They cited two seminars earlier that month sponsored by the Office of Intramural Training and Education (OITE) specifically designed to help fellows craft their C.V.s and résumés and to navigate the Job Fair in the most useful ways.

Choi, Horak, and others also remarked on the advisability of checking the online postings that are generally made available a few weeks before the Job Fair in order to know exactly what to ask representatives about specific job opportunities.



Fellows await their turn at the Genentech (headquartered in South San Francisco) table

Melinda McFarland, NIMH, speaks with a representative from SAIC, Frederick, Md.

Thomas Paul, an NCI fellow and Job Fair committee co-chair with Catherine Kuo of NIAMS, declared the Job Fair a success, with a "record number of exhibitors, who were "thrilled with the quality and quantity of applicants."

Sponsored by OITE and the Office of Research on Women's Health, the Job Fair is an annual event held in conjunction with the NIH Research Festival. It provides a "unique opportunity for NIH fellows to meet face-to-face with potential employers," Paul observed.

"In today's environment, where most job applications are done online, the personal interaction and one-on-one networking fellows encounter at the Job Fair can make them stand out amongst a sea of applications that a company receives. . . . [not] just a résumé, but a person behind a body of work and experience," Paul said. ■

Jennifer Seiler, NCI, discusses job opportunities with a rep from BioReliance (North American headquarters, Rockville, Md.)

Ejaz Shamim, NINDS, hands out information about the Fellows Committee

The Embedded Librarian: NIH Informationists Click into Chemistry and Tech Transfer

by Cindy Clark

The information needs of NIH researchers tend to be highly specialized. Consider the working world of chemists and the technology-transfer specialists who actively promote discoveries made at NIH. Like many specialists, they have a specific language that condenses communication.

When NIH chemists, investigators, or analysts need to find information, informationists who understand their language can be an enormous help.

Now in its sixth year of collaborating with NIH researchers, the NIH Library's Informationist Service employs 14 information specialists who work with research groups across the spectrum of NIH institutes, divisions, and programs.*

"In the past," NIH Library Director Suzanne Grefsheim said, "[our] informationist services focused primarily on clinical researchers, but we always thought basic scientists such as chemists would find the service beneficial. Before reaching out to this community, however, it was a matter of hiring librarians with the subject expertise needed to provide the relevant customized service."

What scientists such as chemists might appreciate from an informationist, she said, "might best be characterized as knowledge management—database development, such as linking structures to relevant citations; data and document curation; and even text and data mining."

An informationist might well introduce the research team to new software tools—such as the electronic lab book—and other new information resources, she said.

An informationist might also search databases that require special expertise, such as patent databases. Their specialization enables informationists not only to "find the answers but also to anticipate the questions," Grefsheim observed.

As for the future, Grefsheim anticipates a growing demand from both clinical and basic researchers for the services of bio-informationists, especially to organize and analyze the massive amounts of genetic information flowing from the Human Genome Project and similar initiatives.

* For background information on the NIH Library Informationist Service, see "The 'Embedded Librarian': NIH Informationists Become Team Players," *The NIH Catalyst*, Nov.–Dec. 2005, available online at **<http://www.nih.gov/catalyst/2005/05.11.01/page8.html>**.

Good Chemistry

Barbara Brandys joined the NIH Library in 1997 and became an informationist in 2004. She provides information services to various NIH groups, including the Drug Information Service at the Clinical Center.

After receiving her undergraduate degree in chemistry, she worked as a science teacher and then as a chemist in the private sector. Brandys speaks several languages, including English, Hebrew, and Polish, and also serves as a volunteer translator in the Clinical Center.

Larry Keefer, head of the Chemistry Section and chief of the NCI Laboratory of Comparative Carcinogenesis (LCC), has worked with Brandys for more than five years. "One of the challenges we were struggling with was setting up a website for compounds. We needed the structures and data uploaded," said Keefer.

Brandys joined on to convert the structures to images and enhance links to relevant data and publications for the *Nitric Oxide Donors* database.

The LCC patents compositions of matter with the intention of making them widely available for pharmacological screening. If a compound has a commercial application, say, as an effective anticancer agent, a material transfer agreement can be signed and licensing can be obtained through NIH.

Brandys' knowledge of chemistry and information management allows her to search and locate accurate compound properties and make expert contributions to the LCC website: **<http://home.ncifcrf.gov/lcc/nitricoxide/default.asp>**.

Michael Walden

Informationist Barbara Brandys displays chemistry structure data she enhanced for Larry Keefer's group at the Laboratory of Comparative Carcinogenesis, NCI

Marti Welch

Larry Keefer's NCI-Frederick lab group: (sitting, left to right) Joseph Hrabie, Larry K. Keefer, Joseph Saavedra; (standing, left to right) Carlos Velazquez, Michael Citro, Daniela Andrei, Geoffrey Lynn, Ana Maciag, and Harinath Chakrapani

Keefer is impressed with Brandys' work. He said that "when the group publishes an article with a new compound in it, she pulls chemical parameters from the methods section and uploads them to the database."

Brandys specializes in chemistry/structures, drug information, and toxicology searches and provides chemistry resources seminars to chemistry-focused groups within NIH. Recently, Brandys started to work on the design and development of a new compound database for the Imaging Probe Development Center. ■

At Home in the IPDC and OTT

Josh Duberman has been an informationist at the NIH Library since May 2005. He has a Masters in Library Studies and a bachelor's degree in chemistry, with extensive information research experience in the private sector, as well as experience as a working scientist in both government and private laboratories.

Duberman has several patents and has written numerous articles for professional publications about the information industry, searching techniques, and information resources. His areas of expertise and research include intellectual property, chemistry, biotechnology, pharmaceutics, engineering, competitive intelligence and technology transfer resources, and information-retrieval issues.

Duberman often works with Gary Griffiths, director of the Imaging Probe Development Center (IPDC), and his staff. An NIH core chemistry facility set up as part of the NIH Roadmap for Medical Research, the IPDC is dedicated to the production of imaging probes for all requesting intramural scientists.

Griffiths said, "We have been very busy with multiple collaborations now ongoing with around a dozen institutes and centers, indicating the demand for such agents here at NIH. A result of this is that we tend to perform syntheses across the full range of chemical compositions, which is a very broad knowledge range indeed. Because of our mandate and our multiple project areas, we have unique, ongoing, and important needs for excellent library services and support. The NIH library staff has helped us substantially on several projects."

Among Duberman's accomplishments at IPDC, Griffiths said, are helping to "decipher multiple patent and literature methods for the chemical syntheses of certain complex dye molecules, thereby allowing our scientists to choose the best method from these multiple alternatives. This in turn saved us time, effort, and resources."

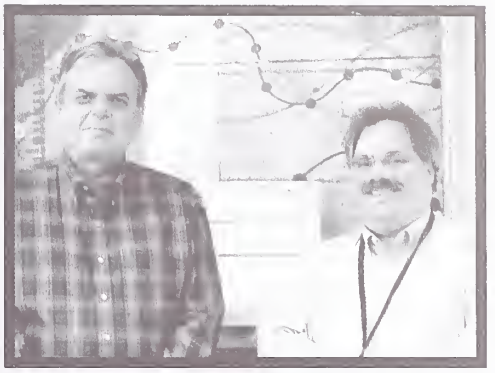
Duberman acknowledged that his continuing education, and especially his study of fluorescence and dye chemistry, made it possible for him to provide the kind of in-depth information services relevant to Griffiths' group. Duberman has also conducted chemical substructure patent searches, called Markush searches, in pursuit of potential IPDC inventions.

Promoting the work of NIH chemists is the purview of Steven Ferguson, director of the Division of Technology Development and Transfer (DTDT) of the Office of Technology Transfer (OTT). He and his staff, as well as students taking the popular FAES course Biomedical Business Development for Scientists, take advantage of Duberman's wealth of chemistry and information research expertise.

Topics covered in Duberman's office and classroom presentations, Ferguson said, include "patent searching" and "how to use public search engines to find information on pharmaceuticals and vaccines."

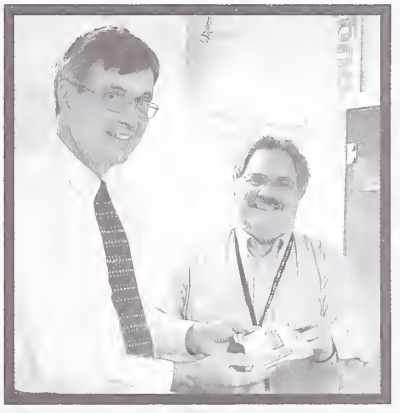
Ferguson's division is responsible for assessing the technology products market. Approximately 200 products on the market, mostly laboratory reagents, were licensed by NIH. About 20 are vaccines and therapeutics. The division uses outside law firms to conduct complete patent searches.

Keeping informed about NIH Library resources and services is a valuable aspect of the Informationist Service. "It's just amazing the kinds of things you can do from your desktop these days," said Ferguson. "It's quite exciting. The new challenge is how to manage it, sort it, and filter it." ■



Michael Walden

He gets around: *Informationist Josh Duberman with (left) IPDC Director Gary Griffiths at the NIH Library's new online catalog access station, and (below) . . .*



Michael Walden

. . . with DTDT Director Steven Ferguson amidst a display of technology transfer successes at OTT's Executive Boulevard site

To read more about informationists, visit the NIH website at **<http://nihlibrary.nih.gov/LibraryServices/Informationists.htm>**.

For more information about the Informationist Service, contact Susan Whitmore at (301) 496-1157 or **<whitmors@mail.nih.gov>** or Suzanne Grefsheim at (301) 496-2448 or **<grefshes@mail.nih.gov>**.

PRAT Fellowship Applications Due January 30, 2008

The NIGMS Pharmacology Research Associate (PRAT) program is now accepting applications for positions to begin October 2008. This competitive research fellowship program supports training at NIH or FDA laboratories for postdoctoral candidates. The program focuses on training in the pharmacological sciences and related research areas such as molecular pharmacology, signal-transduction mechanisms, drug metabolism, immunopharmacology, chemistry and drug design, structural biology, endocrinology, bioinformatics, and neuroscience.

PRAT fellowships are three-year appointments at competitive salaries. Some supply and travel funds are provided to help support research in preceptors' laboratories. Applicants must identify a preceptor in their application. Preceptors may be any tenured or tenure-track scientist at NIH or FDA who has agreed to host the applicant. Applicants must be citizens or permanent residents of the United States and have been at the NIH or FDA for no more than one year at the time they submit their application.

Applications for the 2008 PRAT Fellowships will be accepted through **January 30, 2008.** For more information or application materials, contact the PRAT program assistant at (301) 594-3583 or e-mail **<prat@nigms.nih.gov>**. ■

Graduate Students Present Their Research and Explore Their Options at NIH

by Christopher Wanjek

Nearly 250 graduate students from around the country—selected from about 750 applicants—attended the second annual NIH National Graduate Student Research Festival October 11–12. They came here to learn about NIH and its potential postdoc positions and to present their research. The Catalyst *took a closer look at two of the posters.*

Genomic Variants and Acyclovir Efficacy

Acyclovir is the drug of choice for the treatment of herpes virus hepatitis. Its efficacy, however, varies greatly among patients, a frustrating problem not uncommon in pharmacology.

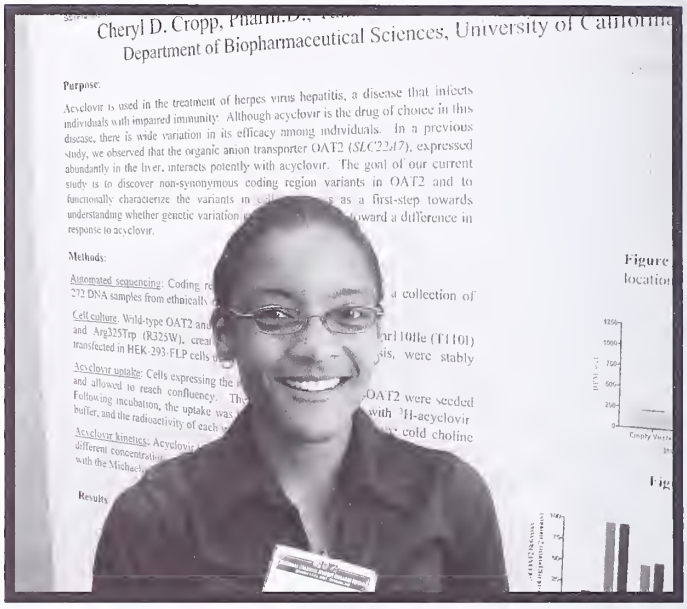
Cheryl Cropp and her colleagues at the University of California, San Francisco, are narrowing in on one possible reason—variants in the organic anion transporter OAT2 (SLC22A7). The group's two-year detective work continues to reveal surprising twists.

Cropp's project has examined the DNA of 272 healthy volunteers in the Bay area. Earlier work revealed that OAT2 in the liver interacts readily with acyclovir.

From the donated DNA, she found six variants of OAT2: four singletons and two polymorphic variants. (These two polymorphic, nonsynonymous variants were found only among African American donors, a finding of possible significance to be investigated later, she said.)

The OAT2 protein threads in and out of the cell membrane. One of the nonsynonymous variants, Thr110Ile, is located in the extracellular environment, and the other, Arg325Trp, is in the cytoplasm, perhaps interfering with the uptake and intracellular function of acyclovir, respectively.

Cropp found that the variants reduce the uptake of acyclovir by 50 percent compared with the wild-type OAT2. The group continues to develop tags to trace acyclovir uptake and utilization and hopes to increase its DNA sample size. Cropp's work could lead to screening for acyclovir use.



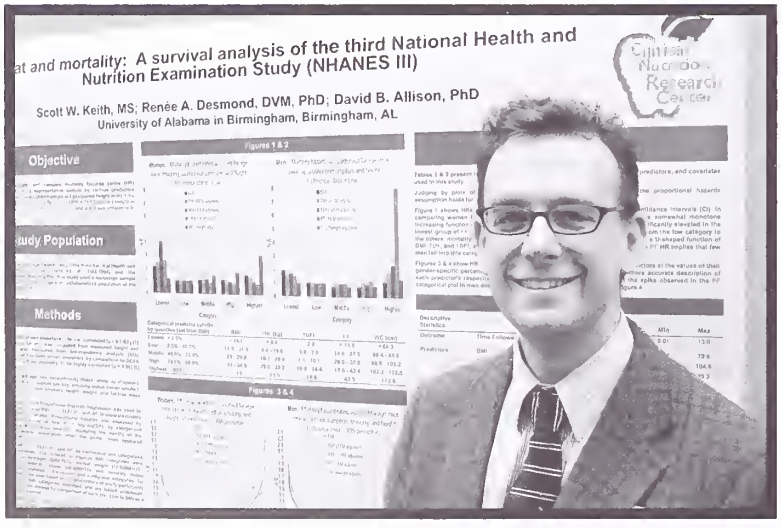
Christopher Wanjek

Cheryl Cropp

Cropp has a doctorate in pharmacy and is in her final year of a Ph.D. program in pharmacogenetics. She was impressed by the opportunities for interaction and collaboration at NIH.

During her visit to the Graduate Student Research Festival, she toured NEI facilities and realized a potential connection between her work and therapies to treat herpes-related eye diseases. ■

Body Fat and Mortality



Christopher Wanjek

Scott Keith

As humans across the globe continue to pack on extra weight, researchers remain unsure how best to gauge the accompanying potential risk of diseases and death.

Scott Keith and his colleagues at the University of Alabama in Birmingham have examined the relationship between estimated total body fat (TBF) and mortality, and they compared this to other obesity biomarkers, such as body-mass index (BMI), percent fat, and waist circumference. Keith utilized bioimpedance analysis measurements in the NHANES III database.

BMI—weight in kilograms divided by squared height in meters—is the most common measure of obesity, adopted by the World Health Organization, the United Nations, and other international bodies. But BMI is limited as a health-risk predicter. For example, higher BMI in women appears to confer a greater risk of death than in men; and certain ethnic groups, such as southeast Asians, can maintain BMIs associated with being overweight but remain healthy. Earlier studies have shown that various measures of obesity and body fat yield inconsistent mortality risk results.

Keith found that TBF is a more powerful tool for predicting mortality risk than BMI and other measures, particularly because TBF is independent of sex. Waist circumference, however, is a strong predictor of mortality risk in women.

He also found that percent fat (TBF divided by weight in kilograms) and TBF index (TBF divided by squared height in meters) were not interchangeable with TBF in terms of predicting mortality risk. While noting that more research is needed, he said that TBF might soon be used to complement the widespread use of BMI.

Keith is in his final year of a doctorate program in biostatistics. His research has involved developing and applying statistical methodology to analyze medical and epidemiological data.

"Opportunities [at NIH] are more advantageous than I'd predicted," he said during his visit to the festival. ■

On Tenure Track

Carmen Williams joined the NIEHS in September, where she heads the Reproductive Medicine Group in the Laboratory of Reproductive and Developmental Toxicology.

Her lab has two major interests. One is the regulation of endometrial function, which is relevant to infertility as well as endometriosis and endometrial cancer. The other is the study of gametes and preimplantation embryos to better understand infertility and to provide information relevant to contraceptive development.

Her past research on these topics—while holding various titles at the University of Pennsylvania in Philadelphia, from infertility fellow to assistant professor—mainly used mouse models. Her lab now also uses human sperm and endometrium samples. As a self-described fertility doc with a doctorate in cell biology, Williams said she hopes to serve as an information resource on women's reproductive health for the NIH intramural staff.

A key protein involved in implantation in the mouse is epithelial membrane protein–2 (EMP2), a molecular facilitator thought to organize other proteins into cell surface signaling complexes, altering the adhesiveness of the endometrium.

Carmen Williams

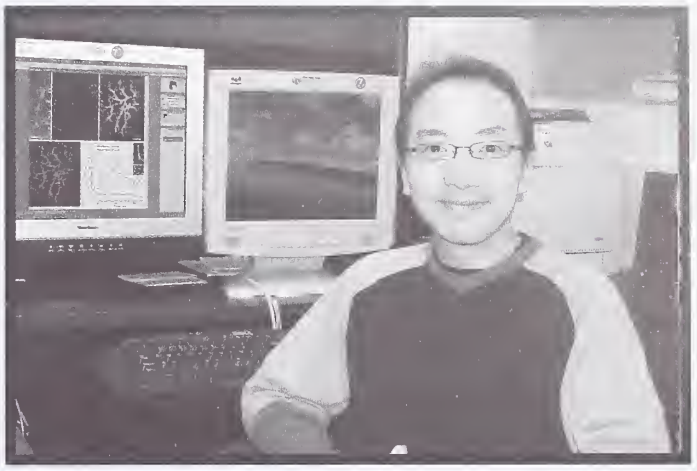
EMP2 localizes on the surface of the uterine epithelium at the time of implantation, and reduced amounts hinder implantation. Conversely, a higher expression of EMP2 is seen in poor-prognosis endometrial cancer, the most common gynecologic cancer in the United States. Williams is collecting endometrial biopsies from women with and without fertility problems to futher examine the role of EMP2 in infertility and endometriosis.

As for male infertility, Williams said that surprisingly little was known before 2002 about how a sperm fertilizes an egg. Then a British group identified a key sperm molecule, phospholipase C-ζ (PLCζ), which initiates calcium oscillations required for fertilization when sperm enters egg.

Williams' group demonstrated how this molecule is biologically significant in mice. Because a repetitive pattern of calcium oscillations is required for successful pre- and postimplantation development, suboptimal PLCζ function could explain some cases of infertility, Williams said.

If this hypothesis is correct, she envisions a test to identify couples who would benefit from treatment of this defect. "There is a huge gap in our understanding of male infertility," Williams said.

—Christopher Wanjek



Yvonne Evrard

Wei Li

Wei Li joined the Unit of Retinal Physiology, NEI, in August 2007. He began his study of the physiology and circuitry of the mammalian retina as a postdoctoral fellow in Chicago in the Northwestern University laboratory of Steven DeVries, where he focused on the cone photoreceptors in the retina and their associated neurons.

Mammalian vision begins with two types of light-sensing neurons—rods, which function predominantly in low-light conditions, and cones, which function in daylight and are responsible for color vision. Both receive visual signals and send them on to the retinal neurons and ultimately to the brain. One research avenue Li is pursuing at NEI is determining how retinal neurons are wired and how they function.

Using direct synaptic stimulation or natural-light stimulation to take recordings from neurons, and combining these with anatomical connections, Li hopes to determine how these visual cues are processed in the retina and passed to the higher visual centers of the brain.

Most mammals, including humans, have rod-dominated retinas; but the ground squirrel—Li's model organism—is unique in that its retina is composed predominantly of cone photoreceptors, allowing him to address more specific questions of mammalian color vision.

Humans and some primates have a trichromatic (blue-green-red) system of cones; most other mammals have only a blue-green cone system. Li is especially interested in elucidating the neural connections and and signaling process of the blue cones, which are the most evolutionarily conserved but least well understood.

Li is also using his ground squirrel model to examine degeneration and photoreceptor-protection mechanisms. During a squirrel's hibernation, retinal neurons undergo degenerative-like changes, including detachment of the photoreceptor synaptic component from the membrane and retraction of neuronal structures. Once the squirrel comes out of hibernation, however, the retina completely recovers within five days.

This hibernation scenario, Li says, provides a unique window into natural photoreceptor-protection mechanisms in adverse environments. In addition, these studies offer a system in which to study synaptic regeneration and prevention of further degeneration after retinal damage.

—Yvonne Evrard

Recently Tenured

Montserrat Garcia-Closas *received an M.D. degree from the University of Barcelona in Spain in 1990 and an M.P.H. in quantitative methods and a Dr.P.H. in epidemiology from the Harvard School of Public Health in 1993 and 1996, respectively. She joined the Hormonal and Reproductive Epidemiology Branch, NCI, in 1996 as a postdoctoral fellow and is now a senior investigator in that branch.*

Montserrat Garcia-Closas

I am interested in the application of biomarkers in molecular epidemiologic studies of breast, ovarian, endometrial, and bladder cancers, with special emphasis on the study of genetic susceptibility and etiologic heterogeneity of breast cancer.

I have also conducted a series of methodological investigations to address relevant scientific questions derived from molecular epidemiologic studies such as assessment of false-positive findings, collection and use of buccal cell DNA, and creation of pilot studies for biomarker development.

The discovery of susceptibility genes for cancer holds great promise for improving risk assessment and developing targeted preventive strategies. It also provides an opportunity to dissect the complex etiology of each cancer.

The importance of susceptibility genes in risk assessment is being evaluated in the Polish Breast, Ovarian, and Endometrial Cancer studies, a set of parallel, molecular epidemiologic studies targeting these three tumors in women and including about 2,500 breast, 500 endometrial, and 300 ovarian cancer cases and more than 2,500 control subjects. This complex study combines detailed exposure assessment and comprehensive collection of biological specimens.

In exploring genetic susceptibility of breast cancer, I have evaluated variation in genes in several candidate pathways, including DNA repair, apoptosis, and regulation of telomeres.

With the Breast Cancer Association Consortium, a consortium of studies including more than 20,000 breast cancer cases, we were able to uncover convincing evidence for modest associations between breast cancer risk and two common genetic variants, caspase 8 (*CASP8* D302H) and transforming growth factor–β1 (*TGFB1* L10P).

In addition, I am collaborating with two efforts to identify genetic susceptibility markers through genome-wide association studies (GWAS) at the University of Cambridge, U.K., and the Cancer Genetic Markers of Susceptibility project. Recent publications from these studies have identified at least five novel genetic susceptibility markers for breast cancer. Numerous other variants are likely to be identified in the coming years using this approach.

Ongoing work after the initial identification of markers includes:

- Fine mapping and functional studies to identify causative markers
- Evaluation of how lifestyle factors affect the risk from genetic variants
- Learning how different genetic variants interact with each other in affecting risk
- Learning whether genetic variants are differentially associated with different types of breast cancer with different clinical and pathologic features at presentation
- Learning how variants affect survival

My work related to etiologic heterogeneity of breast cancer in the Polish breast cancer study has demonstrated substantial heterogeneity in breast cancer risk factors by histopathological tumor characteristics of clinical relevance, particularly tumor grade and size.

In this study, we are using tissue microarray blocks to obtain standardized, rapid, and cost-effective immunohistochemical characterization of many tumors. This process allows us to evaluate relationships between tumor markers with known breast cancer risk factors and newly discovered genetic markers.

Fran Pollner

Kent Hunter

I have also worked on the evaluation of genetic susceptibility factors for bladder cancer and the interaction of those factors with environmental and occupational exposures.

Because of the central role of tobacco smoking in bladder carcinogenesis, I initially focused on polymorphisms in genes involved in carcinogen metabolism and DNA repair. Data from this study provided compelling evidence for the associations of *N*-acetyltransferase 2 (NAT2) slow acetylator and glutathione *S*-transferase null genotype with increased risk of bladder cancer.

The data also supported an interaction between smoking and NAT2 genotype, which is one of the few consistent gene-environment interactions described to date. In addition, I showed that common variants in the nucleotide excision repair pathway are likely to alter bladder cancer risk.

More recently, we used a highly multiplexed genotyping platform to explore associations between variants in cancer-related genes and bladder cancer, which resulted in the identification of novel genes that may be involved in bladder cancer etiology.

I plan to follow up on findings from our candidate gene investigations in the recently formed International Consortium of Bladder Cancer Study. In addition, we are planning to carry out a GWAS of bladder cancer in collaboration with other intramural and extramural studies.

Kent Hunter *received his Ph.D. in biology from the Massachusetts Institute of Technology in Cambridge in 1991. He was an associate member at the Fox Chase Cancer Center in Philadelphia before joining the Laboratory of Population Genetics, NCI, in 1999. He is currently a senior investigator in the Laboratory of Cancer Biology and Genetics, NCI.*

The focus of my laboratory is the study of inherited metastasis susceptibility in breast cancer. Previously, metastasis was thought to be the result of sequential random somatic alterations that occurred in tumor cells as tumors evolved. Thus, metastatic disease was was viewed as a random process, whose appearance could not be predicted before the development of the primary tumor.

While somatic mutation is clearly a part of metastatic progression, work in my laboratory has demonstrated that there is also a significant inherited predisposition to developing secondary tumors. Using a highly metastatic mouse mammary tumor model and a simple breeding scheme, we demonstrated that the genetic background upon which the primary tumor arose had a significant

impact on the number of subsequent lung metastases.

These results suggest that in addition to the somatic mutations, activating or inactivating specific genes that have been previously studied in metastatic disease, polymorphisms that subtly effect gene function also play an important role in breast cancer progression.

Using an integrated genomic and complex trait genetic mapping strategy, my lab has begun to identify some of the genes that may contribute to metastasis susceptibility. The first gene we identified is the signal transduction regulator signal-induced proliferation-associated gene *Sipa1*.

We identified an amino-acid polymorphism in this protein in mice that reduces its enzymatic function. Modeling the effect of this polymorphism by halving the endogenous levels of *Sipa1* mRNA significantly reduced the metastatic capacity of a highly malignant mammary tumor cell line, suggesting that subtle variations in this gene's activity may play a role in human breast cancer progression.

In addition to the mouse modeling experiments, we have also performed pilot epidemiology experiments to directly investigate SIPA1 in humans. In collaboration with Hoda Anton-Culver at the University of California at Irvine, we demonstrated that polymorphisms in SIPA1 were associated with markers of poor outcome in humans, consistent with our hypothesis that this gene may be one of the factors that establish metastatic susceptibility in humans.

There are several important implications of our research. First, our work suggests that modulating gene function by relatively minor amounts may have significant impacts on cancer progression.

This possibility leads to the further hypothesis that one may subtly change the molecular state of the cell, rather than use a cytotoxic agent, to prevent relapse in breast cancer patients.

Moreover, the existence of polymorphisms in the germline DNA that establish susceptibility to metastatic progression should enable the development of a blood-based prognostic test to identify those patients at risk for disseminated disease. We hope that this test will ultimately result in better tailored treatment for patients, reducing the number of relapses and cancer-related deaths.

L. Aravind (aka Aravind Iyer) *received his Ph.D. in 1999 from Texas A&M University, College Station. He worked as a staff scientist at NCBI from December 1999 to December 2002 and is currently senior investigator in the Computational Biology Branch, NCBI.*

Fran Pollner

Aravind (Iyer)

I am an evolutionary biologist, and I use computational methods to decipher biological functions from genome sequences.

The past decade has been an exciting and definitive period in modern biology. Thanks to the advances in sequencing technology, we are in possession of the genome sequences of not just humans, but organisms spanning the entire tree of life. With the whole script of an organism's biology spread before us, we are ready to obtain unprecedented glimpses of life's workings. However, to apprehend these views, we need to be effective readers and interpreters of this script.

My group has primarily worked towards this objective, developing and using computational methods for comparing protein sequences and structures and analyzing biological networks.

To obtain a reasonably complete approximation of biological function, my group investigates different evolutionary problems at multiple organizational levels. At the "microscopic" level, we study protein domains in order to identify their structural and functional determinants. At the "mesoscopic" level, we study the various interactions between different proteins or proteins and nucleic acids in the context of entire biological functional pathways. Finally, at the "macroscopic" level we attempt to reconstruct salient aspects of organismal biology from whole genome sequences.

Our studies at the microscopic level have strongly focused on the discovery of new protein domains and the generation of testable hypotheses regarding their functions.

Some of our major findings include the computational discovery of new peptidases involved in deubiquitination; proteins involved in viral and cellular DNA packaging and segregation; and novel enzymes and factors involved in chromatin dynamics, RNA modification, and post-transcriptional gene silencing.

Overall, we are moving toward generating a complete natural classification of the protein universe.

Our studies on the next level of biological organization have followed the evolutionary trajectories of entire functional systems involved in RNA and DNA metabolism, apoptosis, ubiquitin signaling, transcriptional control, and chromatin-level regulation.

More recently, we have investigated how whole biological networks, especially those involved in transcription, evolve as their component nodes undergo diversification.

Especially striking is our finding that despite conservation of target genes, the major specific transcription factors are subject to massive lineage-specific displacement via new innovations. Different lineages may therefore differ dramatically in their transcription factors despite retaining a similar complement of target genes, suggesting that the evolutionary turnover in transcription factors is a major player in phenotypic diversity.

Our genome-scale analysis has led to the understanding of several aspects of biology at the organismal level, such as the parasitic adaptations of apicomplexans and large DNA viruses, the origins of eukaryotic cellular complexity, and sensory and signaling strategies of organizationally complex bacteria.

In particular, we have made several efforts toward understanding the biology of apicomplexans parasites, which are causative agents of malaria, toxoplasmosis, a fatal lymphoma in cattle, and cryptosporidiosis. We computationally predicted novel *O*-linked protein glycosylation pathways that are likely to modify surface molecules and play a critical role in the way these parasites interact with their host.

Our discovery of apicomplexan-specific transcription factors has also provided a means to unravel the hitherto-unknown mechanisms of gene regulation in these organisms.

In conclusion, we hope to continue to exploit computational methods and evolutionary principles to arrive at a definitive understanding of the major biological systems. This in turn would provide vital insights regarding normal as well as disease states in humans and other organisms.

Recently Tenured

Zu-Hang Sheng *received his Ph.D. from the University of Pennsylvania in Philadelphia in 1993. He completed his postdoctoral training in neuroscience at the University of Washington, Seattle, before he came to NINDS as a tenure-track investigator in 1997. He is currently a senior investigator and chief of the Synaptic Function Section, NINDS.*

Our research is focused on molecular and cellular mechanisms underlying (1) the axonal transport of synaptic components and organelles essential for the assembly of synapses, and (2) the regulation of synaptic vesicle (SV) priming for fusion. Such mechanisms are crucial for the initial establishment of presynaptic terminals and for the modulation of synaptic function.

Fran Pollner

Zu-Hang Sheng

We initially identified Snapin as a SNAP-25–binding protein. The physiological role of Snapin in SV exocytosis was examined and further confirmed by microinjection or overexpression of Snapin in presynaptic neurons in culture and by overexpression of Snapin in hippocampal neurons.

Our studies using *snapin*-knockout mice in combination with genetic rescue experiments provide evidence that Snapin modulates neurosecretion in chromaffin cells by stabilizing the structural coupling of the calcium sensor synaptotagmin-I to the vesicle fusion machinery SNARE complex, a critical step for priming docked vesicles for fusion. The deletion of Snapin leads to a marked reduction in the amount of synaptotagmin-I–SNARE complex and defective exocytosis in the *snapin* (-/-) chromaffin cells.

Individual SNAREs are distributed to specific membrane compartments along secretory and endocytic pathways and contribute to the specificity of membrane trafficking. In addition to its association with SVs, our ongoing study suggests that Snapin is also involved in an endosome-lysosome trafficking pathway, possibly through interactions with endosomal SNAREs.

Syntaphilin (SNPH) is a neuron-specific protein initially identified as a candidate inhibitor of presynaptic function. We recently generated mouse mutants with a homozygous deletion for the *snph* gene, leading to the discovery of a novel role for SNPH as a docking receptor of axonal mitochondria.

Our findings indicate that SNPH is targeted to and required for maintaining a large portion of axonal mitochondria in stationary state through an interaction with the microtubule-based cytoskeleton. The deletion of the *snph* gene in mice dramatically increases mitochondrial motility, reduces their density in axons, and consequently influences short-term facilitation during prolonged high-frequency stimulation, probably by affecting calcium dynamics at presynaptic boutons.

Our studies elucidate a mechanism underlying the docking of axonal mitochondria and provide evidence that the increased motility and/or reduced density of axonal mitochondria have a significant impact on presynaptic function.

We initially identified syntabulin as a syntaxin-binding and KIF5B motor-adaptor protein that mediates anterograde transport of syntaxin-1 to neuronal processes. Recently, we showed that the syntabulin-KIF5B transport complex plays a critical role in axonal delivery of the active zone (AZ) components essential for presynaptic assembly.

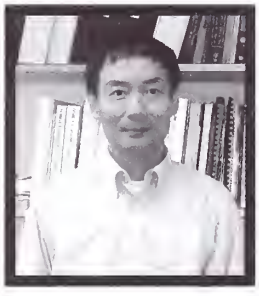
Using time-lapse imaging in live hippocampal neurons, we demonstrate that syntabulin comigrates with AZ precursor vesicles along axonal processes. The knockdown of syntabulin or interference of its interactions with either KIF5B or syntaxin-1 in developing neurons results in the deficient trafficking of the AZ components to nerve terminals, reduces the density of release sites, impairs synaptic transmission, and inhibits the activity-induced formation of new presynaptic boutons.

Our findings suggest a mechanism through which long-term presynaptic plasticity is regulated by the syntabulin-KIF5B-mediated axonal transport of the AZ components, thus contributing to the activity-induced presynaptic assembly.

I believe that our continued application of these multidisciplinary systems analysis of genetically engineered mice will contribute to an understanding of the molecular mechanisms that are crucial for regulation of activity-dependent presynaptic plasticity by (1) the anterograde axonal transport of presynaptic components, (2) the axonal mitochondrial trafficking and docking, and (3) the priming and regulation of SV exocytosis.

Given that defects in axonal transport of presynaptic cargos and mitochondria in neurons have been implicated in the pathogenesis of neurodegeneration, our studies will yield fundamental information that may have an impact on the understanding of human neurodegenerative disorders.

Yihong Yang *received his Ph.D. degree in biophysics from the University of Illinois at Urbana-Champaign in 1995, under the supervision of Nobelist Paul Lauterbur, who invented magnetic resonance imaging (MRI). He did postdoctoral work at NIH in the Laboratory of Diagnostic Radiology Research from 1995 to 1998 and then became an assistant professor in the Functional Neuroimaging Laboratory at the Weill Medical College of Cornell University in New York before joining the Neuroimaging Research Branch, NIDA, in 2002 as an investigator. He is currently a senior investigator and chief of the MRI Physics Section in that branch.*



Yihong Yang

MRI and spectroscopy are noninvasive, versatile techniques that are ideal for providing system-level information on humans and animals. My research at NIDA has focused on the development of advanced magnetic resonance techniques to study brain structure, function, and metabolism, particularly as they are related to the effects of substance abuse on the brain.

My group has developed novel functional MRI (fMRI) methods with enhanced detection and quantification power to observe brain activation elicited by cognitive tasks or administration of drugs.

These fMRI techniques are able to measure multiple functional signals simultaneously (for example, cerebral blood flow, blood volume, and blood oxygenation) and to quantify local oxygen consumption in the brain. Neuronal activity accompanied by metabolism can then be separated by potential vascular artifacts in neuropharmacological studies using the comprehensive information provided by these techniques.

We have conducted research on functional connectivity of the brain using "resting-state" fMRI, in which intrinsic interactions between brain regions are reflected by synchronized fluctuations of the fMRI signals.

Compared with healthy control subjects, cocaine users showed significant reduc-

tion of functional connectivity in the "reward circuitry" of the brain, which was heavily involved in addiction processes.

This study revealed for the first time the relationship between functional brain connectivity and chronic drug abuse, and pointed to a powerful new tool to study drug-induced neuronal dysfunction or dysregulation.

Magnetic resonance spectroscopy (MRS) can be used to measure metabolite and neurotransmitter concentrations in vivo. My group has developed a new MRS method that provides well-resolved glutamate, glutamine, and GABA signals. We performed MRS experiments on cocaine users and healthy control subjects that revealed significantly lower glutamate levels in the anterior cingulate cortex of the cocaine addicts, a finding potentially associated with chronic drug addiction.

We have also developed several new diffusion-based MRI methods for better delineating complex brain white matter structures. These methods can be used to improve fiber-tracking techniques by identifying multiple fibers coexisting in an image voxel—not possible with traditional diffusion tensor imaging. Using a group-level analysis on diffusion-based imaging data, we assessed structural integrity of neural circuits relevant to drug addiction.

Recently, we developed an animal model to investigate the underlying mechanisms of resting-state fMRI by integrating electrophysiological and fMRI signals in the resting rat brain.

Our results demonstrated that, unlike the evoked fMRI response that correlated with power changes in high-frequency bands, power coherence in low-frequency bands (particularly the delta band) correlated with the resting-state fMRI signal in a region-specific and anesthesia dose–dependent fashion.

These results provided new insights into the linkage between neuronal activity and hemodynamic-based fMRI signal at rest.

Most existing fMRI techniques measure neuronal activity indirectly through hemodynamic responses coupled to neuronal and metabolic changes. To avoid potential vascular effects of drugs, we used Mn^{2+}-enhanced MRI to detect neuronal activity reflected by an influx of Ca^{2+} ions due to action potentials.

In experiments on rats given cocaine, we showed that this noninvasive method detected brain activations as consistently as nonhemodynamic invasive methods and thus appears to be a very promising tool for mapping drug-induced neuronal activity. ■

Demystifying Medicine for Ph.D.s, 2008

When: every Tuesday from **January 8 to May 13**, 4:00 to 6.00 p.m.
Where: Building 50 ground-floor auditorium. All presentations will be videocast and archived.

For academic credit, register with FAES: **<http://www.faes.org>**; otherwise, register at the Listserv: **<http://list.nih.gov/archives/demystifyingmed.html>**.

The schedule can also be seen at <http://www1.od.nih.gov/oir/DemystifyingMed/index.html>.

Date	Speakers	Subject
	January	
8	John Coffin (NCI) Henry Masur (CC)	HIV/AIDS: the ultimate chameleon
15	Michael Sack (NHLBI) Bob Balaban (NHLBI)	Heart failure: advances regarding a major clinical problem
22	Meral Gunay-Aygun (NHGRI) Carolyn Ott (NICHD)	Cystic diseases and cilia: a new frontier
29	Harvey Klein (CC) Harvey Alter (CC)	Diseases from the blood bank: progress and the future
	February	
5	Warren Strober, NIDDK Peter Mannon, NIAID	Inflammatory bowel disease: what is the target?
12	Thomas Wellems (NIAID) John Robbins (NICHD)	Malaria: big killer and big advances
19	Nora Volkow (NIDA) Monica Skarulis (NIDDK)	Hunger, appetite, obesity, addiction: the new pandemic
26	Abner Notkins (NIDCR) Phillip Gorden (NIDDK)	Diabetes and autoimmunity: turning against self
	March	
4	Amy Agrawal (CC) Philip Murphy (NIAID)	West Nile virus: a new threat
11	Les Biesecker (NHGRI) Julie Sapp (NHGRI)	Genetic screening: finding Mendelian disease genes
18	Andrew Singleton (NIA) Katrina Gwinn-Hardy (NINDS)	Neurologic diseases in the genome era
25	Francis Collins (NHGRI) Sharon Milgram (OD)	Cystic fibrosis: a common inheritable disease with many unknowns
	April	
1	William Gahl, Marjan Huizing, Amanda Helip-Wooley, Wendy Westbroek (NHGRI)	Lysosomal diseases: patients and problems
8	Ellen Sidransky (NIMH) Chris Austin (NHGRI)	Gaucher's disease: treating a genetic disease
15	Leighton Chan (CC) Walter Koroshetz (NINDS) and DOD colleagues	Traumatic brain injury: mechanisms, treatment and challenges
22	Howard Fine (NCI) and colleagues	Brain cancer: problems and progress
29	Ezekiel Emanuel (CC) Win Arias (NICHD/OD)	Liver cancer: a global problem. Who gets THE liver transplant?
	May	
6	Giuseppe Giaccone (NCI) Lyuba Varticovski (NCI)	Lung cancer: clinical progress and the cancer stem cell paradigm
13	**Finale: Minisymposium: What does the future hold for Ph.D.s ? TBA**	

CATALYTIC REACTIONS?

If you have a photo or other graphic that reflects an aspect of life at NIH (including laboratory life) or a quotation that scientists might appreciate that would be fit to print in the space to the right, why not **send it to us via e-mail: catalyst@nih.gov>; fax: 402-4303; or mail: Building 2, Room 2E26.**

Also, we welcome "letters to the editor" for publication and your reactions to anything on the *Catalyst* pages.

In Future Issues...

- IRP Research Roundup
- Sister Study
- Mercury Cleanup

Combined Federal Campaign Kickoff To the Cowboy Two-Step

Christopher Wanjek

The final 3: *From a field of IC heads who "wannabe" NIH dance idols, finalists Elias Zerhouni, Alfred Johnson, and Story Landis go all out for the judges (and the CFC kickoff) one last time—it was a close call, but the man in the middle took the honors (we hear he's holding on to his day job)*

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 2, Room 2E26, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

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