FOSTERING COMMUNICATION AND COLLABORATION

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New Technology

GAUGING DEMAND FOR ATLAS' STRENGTHS

by Fran Pollner

About 10 years ago, Mike Green recalls, a harmonic convergence of sorts occurred in the positron-emission tomography (PET) community. A small group of investigators had the same idea at the same time—that PET could be used not just to diagnose human disease but also as a basic research tool to

probe the physiology and biochemistry of living systems such as the rat and mouse.

Green and his colleagues spent the next decade fashioning the technology to achieve the spatial resolution necessary to image such small subjects. Chief of the Imaging Physics Laboratory in the CC Department of Nuclear Medicine, Green enlisted the aid of the master builders in the ORS precision instrument and electronics shop, the software whizzes at CIT, the electronics wizards at The Thomas Jefferson National Accelerator Facility in Newport News, Va., computer scientists in Spain, and the brainpower of his lab colleagues, most notably staff scientist Jurgen Seidel.

With Clinical Center support, a three-year research and development project was launched that culminated last fall in ATLAS (Advanced Tech-

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Bench-to-Bedside: Childhood Batten Disease

SERENDIPIDITY AND TEAMWORK POINT THE WAY TO APPROACHES TO RARE LYSOSOMAL STORAGE DISORDER

by Celia Hooper

Infantile neuronal ceroid lipofuscinosis (INCL) is the tongue-twisting name for a horrific, but fortunately rare, lysosomal storage disease, also known as childhood Batten disease. Children born with the defect are normal at birth, but by around 1 year, show symptoms of retinal damage and neurodegeneration that will leave them blind by age 2 and brain dead by age 4. Children with INCL persist in a vegetative state until death at age 8 to 12 years.

Into this hopelessness NICHD's Anil Mukherjee and his colleagues have brought a tiny ray of light, thanks to a pilot project spawned by a Bench-to-Bedside Award. Very preliminary results from their treatment of two infants with INCL indicate that an experimental drug, cysteamine bitartrate (Cystagon), may stop the disease's progressive retinal and brain damage. Definitive data will be collected over the pilot study's four years of observation on five infants.

"The Bench-to-Bedside Award helped us enormously," Mukherjee told *The NIH Catalyst* in an interview. "We used the award to complete some of our basic laboratory research (Z. Zhang, J.DeB. Butler, S.W. Levin, K.E. Wisniewski, S.S. Brooks, and A.B. Mukherjee. *Nature Medicine* 7:478–484, 2001) and then embarked on this protocol." Mukherjee says the B-to-B award "provided an added incentive to initiate the pilot study," which evaluates Cystagon for the treatment of INCL.

Mukherjee worked on the study with Zhongjian (Gary) Zhang from his laboratory, James Sidbury, Jr., former NICHD

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From Bench to Bedside Under the NIH Canopy

The idea arose from the perceived need to reinvigorate the atmosphere for clinical research at NIH; it was given form in 1998 when the NIH Clinical Research Revitalization Committee established the Bench-to-Bedside Award, a vehicle to encourage intramural collaboration between basic and clinical researchers at NIH—not just within but across the institutes.

The awards were supported by Clinical Center carryover funds for the first two competitive cycles, after which the institutes agreed to continue the program with their own funds. Projects are funded for one to two years for up to $100,000 a year. For more information, including criteria for research proposals, visit <**http://www.cc.nih.gov/ccc/btb/awards.html**>.

The Bench-to-Bedside initiative, says CC Director John Gallin, capitalizes on the unique opportunity NIH provides to realize the clinical implications of basic research discoveries—especially in the context of conditions that cross institutes and laboratories. The projects, he notes, have stimulated new clinical protocols and in a few cases have resulted in new therapeutic approaches.

Thus far, 32 Bench-to-Bedside proposals have been funded (see page 9). ■

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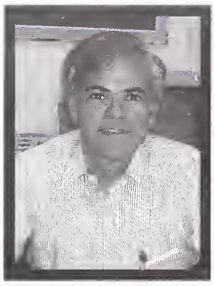
Travel to Scientific Meetings: The Road Ahead



Michael Gottesman

Other than space and parking, there are few issues so likely to inspire spirited discussion by scientists at NIH than restrictions on travel to scientific meetings. Professional meetings and publications form the core of scientific communication and provide critical opportunities for scientists to present their work and become acquainted with colleagues—something especially important for trainees.

The numerous ways in which scientific meetings contribute to the vitality of modern science and improve efficiency of information transfer were the subjects of a recent white paper written by the Office of Intramural Research to inform the discussion about the importance of meeting travel.

Last year, the Department of Health and Human Services (DHHS), of which NIH is a part, began to review travel to scientific meetings as part of its responsibility to ensure appropriate stewardship of public funds.

To be sure that all scientific meeting travel is essential, the DHHS decided that it had to review and approve travel to meetings any time it involved five or more NIH scientists. This proactive approach to management of travel to scientific meetings created logistics problems for the NIH and DHHS.

After discussions, we agreed last December that a new approach, based on development of a defined budget for both domestic and foreign travel to scientific meetings, would supersede the "rule of five" for the NIH.

Based on needs projected from last year's budget, NIH prepared a budget for scientific meeting travel for the current fiscal year. That budget was vetted and approved by the DHHS and distributed among our institutes and centers.

The institute budgets were created with an eye to providing enough funds to support essential scientific meeting travel for all intramural and extramural investigators—assuming last year's average costs for domestic and foreign meetings, with a modest increase for security costs.

Senior investigators would typically travel to more meetings than junior investigators; postdoctoral fellows, graduate students, and some postbacs would be funded for travel to a meeting approved by their supervisors. Trainees who win a competitive $1000 FARE (Fellows' Award for Research Excellence; see announcement, page 3) will have the opportunity to attend an additional meeting.

To optimize the use of travel funds, there is a premium on finding the least expensive ways to get to meetings and to secure accommodations while there. Funds saved by keeping expenses down will allow for additional essential travel to meetings. Here are some considerations for meeting travel budgets:

- Where feasible, car travel and shared rooms make sense.
- Registration fees do not count against meeting travel ceilings.
- Check out the December 2001 "News To Use" accessible from the website below for information on securing the most economical travel arrangements (see "NIH Lowest Airfare Guarantees. . . and Exceptions," beginning on the bottom of page 4): **<http://www.nih.gov/od/ors/publications.htm>**.

> . . . . we agreed last December that a new approach, based on development of a defined budget for both domestic and foreign travel to scientific meetings, would supersede the 'rule of five' for the NIH.

Within each institute and center, travel ceilings will be allocated to laboratories, branches, and programs, or other units of appropriate size to support efficient distribution of funds. Leadership at these levels will determine the optimal use of travel funds, in keeping with the travel needs of scientific staff and trainees.

We believe that planning travel through this approach can work for NIH. Throughout this fiscal year, we will take stock of how this system is working and make adjustments as needed to be sure that essential travel to scientific meetings is supported in the most efficient way.

—Michael Gottesman
Deputy Director for Intramural Research

Fellows Career Workshops: Life After NIH

Here are two more workshops for fellows who are setting their sights on post-postdoctoral life.

■ **Monday, April 22: Negotiating a Job Offer**, 8:30–11:30 a.m., and, again, from 1:30–3:30 p.m. Participants will learn to identify and discuss key issues such as salary and start-up funds with potential employers, as well as how to obtain a desirable employment package and the resources required to be successful in a new job.

■ **Monday, May 13: Advancing Your Career**, 8:30–11:30 a.m. This workshop addresses the basics of employee promotions and strategies for success in the workplace (academia and industry). Participants will learn to hone hiring, supervisory, and mentoring skills. There are also break-out sessions on conflict management and a mock tenure review.

Workshops will be held in **Lipsett Amphitheater (Building 10)** and are open to all fellows at NIH. There is no pre-registration, but seating is limited and available first-come, first-seated.

The workshops are sponsored by the NIH Fellows Committee, the Office of Education, and the Office of Research on Women's Health. For more info, contact Debbie Cohen at <**dec@helix.nih.gov**> or Margaret Mentink-Kane at (301) 594-2345 or <**mmentink@niaid.nih.gov**>.

Where the Jobs Are

Networking is a key to finding job opportunities. It can provide preliminary information about a particular company, agency, or university. It can point to new career directions. Fellows are typically at a point in their careers when it is both most critical to network and most difficult to find the time.

For this reason, Christine Brennan, NINDS, and Yvonne Szymko, NIDCD, created JobNet.

The original idea was to establish an alumni database through which former NIH fellows with established careers could provide advice exclusively to current fellows. The JobNet database has recently been expanded to include career contacts from all backgrounds; however, it still can be viewed only from within NIH.

Volunteer contacts have information on research and nonresearch careers, in fields as diverse as scientific writing, regulatory affairs, teaching, signal transduction, immunology, and bioinformatics. They often have positions available at their facilities and are eager to hear from NIH fellows.

Fellows can visit the JobNet site at

<**http://felcom.nih.gov/Careers/Jobnet/index.html**>

to view the current career contact listings; scientists in permanent positions can complete a form to volunteer as a career contact. Members of the NIH Fellows' Committee maintain the site by advertising, recruiting volunteers, and periodically updating the site. Fellows interested in helping maintain or adding to this resource can contact Diane Lawrence or Joanna Kirman at

<**lawrencd@ninds.nih.gov**> or <**jkirman@mail.nih.gov**>.

Fellows Sought To Help Organize FARE 2003 Competition . . .

Once again, the Fellows committee (FELCOM) is scouting for fellows to run this year's Fellows Award for Research Excellence (FARE)—FELCOM's yearly abstracts competition.

Fellows who take on this task will work hardest in May and early June, when the competition and judging take place, but will be devoting time from March, when the planning begins, through September, when the awards ceremony takes place.

If interested, contact

<**felcomwebmasters@nih.gov**>.

. . . and To Compete

Fellows interested in entering the FARE competition are invited to submit their application, including abstract, electronically from May 1–31 via

<**http://felcom.nih.gov/FARE**>.

Winners receive $1,000 for travel to present their research at a scientific meeting. Abstracts are judged on the basis of scientific merit, originality, and experimental design. The travel award must be used between Oct. 1, 2002, and Sept. 30, 2003.

The competition is open to postdoctoral IRTAs, untenured visiting fellows, and other fellows with fewer than five years of total postdoc experience at NIH, as well as doctoral candidates doing dissertation research at NIH. ■

ATLAS
continued from page 1

nology Laboratory Animal Scanner)—"the most technically sophisticated small animal PET scanner in the world at this moment," says Green, noting that every step of the process to create ATLAS—benchtop experiments, computer simulations, structural designs—is public information. There are probably about a dozen labs worldwide now working on ATLAS-type scanners.

ATLAS is currently housed in Green's lab, back-to-back with a micro-CT scanner. Spatially registered and superimposed CT and PET images of the same animal will help characterize the biodistribution of the PET tracer and correct the PET image data for radiation attenuation.

PET Digs

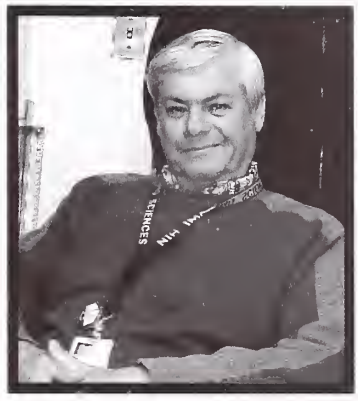
A question before the NIH intramural research community is whether ATLAS will take up residence in the newly opened Mouse Imaging Facility (MIF)—the multimodality small-animal imaging suite located in another part of the Clinical Center (see "Mighty Machines for Mini-Models," *The NIH Catalyst*, January–February 2002, page 1).

In Green's view, the question boils down to whether the unique information obtainable with PET imaging is enough in demand by NIH scientists to warrant its becoming another collectively supported resource within the MIF.

The support required is substantial, Green observes—at the least additional radiochemistry personnel, space, and equipment to produce the theoretically unlimited number of radiopharmaceuticals that investigators might wish to use in small-animal PET imaging.

Bill Eckelman, chief of the CC PET Department, and his staff have been providing the radiopharmaceuticals for the research projects that Green and collaborating investigators from other NIH institutes have been conducting during the decade-long research and development effort leading to the ATLAS machine. This work has taken advantage of the existing cyclotrons and radiochemistry facilities of the CC PET Department in the basement of the Clinical Center.

Radiopharmaceuticals administered to an animal and imaged with ATLAS consist of a positron-emitting isotope attached to a compound whose transport in the body follows a particular biochemical pathway. For example, labeled compounds are available that allow ATLAS to display and quantify the processing of glucose by the brain, the amount and distribution of various receptor types in the brain, gene expression throughout the body, regional hypoxia in tumors, and the movement of various cell types within the body. If the kind and number of such studies were to increase substantially, the additional material and personnel resources mentioned above and some physical modifications to the MIF would be required.



Fran Pollner

Mike Green

PET Projects

Over the next year or so, demonstration projects will be carried out that will help the community assess whether ATLAS should become a MIF regular. Green has a list of about 10 experiments to be performed with the PET Department and other institute collaborators for this purpose.

One, a "simple experiment," would determine the effect of various anesthetics on the mouse brain distribution of several radiolabeled compounds such as fluorodeoxyglucose (FDG), a popular PET tracer of glucose metabolism in brain, myocardium, and tumors. "This is of particular importance technically," Green notes, because animals must be motionless during imaging and, unless the animal is sacrificed, must be anesthetized during a study.

While live animals must also remain motionless during other forms of imaging, such as MR and CT, anesthetics can potentially distort the "true" time-varying tracer distribution compared to the distribution in the unanesthetized brain. Knowing how much distortion is induced by various anesthetics is important when trying to interpret changes in these distributions.

A study planned by NIMH's Robert Innis will label amyloid plaques in the mouse brain and track distribution and amount in response to drugs. Fluorinated probes produced for this project will be evaluated. If the probes are effective in labeling and monitoring plaque changes, the payoff would be a treatment model for Alzheimer's disease, Green notes.

In general, he says, the scope of small-animal PET experiments is limited only by the imagination of the researchers using the modality—and its value is "unequivocal."

PET, he says, provides "extraordinary sensitivity and specificity in visualizing biochemical processes in vivo. One can choose specific biochemical pathways and examine the behavior of those pathways under various conditions."

It's a better choice for visualizing brain receptors than, for example, MRI, since PET can measure very small tracer amounts of drug that will not alter the pathway under scrutiny.

Whither Small-Animal PET at NIH?

Until now, word-of-mouth has brought investigators to Green's door to explore the possibility that PET might help solve a particular research question.

Investigators in the radiation oncology branch, for instance, sought a means of assessing hypoxia in tumors so they could determine the effect of drugs or other treatments on tumor hypoxia. Fluorine-18–labeled misonidazole, a compound avidly taken up by hypoxic cells, was used as a probe of hypoxia levels.

PET was also harnessed in the service of cell trafficking in a collaborative study published earlier this year and involving NCI researchers Edit Olasz and Steve Katz, the PET Department's Eckelman and Lixin Lang, and Nuclear Medicine's Green and Seidel.

The researchers labeled and followed dendritic cells through the rat lymphatic system, an exercise with implications for research in immunologic processes.

The extent of small-animal PET's distribution through NIH remains to be seen. PET capabilities, Green observes, do not contribute to achieving one of the main objectives behind the MIF, which was "conceived as a high-throughput lab." PET lends itself to hypothesis-driven experiments, he says, not to such endeavors as mass screening for mutations—a major impetus for the establishment of the MIF.

"The user community [at NIH for ATLAS] has not been identified," Green says, "but it soon will be." ■

It Takes a Village

A collaboration of the following NIH and other entities produced ATLAS.

Imaging Physics Laboratory, Nuclear Medicine Department, Clinical Center, NIH
(physics, engineering and custom electronics design, assembly, testing and system integration)
Michael V. Green (chief)
Jurgen Seidel (staff scientist)
Juan Vaquero (visiting fellow electrical engineer)
Injae Lee (visiting fellow physicist)
Chosun Snyder (summer student)
Albert Mao (summer student)

Mechanical Instrument Design and Fabrication Section, SEIB, ORS, NIH
(design, mechanical fabrication and assembly)
Biomedical Engineering Technicians:
James Sullivan (chief)
Paul Fitze
Jimmie Powell
Frank Sharpnack II
Carroll Toms

Computer and Electronics Section, SEIB, ORS, NIH
(electronics layout and fabrication)
Burt Chidakel

Division of Computational Bioscience, Center for Information Technology, NIH
(image reconstruction software development, high performance computing)
Calvin Johnson (staff scientist)
Steve Fellini (systems specialist)

Hospital Universitario Gregorio Maranon, Madrid, Spain
(user interface, image registration software)
Manuel Desco (chief)
Javier Pascau (software engineer)

Thomas Jefferson National Accelerator Facility, Newport News, VA
(custom electronics design, board fabrication and testing)
Fernando Barbosa (staff engineer)
William Gunning (electronics assembly)

A and D Precision Co.
(data acquisition system with custom modifications)
Val Zavarzin (nuclear physicist)

Fran Pollner

IRP Team ATLAS: *(left to right) Steve Fellini, CIT; Calvin Johnson, CIT; Jim Sullivan, ORS; Jimmie Powell, ORS; Carroll Toms, ORS; Mike Green, CC; Jurgen Seidel, CC; Burt Chidakel, ORS (not pictured: Paul Fitze and Frank Sharpnack II)*

Fran Pollner

Cameo Appearance: *The ATLAS small-animal imaging PET scanner was brought into the Mouse Imaging Facility (MIF) March 5 to participate in the MIF open house (which, by all accounts, was a packed event from beginning to end). In the photo above, Mike Green, ATLAS designer and chief of the Imaging Physics Laboratory in the CC Nuclear Medicine Department, explains the ATLAS modus operandi to an interested scientist. The animal is placed in the scanning imaging bed, which is advanced into the aperture of the scanner; the radiopharmaceutical is injected and its distribution tracked by ATLAS detectors. The data acquired are accumulated within the ATLAS computer (under the shelf upon which the scanner stands). This raw data may be sent via the accompanying software to the Biowulf supercomputer in CIT's Building 12A to be converted to useful images. The images are ready to be called up in about an hour; meanwhile, another animal can be placed in the scanner. The investigator may also opt for the cruder images obtainable in ATLAS itself in about 5 minutes. At under 1.8-mm spatial resolution, these images are a far cry from the "uselessly blurred" images of small animals obtainable with the 5-7 mm resolution of a human PET scanner. But for the price of a little more time, Biowulf delivers exquisite images at 1.3-mm spatial resolution.*

Bench-to-Bedside: Lysosomal Disorder
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scientific director (now retired), Rafael Caruso of NEI, Andrea Gropman and Kenneth Fischbeck of NINDS, and the CC's Zenadie Quezado and Nicholas Patronas. Extramural collaborators included Sondra Levin from Walter Reed Army Medical Center, Sandra Hofmann of the University of Texas Southwestern Medical Center in Dallas, Krystyna Wisniewski of the New York State Institute for Basic Research on Staten Island, and Pirkko Santavuori of the University of Helsinki, who first described INCL.

In a recent lecture, NICHD cell biologist Juan Bonifacino said that, from the outset, serendipity has played a leading role in research on lysosomes and lysosomal storage diseases. INCL is no exception. In 1997, as Mukherjee's lab was trying to isolate and characterize a mouse phospholipase A2 gene, they also came upon the gene for another enzyme—palmitoyl-protein thioesterase (PPT). As it turned out, Hofmann's group had already snared the human PPT gene. She and others went on to link mutations in the PPT gene to INCL as Mukherjee and his colleagues went to work on a therapy to replace or compensate for the defective enzyme.

Fran Pollner

Collaborators: *Anil Mukherjee (right) and co-author Zhongjian (Gary) Zhang*

Mukherjee explains that many proteins are modified, post-translationally, by the addition of lipids such as palmitic acid. These adornments may anchor proteins in membranes or be essential for their function in other ways. But once the proteins have served their purposes, "the thioester linkage must be disrupted and the lipid must be cleaved from these proteins for recycling or degradation," Mukherjee says. "Thus PPT plays a critical role." Unable to degrade the protein-lipid complexes (ceroid), the cells of INCL patients—including key retinal and brain cells—just keep accumulating ceroid in lysosomes.

Mukherjee saw INCL's Achilles' heel in PPT's flimsy thioester linkage, which he and his colleagues rationalized would be susceptible to nucleophilic chemicals. They screened a list of such drugs, tested three for their ability to cleave the thioester linkage, and picked two with very few side effects—cysteamine and *N*-acetyl cysteine—to test in vitro in cells from nine INCL patients. Serendipity again had a role in the choices. Just down the hall, Mukherjee's NICHD neighbor, Bill Gahl, had been using cysteamine bitartrate for more than 20 years to treat cystinosis, another hereditary lysosomal storage disease. Gahl's data showed that cysteamine could enter the lysosomes and was likely to cross the blood-brain barrier—properties that would be essential for an INCL therapy.

Results of the lab experiments were spectacular—biochemical and electron microscopic inspections of the cells showed that Cystagon cleared ceroid deposits from the cells. This result paved the way for a clinical trial. "There is no effective treatment for these patients, and INCL is uniformly fatal," Mukherjee says. "Cystagon is definitely worth trying." The scientists fired off their B-to-B proposal.

INCL is a rare disease (1 in 100,000 births) and, thus far, Mukherjee and his colleagues have treated just two of the five patients permitted for their Cystagon protocol. The signs are as positive as could be hoped at this early stage (6 months for one patient, almost a year for the other). Patients' white blood cells are free of ceroid inclusions and, within the 6 months of treatment, the babies show no signs of further retinal and brain deterioration. "The biology of this disease predicts that retinas and the brain would degenerate further during this period," Mukherjee says, leaving him guardedly optimistic.

Because some damage to the babies' eyes and brains had already occurred before the disease was recognized and the infants were brought in for treatment at age 2 years, Mukherjee and his colleagues do not know the extent to which the infants will achieve normal developmental milestones. "If they still have brain function at age 4, then we have achieved something," says Mukherjee. "And if they regain eyesight, that would indicate the drug is effective."

Ultimately, the key to real success with the drug may lie in the earliest possible treatment—before the onset of symptoms. Scientists in Finland are now giving this twist to the NIH experimental approach. They have given Cystagon treatment to a days-old infant whose parents had previously had a baby with INCL.

Mukherjee says the infant's circumstances are unusual—generally INCL is not anticipated (heterozygous carriers have no symptoms) or recognized until an affected infant shows symptoms later in its first year of life.

As word gets out about the Cystagon clinical trial, Mukherjee keenly awaits results from the next visits of his small patients and the recruitment of additional new patients. But he says that despite the drug's encouraging signs, he's not putting all his eggs in one basket. The research team is now starting to develop strategies for INCL gene therapy. ■

Bench-to-Bedside: Looking for Genomic Markers of Cancer

NCI Investigators Seek Earlier Alert to Breast Cancer Than Possible with Mammography

by Rashmi Nemade

Imaging techniques for the detection of breast cancer have been faulted for a lack of specificity—a shortcoming that is magnified in women genetically at high risk for breast cancer.

Women with a mutation in the *BRCA1* and/or *BRCA2* genes have a lifetime risk of breast cancer that is estimated to be between 56 and 85 percent. Many of these women will develop breast cancer at a young age when mammography is least likely to be effective because a small cancer can be obscured by the dense breast tissue of young women.

Innovative approaches to screening for early breast cancer in women at high genetic risk are clearly needed.

That is the crux of the Bench-to-Bedside project on "Genomic Changes in Premalignant, Preinvasive, and Invasive Breast Cancer in Women Genetically at High Risk for Breast Cancer," conducted by Ruthann Giusti, staff clinician in the Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, NCI, and Thomas Ried, a senior investigator in the Genetics Department of NCI.

Approximately 200 women at high genetic risk of breast cancer will be enrolled into a screening study that includes mammography, breast magnetic resonance imaging, positron emission tomography, and breast ductal lavage done annually for four years.

Breast cancer develops from the cells of the breast duct epithelium. Breast ductal lavage (BDL) is a newly developed method of cell collection that introduces a microcatheter into the nipple duct openings and recovers far greater numbers of ductal epithelial cells than is possible by traditional nipple aspiration.

The procedure is minimally invasive, safe, and well tolerated, Giusti says.

Ried and Kerstin Heselmeyer-Haddad, a research fellow in the NCI Genetics Department, are applying fluorescence in situ hybridization (FISH) analysis for specific tumor markers on BDL samples and have designed a probe set to analyze gains in oncogenes such as *myc* or *her* and losses in the *Tp53* tumor suppressor gene.

Rashmi Nemade

Collaborators: *(left to right) Ruthann Giusti, Kerstin Heselmeyer-Haddad, and Thomas Ried hope to establish the value of breast ductal lavage and ductal cell fluid analysis for earlier detection of breast cancer and breast cancer precursors in high-risk women*

FISH allows the analysis of ductal cell fluid for genomic imbalances known to occur in breast cancer—and it is hoped that this study will produce a sensitive, specific, and predictive test for the presence of tumor cells or cells that are committed to malignant transformation.

In a progress report on their project, the investigators note that "It has now been established that the pattern of genomic imbalances is both tumor site and stage specific. In breast cancer and its precursor lesions, the most recurring copy-number alterations map to chromosome 17 and to chromosome arms 1q, 8q, and 20q. The detection of this nonrandom and reproducible pattern of specific DNA gains and losses therefore has potential to complement standard morphological evaluation of cells with genetic markers of tumorigenesis."

"The advantage of this procedure," says Ried, "is that we can combine specific genetic markers with conventional cytopathology studies."

Preliminary studies have demonstrated the accuracy of these probe sets in analyzing cells from nipple aspirates, Heselmeyer-Haddad says, noting that "the FISH procedure can be completed in a matter of hours and with high-throughput automated analysis, one can have the results of a BDL sample the day after cell collection."

Analysis of BDL fluid will not replace the need for mammography, Giusti says, but detection of cancer at its earliest stages may provide patients with more options for clinical management of breast cancer.

Detection of precancerous changes, she notes "may help high-risk patients weigh the timing of the use of chemopreventives or prophylactic surgery to lower breast cancer risk and, we hope, enable us to learn more about the pathogenesis of breast cancer in this high-risk group." ■

A parallel BDL project is among the most recent Bench-to-Bedside awardees: *"Characterization of High-Risk Breast Duct Epithelium by Cytology, Breast Endoscopy, and cDNA Gene Expression Profile"* (David Danforth and Patricia Steeg, NCI).

Bench-to-Bedside: McCune-Albright Syndrome

NICHD, NIDCR Researchers Continue Probing Nature and Treatment of Mosaic Endocrine Disorder

by Fatima Husain

NIH has a long history of research on the McCune-Albright syndrome (MAS).

Penelope Feuillan, Gordon Cutler, and other NICHD researchers pioneered the treatment of the endocrinopathies in MAS patients, specifically those with precocious puberty, more than 15 years ago.

The molecular defect that causes MAS and polyostotic fibrous dysplasia (PFD)—activating mutations in a G-protein α subunit (Gs-α) that normally transmits signals from hormone receptors to effectors such as the enzyme adenylyl cyclase—was discovered by NIDDK researchers Lee Weinstein, Andy Shenker, Allen Spiegel, and others in the early 1990s.

Expanding on the work of the NIDDK scientists, Pamela Robey, Paolo Bianco, Michael Collins, Shlomo Wientroub, and other researchers in the NIDCR Craniofacial & Skeletal Diseases Branch (CSDB) have performed extensive analyses of PFD lesions, describing the complex interactions between mutated and nonmutated cells and characterizing the differences in PFD lesions in different parts of the skeleton.

As Robey recalls, "Andy Shenker and Allen Spiegel walked across the parking lot one day to my office and said, we now know what the mutations are but we don't know why they are causing the bone to form abnormally. That's how my branch got involved. This was in 1994."

Today, CSDB has become a major research center of PFD, with protocols for screening PFD patients and examining various therapies, including bone grafting and drugs.

In the current Bench-to-Bedside project, NICHD and NIDCR scientists, led by Feuillan, a clinical investigator in the Developmental Endocrinology Branch, NICHD, and Robey, chief of skeletal biology and acting chief of skeletal clinical studies, CSDB, NIDCR, are evaluating letrozole, an aromatase inhibitor that blocks the synthesis of estrogen, in treating precocious puberty and PFD in four girls between the ages of 5 and 6.5.

There are many reasons why precocious puberty should be treated as early as possible, the investigators say. The main one is to slow the effects of elevated sex steroid levels on closure of growth plates to attempt to maximize final adult height and minimize PFD-related lesions. Another reason is to minimize early breast growth and menses and ameliorate the associated social stigma.

Letrozole is a more potent and longer-acting drug than the currently used testolactone and can therefore be given twice rather than four times a day, Feuillan notes. It may offer patients an alternative to tamoxifen, which also blocks the action of estrogen and is being tested by other clinical researchers outside NIH. It's also possible, she adds, that some patients could be treated most effectively with both tamoxifen and letrozole.

Robey and her colleagues will evaluate the effect of letrozole in slowing bone maturation and the growth of lesions associated with PFD.

The bone age of patients enrolled in the current study ranged from 7.8–11.0 years. The first patient has completed the first 12 months (6 months on therapy, 6 months off therapy) of the trial and has experienced a decrease in the frequency of menses and in her mean ovarian volume. The other three patients are completing the on-therapy phase. The effect of the therapy on bone growth and lesions will be evaluated as data become available from the others.

Feuillan is guardedly optimistic that the therapy will be successful in delaying puberty and ameliorating the associated dysplasia. She hopes to enroll 15 to 20 patients and anticipates the project in its present form will take another one to two years.

"If letrozole is safe and effective," she says, "I would hope to offer uninterrupted treatment to all families who request it until our patients reach the age of normal puberty." ■

The author wishes to thank Lee Weinstein, NIDDK, for his help in preparing this article.

For more information on MAS, see **<http://www.nichd.nih.gov/publications/pubs/mccunetoc.htm>**.

For more information on PFD, see **<http://csdb.nidcr.nih.gov/csdb/frame_clinical_bkg.htm>**. For information on the current protocol, click on "Related Protocols" within the url above. ■

Fatima Husain

Penelope Feuillan

Fatima Husain

Pamela Robey

MAS Background

The McCune-Albright syndrome (MAS, first described in 1937) is characterized by at least two of a triad of features: (1) polyostotic fibrous dysplasia (PFD) that causes focal damage to bones, (2) hyperpigmentation, and (3) autonomous endocrine hyperfunction.

The endocrine disease includes early puberty with menstrual bleeding, development of breasts and pubic hair, and an increased rate of growth. Precocious puberty may start as early as infancy and is more common in girls—ovaries seem to be more susceptible to the G-protein malfunction associated with MAS.

Apart from the early onset, precocious puberty differs from normal puberty in that the brain centers normally mediating puberty are often not active in MAS and there is autonomous secretion of estrogen from the ovaries.

MAS is believed to be caused by genetic mutations within Gs-α occurring after the egg has been fertilized. This timing results in somatic mosaicism and varying degrees of severity depending on where the mutation is expressed. Apart from the reproductive organs, bones, and skin, predominantly endocrine tissue such as adrenal glands (leading to Cushing's syndrome), thyroid glands (resulting in goiter and other nodules or cysts and hyperthyroidism), and various pituitary populations (leading to acromegaly or gigantism) may be involved.

—Fatima Husain

Bench-to-Bedside Awards: The First Four Rounds

1998

"Pretargeted Therapy of Epithelial Cancers with Radiolabeled Monoclonal Antibody B3" **Ira Pastan, NCI; Jorge Carrasquillo, CC**

"Gene Therapy for X-linked Severe Combined Immunodeficiency" **Jennifer Puck, NHGRI; Harry Malech, NIAID**

"Vasculogenesis Using Progenitor Cells" **Arshed Quyyumi, NHLBI; Toren Finkel, NHLBI**

"Evaluation of Therapeutic Reduction of Raised Elastase Levels by Reducing Neutrophil Numbers or by Direct Anti-elastase Treatment of the Acceleration of the Rate of Healing" **Sharon Wahl, NIDCR; Annette Wysocki, NINR**

"Gene Therapy for Chronic Cancer and Arthritic Pain" **Michael Iadorola, NIDCR; Ray Dionne, NIDCR; J. Klippel, NIAMS**

"Development of Novel Therapies for Sickle Cell Disease" **Alan Schechter, NIDDK; Larry Keefer, NCI; Frederick Ognibene, CC**

"Production of Clinical Grade ITXC to Perform Clinical Trials for Treartment of Muscle Spasm Disorders (e.g., Torticollis and Blepharospasm) at NIH" **Richard Youle, NINDS; Mark Hallett, NINDS**

"Study of the Effect of the Humanized Monoclonal Antibody Against the Interleukin-2 Receptor Alpha Subunit (Il-2Rα, Zenapax®) on Inflammatory Activity in the Central Nervous System in Multiple Sclerosis in a Baseline-to-Treatment, Crossover, MRI-Controlled Single-Center, Phase I/II Trial" **Roland Martin, NINDS; Henry McFarland, NINDS; Tom Waldmann, NCI**

1999

"Phase I Study of 5-aza-2'dexycytidine in Lung Cancer: Tumor Response and Analysis of Altered Gene Expression and Chromatin Structure Following DNA Demethylation in Vivo" **Frederic Kaye, NCI; David Schrump, NCI; Alan Wolffe, NICHD**

"Treatment of Infantile Neuronal Ceroid Lipofuscinosis Patients with Phosphocysteamine" **Anil Mukherjee, NICHD; Rafael Caruso, NEI**

"Tumor-Specific Replicating Vaccinia Virus Expressing Cytosine Deaminase for Therapy of Metastatic Colorectal Cancer" **David Bartlett, NCI; Bernard Moss, NIAID; H. R. Alexander, NCI; Steve Libutti, NCI; Richard Chang, CC; Clara Chen, CC**

"Anti-Tumor Immunotherapy Linking the Mouse to the Human Experience Through the Danger Model" **Polly Matzinger, NIAID; Francesco Marincola, NCI**

"Effect of the Aromatase Inhibitor Letrozole on Estrogen Levels and Fibrous Dysplasia of Bone in Patients with McCune-Albright Syndrome" **Penelope Feuillan, NICHD; Pamela Robey, NIDCR**

"Genotypic and Phenoptypic Dissection of the Smith-Magenis Syndrome: An Interdisciplinary Study of Physical, Cognitive, and Neurobehav-ioral Abnormalities in SMS" **Ann Smith, NHGRI; Thomas Friedman, NIDCD; J. Blancato, Georgetown; Andre Gropman, NHGRI; P. Wolters, CC; Barbara Sonies, CC; Beth Soloman, CC: Andrew Griffith, NIDCD; Judith Rapoport, NIMH; Jay Giedd, NIMH; R. Nicholson, NIMH**

"Inhibition of Angiogenesis in Severe Early Rheumatoid Arthritis" **Hani El-Gabalawy, NIAMS; William Eckelman, CC; Jorge Carrasquillo, CC: Bob Balaban, NHLBI; S. Koenig, MedImmune, Inc.**

"Selective Depletion of Donor Lymphocytes Causing Graft-versus-Host Disease to Improve Outcome of Allogeneic Stem Cell Transplantation" **John Barrett, NHLBI; Elizabeth Read, CC**

"Studies of CD40 Ligand trimer as an Adjuvant for HIV-1 Vaccines" **Genoveffa Franchini, NCI; Ashish Jain, NIAID; Warren Strober, NIAID; Joe Kovacs, NIAID; Robert Seder, NIAID**

2000

"Magnetic Resonance Elastography: A Clinical Technique in the Management of Malignant Acute Hemispheric Stroke with Implications for Patient Intervention by Hemicraniectomy and Duroplasty" **David Moore, NINDS; Emilio Dimitriadis, DBEPS**

"A Phase I/II Pilot Study to Evaluate the Induction of Immune Tolerance in Patients with Sight-Threatening Autoimmune Uveitis Treated with Zenapax and Rapamycin" **Jack Ragheb, NEI; Tom Waldmann, NCI; Robert Nussenblatt, NEI**

"*Use of IL-10 to Improve the Therapeutic Window of Cisplatin"* **Robert Starr, NIDDK; David Bartlett, NCI; R. Alexander, NCI**

"Targeted Delivery of Nitric Oxide by Hemoglobin to Improve Regional Blood Flow in Sickle Cell Disease" **Frederick Ognibene, CC; Richard Cannon, NHLBI; Mark Gladwin, CC**

"Combination Antiviral and Immunomodu-latory Therapy for Chronic Hepatitis B" **Marc Ghany, NIDDK; Barbara Rehermann, NIDDK; Harvey Alter, CC**

"Mutation of Human Growth Hormone (hGH) Sorting Motifs to Facilitate Gene Therapeutics Applications with Salivary Glands in Adult hGH-Deficient Patients" **Bruce Baum, NIDCR; Y Peng Loh, NICHD**

"Genomic Changes in Premalignant, Preinvasive, and Invasive Breast cancer in Women Genetically at High Risk for Breast Cancer" **Ruthann Giusti, NCI; Thomas Ried, NCI**

"Treatment of Smith-Lemli-Opitz Syndrome with Simvastatin" **Forbes Porter, NICHD; Alfred Yergey, NICHD; E. Tierney, The Kennedy Krieger Institute**

"New Treatments for Intractable Pain" **Michael Iadorola, NIDCR; Ann Berger, NIDCR/CC**

2001

"T Cell-Depleting Monoclonal Antibody Campath-1H in patients wioth Inclusion Body Myositis: Correlation of Clinical Response with Changes in Endomysial T-Cell Epitopes, Inflammatory Cytokines, and Costimulatory Molecules" **Marinos Dalakas, NINDS; Roland Martin, NINDS;**

"Alloreactive Natural Killer (NK) Cell Immunotherapy to Improve Outcome of Allogeneic Stem Cell Transplantation" **John Barrett, NHLBI; Elizabeth Read, CC**

"Intracellular calcium Measurement in Adipocytes (ICMA): An Adjunct to the Study of Supplemental Calcium in Overweight Outpatients (SCOOP) Study" **Shamik Parikh, NICHD; Paul Blank, NICHD**

"Impact on Platelet Survival of Donor/Recipient Selection based on Definitive Sequence-Based HLA Typing" **Susan Leitman, CC; Francesco Marincola, CC**

"Characterization of High-Risk Breast Duct Epithelium by Cytology, Breast Endoscopy, and cDNA Gene Expression Profile" **David Danforth, NCI; Patricia Steeg, NCI**

"Potential Involvement of a Brain-Specific Isoform of the Winged Helix Transcription Factor RFX4 in Human Congenital Hydrocephalus" **Perry Blackshear, NIEHS; Darryl Zeldin, NIEHS**

Recently Tenured

Lawrence Brody *received his Ph.D. in Human Genetics from the Johns Hopkins University, Baltimore, in 1991. He was a Howard Hughes postdoctoral fellow at the University of Michigan before coming to NHGRI in 1994. He is currently a senior investigator in the Genome Technology Branch and head of the Molecular Pathogenesis Section, NHGRI.*

Fran Pollner

Lawrence Brody

Identification and evaluation of human gene variants important to health have not kept pace with the rush of gene discovery from the Human Genome Project, and our ability to deduce the function of novel genes remains limited. Research in my section is focused on defining genetic variations that underlie disease and understanding the mechanism by which these variant alleles produce disease.

To accomplish this, we use research tools traditionally associated with diverse disciplines such as genetic epidemiology, evolutionary biology, cell biology, biochemistry, and physiology. Our integrated approach, combining investigation into genetic variation and biological function, allows us to take maximum advantage of the wealth of data produced by the Human Genome Project.

One of our research interests is the study of the genes *BRCA1* and *BRCA2* and their role in inherited breast and ovarian cancer susceptibility. Although the link between these genes and cancer risk is well established, it is based on the averaging of data from the more than 600 different mutations.

We have very limited knowledge of the risk associated in carrying a *specific BRCA1* mutation. In collaboration with investigators at NCI, our laboratory discovered that a small set of specific *BRCA1* mutations are present at a high frequency in the Ashkenazi Jewish population.

This finding allowed us to measure the risk associated with these mutations in an unselected population. We are continuing to study this population in order to better understand the risk of cancer associated with these mutations and to identify additional genes that may modify this risk.

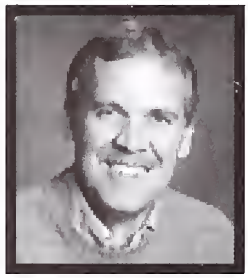
The biological function of the *BRCA1* and *BRCA2* genes remains poorly understood. Work from numerous labs suggests that *BRCA1* controls some aspect of DNA repair. Our own investigations into the function of the *BRCA1* gene revealed that *BRCA1* interacts with the proteins of the chromatin-remodeling complex.

We recently found that *BRCA1* plays an important role in the regulation of the cell cycle. By restoring *BRCA1* to *BRCA1*-minus cells, we were able to demonstrate that *BRCA1* is essential for maintaining the DNA damage checkpoint preceding the G2-to-M transition. *BRCA1* appears to control this activity by directly interacting with the cell cycle regulator.

Our second area of interest is the identification of genes associated with the risk of neural tube defects (NTDs). These defects are a major public health concern, yet the pathogenesis of NTDs is poorly understood. In collaboration with investigators at the NICHD and Trinity College, Dublin, we are searching for genes controlling disease risk in a large series of NTD families.

In contrast to breast cancer, there are epidemiological clues to the identity of the genes responsible for the inherited component of NTD risk. Past research established that perturbations of the metabolic pathways involving folate, vitamin B12, and homocysteine can account for a large fraction of NTD cases. The genes making up these metabolic pathways are logical candidates for putative "NTD genes." We identified human genetic variants in the majority of the genes comprising these pathways. We are currently measuring the frequency of these variants in NTD families and control subjects. In addition to measuring the connection between specific variants and the risk of having an NTD, we plan to measure the biochemical and functional consequences of these variants in experimental models and in the patients who carry them.

Steven Kleeberger *received his Ph.D. in ecology from Kent (Ohio) State University in 1982 and did his postdoctoral research at Johns Hopkins University, Baltimore, where he became a full professor. He was recruited to NIEHS as chief of the Laboratory of Pulmonary Pathobiology in 2001. He also directs a research group in environmental genetics.*



Steven Kleeberger

Epidemiological studies have shown that exposures to outdoor and indoor pollutants are associated with increased morbidity and mortality in cities throughout the United States and other industrialized countries. In particular, ozone—a highly reactive and toxic oxidizing pollutant—and particulates have become prevalent and have received considerable attention.

Because of the potential effect that pollutants may have on public health, identification of the intrinsic and extrinsic factors that may influence the pulmonary response(s) to exposures is an important issue. Over the past few years, my laboratory has focused considerable effort on understanding the role of genetic background as a susceptibility factor in the toxic effects of common air pollutants. Our lab combines state-of-the-art methods in inhalation toxicology, pulmonary physiology, and molecular genetics to address this goal.

Our initial analyses of susceptibility to inflammation and injury induced by ozone indicated that susceptibility was controlled by a locus we termed *Inf-2*. We subsequently identified a quantitative trait locus (QTL) for ozone susceptibility on chromosome 17. Candidate genes for the locus include the pro-inflammatory cytokine tumor necrosis factor–α (*Tnf*). Functional analyses of this locus confirmed *Tnf* as a candidate gene.

More recently, we have identified another ozone susceptibility QTL on chromosome 4 that contains a candidate gene, toll-like receptor 4 (*Tlr4*). *Tlr4* has recently been implicated in innate immunity and endotoxin susceptibility. Functional analyses confirmed an important role for *Tlr4* in oxidant lung injury.

Results of our linkage analyses therefore suggest a genetic commonality between signal transduction pathways involved in determining ozone and endotoxin responsivity. We believe this work will have important implications for understanding mechanisms of oxidant lung injury as well as exacerbation of preexisting diseases, such as asthma.

We have also recently identified susceptibility loci for alveolar macrophage immune dysfunction induced by inhalation of sulfate-associated carbon particles in differentially susceptible inbred mice. A genome-wide linkage analysis identified significant and suggestive QTLs on chromosomes 17 and 11, respectively. Candidate susceptibility genes were identified for mouse and human by comparative mapping. Importantly, both QTLs overlap previously identified QTLs for susceptibility to another common pollutant, ozone. This is the first demonstration that genetic background is an important determinant of responsiveness to particle-induced immune dysfunction—which has important implications for understanding the epidemiological associations between particulates and morbidity and mortality.

Our research focus has expanded to include genetic pathogenesis of hyperoxia-induced acute lung injury. Hyperoxia exposure induces lung responses that resemble subphenotypes of acute respiratory distress syndrome (ARDS), a major lung disease characterized by noncardiogenic edema and inflammation. We have developed a mouse model of susceptibility to hyperoxia exposure and identified significant and suggestive susceptibility QTLs on chromosomes 2 and 3, respectively. We have further identified—within the chromosome 2 QTL—a candidate susceptibility gene, NF-E2 related factor 2 (*Nrf2*), an essential nuclear transcription factor involved in antioxidant gene expression and regulation. We used sequence analysis to identify the polymorphism in the *Nrf2* promoter that confers differential susceptibility to hyperoxic injury in this model. Functional analyses with a *Nrf2* knockout mouse confirmed this observation. We believe this model has important implications for developing treatments for oxidant lung injury and ARDS.

Another aspect of my research is focused on gene-environment interaction and the pathogenesis of disease in human populations. In this work, we are participating in genetic analysis of asthma pathogenesis in a large population-based case-control study in Finland. We are also collaborating with Francine Kaufmann and Rachel Nadif of INSERM to identify the genetic basis of susceptibility to pneumoconiosis in a cohort of coal miners in France. These studies look at the interaction of polymorphisms in innate immunity and pro-inflammatory genes with occupational exposures to coal dust. We are also examining the role of innate immunity genes in determining susceptibility to HIV infection and AIDS progression in the NIH-sponsored Multicenter AIDS Cohort Study.

The integration of predictive animal genetic models with population-based epidemiological studies positions my laboratory to take advantage of the exciting progress in genome sequencing and proteomics, the better to tackle the etiology of environmental lung diseases, identify genetically susceptible individuals, and design interventions.

Ward Odenwald *received his Ph.D. from the combined Johns Hopkins University–NIH FAES program in 1987 after having joined the NINDS Medical Neurology Branch in 1978. He is now a senior investigator for the Neurogenetics Unit, LNC, NINDS.*

The long-term goal of my research is understanding the molecular events that guide cell fate decisions in the developing nervous system. Over the last four years, my unit's studies on Drosophila CNS cell-fate patterning reveal that a transcription factor regulatory network acts temporally during stem cell lineage development in all CNS ganglia.

Fran Pollner

Ward Odenwald

We have discovered that during neuroblast (NB) lineage formation many NBs undergo sequential transitions in the expression of the transcription factors: *hunchback* (*hb*), the *POU domain 1* and *2* genes (*pdm1/2*), *castor* (*cas*), and *grainy head* (*grh*). As a result of the *hb->pdm1/2->cas->grh* (H-P-C-G) NB expression, sequentially formed basal (inner and first born)-to-apical (outer and last born), multilayered neuronal subpopulations arise that share expression of one of these factors.

Our loss-of-function analysis of four of the five H-P-C-G network components (*hb*, *pdm1/2*, and *cas*) indicate that this temporal regulatory cascade is required for the proper development of many, if not all, neuronal subtypes.

Given the global nature of the H-P-C-G cascade, we hypothesize that these transcription factor expression domains represent fundamental branch points in a genetic circuit controlling temporally sensitive functions common to the development of all CNS ganglia. Our analysis of *POU* gene regulation by Cas demonstrates that Cas performs at least two essential roles.

First, Cas insulates the developmental program(s) of the sublineages that express it by repressing neural identity regulators (such as *pdm1/2*) that participate in the cell fate decisions of neuronal subtypes born earlier. Second, Cas promotes neural identity decisions within its sublineages by directing and promoting expression of other neural identity *POU* genes.

Our in vitro analysis of the regulatory mechanisms controlling the orchestrated expression of the H-P-C-G network factors indicates that once NBs initiate lineage formation, no additional extrinsic cues from either the neuroectoderm or underlying mesoderm are required. Given the remarkable conservation observed in metazoan transcription factor networks and the identification of putative H-P-C-G cognate genes in mammals, we believe that all or part of the temporal regulatory network may function during vertebrate CNS development. In order to fully understand the biological significance of the H-P-C-G cascade and how its transcription factors integrate with upstream cell fate decisions, we need to identify the downstream targets of these regulators and to understand the time-dependent signals that regulate it.

To identify new components of the network, we have recently carried out differential gene expression, enhancer-trap, and mutant screens for novel genes that are either dynamically expressed during NB lineage development or required for proper expression of the H-P-C-G network. Our developmental expression screen of cDNAs prepared from staged embryonic fruit fly heads has thus far identified 57 new genes that are dynamically expressed during NB lineage development. Our experimental approach is now aimed at understanding their roles in NB lineage development. These genes—plus 1,837 other genes identified in the screen—are described at our website:

<http://sdb.bio.purdue.edu/fly/brain/ahome.htm>.

Recently Tenured

Ignacio Rodriguez *received his Ph.D. in 1986 from the University of Miami in Coral Gables, Fla. He did his initial postdoctoral training at the Bascom Palmer Eye Institute in Miami, Fla. He joined the National Eye Institute in 1987 and continued his postdoctoral training in several laboratories. He is now a senior investigator at the Laboratory of Retinal Cell and Molecular Biology, NEI.*

My main interests are in understanding the molecular mechanisms of age-related diseases. I am particularly interested in those affecting the eye, more specifically, age-related macular degeneration (AMD). AMD is the leading cause of blindness in the United States, responsible for severely reducing the quality of life of millions of our senior citizens.

Fran Pollner

Ignacio Rodriguez

This disease is complex and scientifically very challenging because it involves aging, genetics, and environmental factors. As biological systems, humans are not designed to live much beyond middle age. Thus, there are fundamental design flaws in our biology that make certain organ systems susceptible to age-related malfunctions. The eye is a particularly interesting system to study because AMD and cataracts are often found in individuals who have survived into their late 60s and 70s in relatively good health.

I originally trained as a carbohydrate biochemist and started working in vision research during my first postdoctoral fellowship at the Bascom Palmer Eye Institute. After joining NEI in late 1987, I trained as a molecular biologist, initially in the Laboratory of Ophthalmic Genetics and Visual Function Branch and later in the Laboratory of Mechanisms of Ocular Diseases (LMOD). While at the LMOD, I identified a splice site deletion in the guinea pig z-crystallin gene responsible for a hereditary cataract. I joined the Laboratory of Retinal Cell and Molecular Biology in 1990 and began cloning retina-related genes and genes differentially expressed between the macular and peripheral regions of the retina. This research led to the cloning and characterization of 10 novel human genes and several orthologs in other species.

My research program is now focused on determining the pathogenesis mechanism(s) of AMD. We have been searching for common age-related factors that may be adversely affecting the health of the retinal pigment epithelium (RPE) and the choriocapillaris. The observed accumulation of lipids and lipoproteins in the back of the eye as a function of aging is particularly interesting because of the known cytotoxic effects of oxidized LDL and oxysterols. The RPE expresses a variety of receptors capable of internalizing LDL and is believed to shuttle cholesterol to the photoreceptors. We have found that oxidized LDL and certain oxysterols are highly cytotoxic to cultured human RPE cells. We hypothesize that this lipid accumulation in Bruch's membrane leads to its hyperoxidation, which can adversely affect the RPE and choriocapillaris.

The immune system may also play a role in the pathogenesis of AMD given that scavenging macrophages that encounter the oxidized LDL in Bruch's membrane experience cytotoxicity. This could lead to an inflammatory response that may contribute to the drusen deposits observed during the early stages of the disease.

Oxysterols have potent pharmacological properties and are likely the most cytotoxic substances associated with LDL and other lipoproteins. Thus, we have been cloning and characterizing oxysterol-binding proteins (OSBPs). We recently characterized a novel OSBP, OSBP2, which is almost exclusively expressed in retina and has a high affinity for one of the most toxic oxysterols, 7-ketocholesterol. We have also identified and cloned 10 additional and previously uncharacterized OSBP genes.

The OSBPs, although highly similar in structure, have different expression patterns. In addition, 11 of the 12 known OSBPs contain Pleckstrin homology (PH) domains. These PH domains are known to bind phosphinositols and are likely serving to target the OSBPs to different cellular organelles. Future work will focus on elucidating the function(s) of the OSBPs and determining whether they mediate the pharmacological effects of oxysterols.

William Simonds *received his M.D. from the University of Pittsburgh in 1981 and did postdoctoral work in the Laboratory of General and Comparative Biochemistry and Laboratory of Molecular Biology in NIMH before joining the Molecular Pathophysiology Branch of NIDDK in 1987. He is now a senior clinical investigator in the Metabolic Diseases Branch, NIDDK.*

My basic science interests have centered on biochemical and signal-transducing properties of the G protein–βγ complex and related heterodimers. The tightly associated βγ complex of heterotrimeric G proteins is required for activation of G-protein pathways by seven transmembrane-spanning receptors in response to such diverse extracellular stimuli as neurotransmitters, hormones, pheromones, light, tastants, and odorants.

Fran Pollner

William Simonds

Activation of G proteins by receptors results in dissociation of the α subunit (in GTP-bound form) from the βγ complex. The Gα-GTP and/or the free Gβγ complex can regulate a variety of downstream effector molecules depending on the cellular context. The βγ complex regulates the function of ion channels, isoforms of adenylyl cyclase (AC), isoforms of phospholipase C-β (PLC-β), and MAP kinase (MAPK) pathways and thus mediates critical cellular functions such as chemotaxis, response to growth factors, and regulation of neurotransmitter release.

Despite the multiplicity of potential Gβγ dimers implied by the existence of multiple genes encoding Gβ and Gγ isoforms, Gβγ heterodimers had been considered functionally interchangeable. This view prevailed until my colleagues and I provided the first clear demonstration of functional specialization among Gβ subunits in an analysis of the effector properties of brain-specific Gβ5. Gβ5-Gγ2 complexes stimulated PLC-β like Gβ1-Gγ2, but unlike the latter complex, Gβ5-Gγ2 failed to activate MAPK, Akt/PKB, and AC type II.

We hypothesized that the unique functional properties of Gβ5 might indicate

its specialization to interact with novel effectors. This was borne out when we purified native Gβ5 complexes from mouse brain and discovered tightly bound regulator of G-protein signaling (RGS) proteins 6 and 7, not Gα or Gγ subunits. This unexpected finding complemented work from other laboratories describing Gβ5-RGS protein complexes in the retina and identifying a subset of RGS proteins containing a "Gγ-like" domain that could mediate binding to Gβ5 in a Gγ-like fashion.

Using neuron-like PC12 cells, we discovered that Gβ5 and RGS7 proteins are expressed in neuronal cell nuclei, a novel subcellular localization for G proteins that we confirmed in mouse brain.

These findings raise the possibility of information transfer by Gβ5-RGS protein complexes between the plasma membrane and nuclear compartments in neurons. We are now looking for the signals that may regulate the function and/or subcellular localization of Gβ5-RGS protein complexes in brain and trying to learn whether these complexes might regulate transcriptional events in a signal-dependent fashion.

My clinical interest involves familial isolated hyperparathyroidism (FIH) and related disorders. We recently reported a series of 36 FIH kindreds seen in the Clinical Center over the past 30 years. In these 36 families, genomic DNA mutational and biochemical testing largely excluded multiple endocrine neoplasia type 1 as an etiology. Among the total set of families, we identified three kindreds with the hyperparathyroidism–jaw tumor syndrome (HPT-JT), five kindreds with mutations in the gene for the calcium-sensing receptor, and 28 kindreds for whom no syndromic diagnosis could be made.

Future testing for *HPT-JT* mutations should clarify whether any of the 28 nonsyndromic families have currently unrecognized causes of FIH. We are working with colleagues at NHGRI and others in an international consortium to identify the gene on chromosome 1 responsible for the HPT-JT familial cancer syndrome.

Stanislav Tomarev *received his Ph.D. from the Russian Academy of Sciences in Moscow in 1977 and worked at the N.K. Koltzov Institute of Developmental Biology of the Russian Academy of Sciences as a group leader and leading investigator before joining the NEI Laboratory of Molecular and Developmental Biology in 1989. He is now a senior investigator in the same laboratory.*

Fran Pollner

Stanislav Tomarev

My scientific interests are in the area of molecular mechanisms underlying glaucoma. Glaucoma is the second leading cause of blindness in the United States and affects between one and two percent of people over the age of 40. Glaucoma is a group of neurodegenerative disorders characterized by the death of retinal ganglion cells and by a specific deformation of the optic nerve head, known as glaucomatous cupping. This disease is also often associated with elevated intraocular pressure (IOP). The molecular mechanisms underlying neuronal damage associated with elevated IOP are not understood.

My group is developing mouse and rat models of glaucoma using genetic approaches. In 1997, my colleagues and I isolated and characterized the mouse myocilin gene. Mutations in this gene lead to autosomal dominant juvenile open-angle glaucoma with elevated IOP in humans and may account for 2.6–4.3 percent of adult forms of open-angle glaucoma.

Using myocilin knockout mice, we demonstrated that the absence of myocilin does not lead to glaucoma and therefore glaucoma-causing mutations most probably act by a gain-of-function mechanism. Overproduction of myocilin in the ocular tissues, using myocilin-containing BAC clones, also fails to produce glaucomatous changes. We demonstrated that mutations in the mouse myocilin gene, corresponding to glaucoma-causing mutations in the human myocilin gene, reduce secretion of the protein. We are currently generating transgenic mice expressing mutated forms of myocilin in the ocular tissues and we believe that this approach will produce a useful new animal model of glaucoma.

To understand mechanisms of myocilin action, we are looking for proteins that interact with myocilin. We identified a new secreted protein, named optimedin, that interacts with myocilin. Both optimedin and myocilin contain the olfactomedin domain that was previously identified in several biologically active proteins, and we demonstrated that myocilin and optimedin interact through this domain. We showed that the presence of mutant myocilin interferes with secretion of optimedin in transfected cells. We are testing the hypothesis that changes in the expression level and distribution of optimedin in eye tissues in myocilin-related glaucoma contribute to the pathology of myocilin-related glaucoma.

My colleagues and I are using rat models of pressure-induced optic nerve damage to identify changes in the retina at different stages of retinal damage. We demonstrated that molecular pathways activated by elevated IOP overlap those induced by optic nerve transection. We showed that elevated IOP may stimulate a group of genes normally associated with inflammation and the immune response. These findings suggest new possibilities for treating glaucoma.

We are also looking for glaucoma-associated genes in the cDNAs from the human trabecular meshwork—a key component of the aqueous humor outflow pathway in the eye that is essential for maintaining normal IOP. This approach has allowed us to identify several new candidate genes that we are probing by examining families of patients with glaucoma.

We hope that by elucidating the molecular changes in the eye associated with glaucoma, we may improve diagnostic tools and treatments for this blinding disease. ■

Long Term Care On Menu

Long-term care (LTC) insurance is now an option for federal employees. Coverage includes but is not limited to care in a nursing home, assisted living facility, home, or hospice, as well as respite care.

Early enrollment runs from March 25 to May 15, 2002. Regular open enrollment runs from July 1 to Dec. 31, 2002.

A forum on the federal LTC insurance program, held at Natcher March 6, can be viewed at **<http://videocast.nih.gov/PastEvents.asp>**.

Questions can be directed to institute and center human resources offices. ■

Xenogenization a Major Focus Of New Fogarty Scholar

A major research focus of Jacob Hochman of the Hebrew University of Jerusalem has been xenogenization (a process by which tumor cells are rendered immunogenic instead of tumorigenic), growth regulation, and metastasis of malignant lymphoma. His unique mouse model of these processes is the only one currently available that can also be used for molecular analysis of the infiltration of lymphoma through the blood-brain barrier into the brain and subsequent migration along the optic nerve sheath into the eye.

Among Hochman's collaborators over the years are NIH scientists who have pressed for his nomination to be a Fogarty Scholar and with whom he will be working during his Fogarty tenure here (divided over several years; this year, from June to September).

■ With John Hanover, chief of the Laboratory of Cell Biochemistry and Biology, NIDDK, Hochman plans to continue studies on the role of nucleolar MMTV env-precursor proteins p14 and p21 in regulating tumorigenicity and immunogenicity of the experimental lymphoma model as well as in other lymphomas harboring MMTV genes.

■ With Michael Gottesman, chief of the Laboratory of Cell Biology, NCI, he plans to use MDR vectors for the selective overexpression of p14 and p21 in recipient cells of differing backgrounds and study their tumorigenic and immunogenic potential. The contribution, direct or indirect, of the MDR P-glycoprotein to metastasis in the lymphoma model to the brain and eyes will also be assessed.

Fran Pollner

Jacob Hochman

■ With Michael Bustin, chief of the Protein Section, Laboratory of Metabolism, NCI, he plans to study the possible interaction of p14 and p21 with chromatin.

■ With Larry Wahl, chief of the Immunopathology Section, NIDCR, Hochman has initiated study of the role of matrix metalloproteinases in the metastasis of lymphoma cells into the brain.

■ Collaborating with NEI investigators, he will investigate lymphoma metastasis into and within the different compartments of the eye. ■

From Bench to Business

A program designed to introduce NIH scientists to entrepreneurship in biotechnology and science will be held Thursday, April 18, from 1:00 to 5:15 p.m. in the Masur Auditorium. A reception will follow.

"Moving from the Bench into Business: Entrepreneurship in Science" is aimed at anyone who has ever thought about starting a business after leaving NIH or transferring their discoveries to others for this purpose. The program will include:

■ The essential elements of a successful business plan
■ Presentations by entrepreneurs who transitioned from the bench to business
■ Resources available to young companies
■ Insight into the differences in workplace cultures
■ The opportunity to network with interested colleagues and biotech entrepreneurs

Admission is free, but registration is requested. Log on to <**http://www.otir.cancer.gov/registration**>. The event will also be webcast at <**http://www.videocast.nih.gov**>. Sign language interpretation provided on request—call Christy Meek at (301) 451-6835. This program is sponsored by the Macklin Business Institute Center for Entrepreneurship of Montgomery College, NCI Office of Technology and Industrial Relations, NCI Center for Cancer Research, NCI Technology Transfer Branch, NCI Fellowship Office, NIH Office of Education, NIH Foundation for Advanced Education in the Sciences, and the NIH Graduate Program Partnerships Office. ■

CME Credit Available Online

Physicians can now earn CME credits online, and at no cost, thanks to The Oncologist CME Online service.

This comprehensive collection of CME-accredited courses focuses on cancer, as well as risk management and supportive care. Physicians generally, and oncologists specifically, who are interested in fulfilling state board certification requirements without leaving their desks are invited to explore this service. The Oncologist CME Online also offers a portal to other online CME activities hosted by NIH.

Each registered user receives a unique online personal folder ("My CME") that maintains a collection of completed CME courses as well as access to printable certificates for completed courses. Completed course credits are forwarded to the Accreditation Council for Continuing Medical Education for official acceptance and are then available to state board licensure agencies.

The NIH/Foundation for Advanced Education in the Sciences designates this educational activity for a period of 3 years in category 1 credit towards the AMA Physician's Recognition Award.

Courses submitted for CME credit on or before December 31, 2002, will be recorded in 2002. Courses submitted for CME credit on or after January 1, 2003, will be recorded in 2003.

For more information, visit The Oncologist CME Online service website at <**http://cme.alphamedpress.org**>.

To register, click onto <**http://cme.alphamedpress.org/subscriptions/subscaution.shtml**>.

New Fellowship

The Foundation for the NIH and NCI will present the first annual Sallie Rosen Kaplan Fellowship for Women Scientists in Cancer Research Training Award (CRTA) on **Thursday, April 4, 2002, at 4:00 p.m. in the Natcher Building, Room A.**

In this inaugural year of the program, the award will be made to extend the fellowship of a current postdoctoral CRTA recipient by one year. The intent of the extension is to allow the recipient to continue productive research that would otherwise be cut short were the fellowship appointment to end as scheduled.

In future years, NCI will hold an annual, national competition through which one or more starting CRTA postdocs will be honored as Sallie Rosen Kaplan Fellows. ■

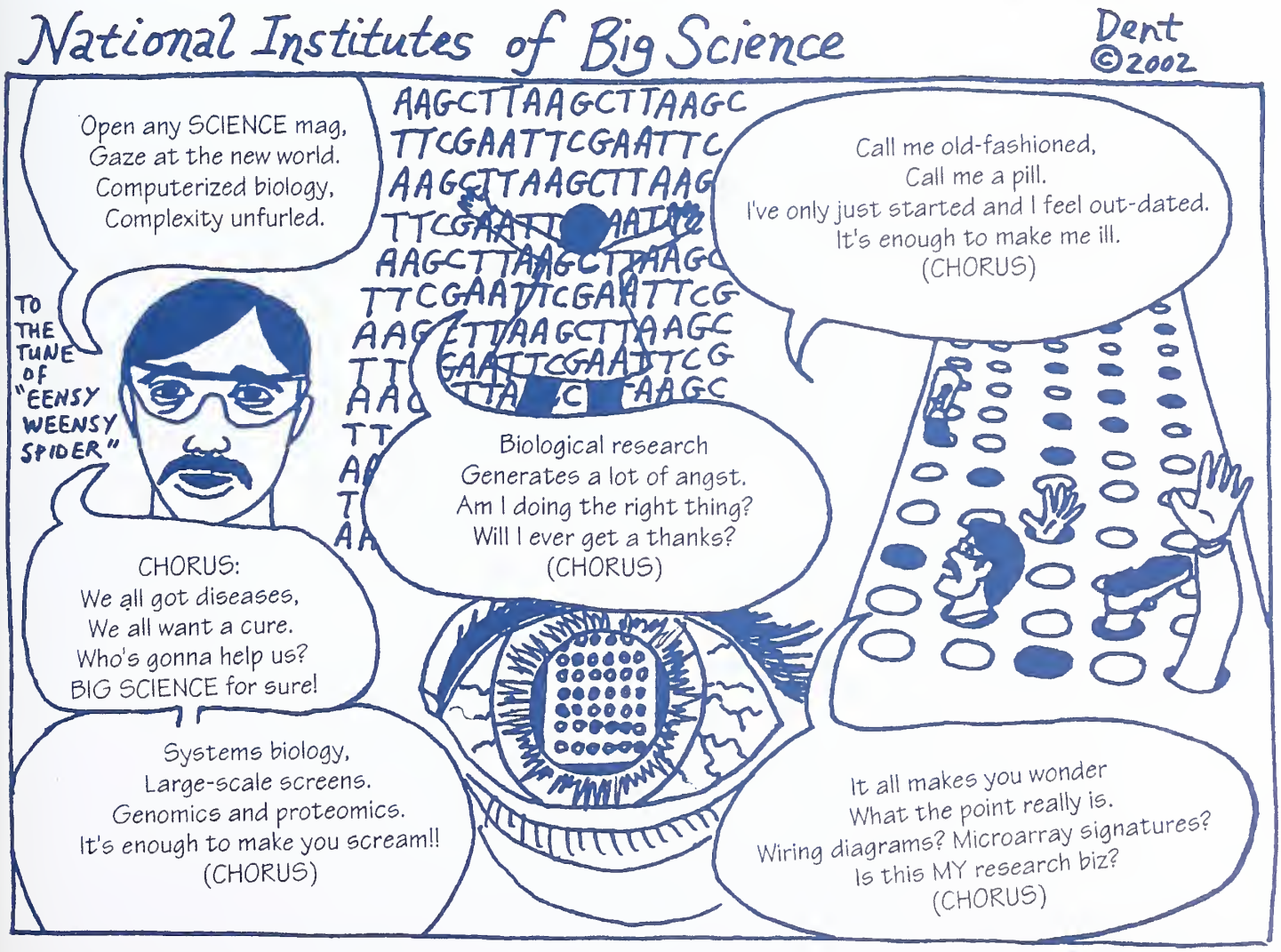
To Know What's Going On at NIH . . .

. . . Click onto the **DDIR's Web Board**. It comes out about once a month with news about key intramural policy changes, fellowship opportunities, tenure-track positions, interest group news, upcoming lectures and workshops, electronic resources, and cool pix of intramural research.

To view the newsletter, go to the site's new url:
<http://ddir.nih.gov/ddir/>.

To subscribe, send an e-mail message to
<Listserv@list.nih.gov>.

Substituting your name for Jan Doe's, your message should say: Subscribe DDIRBB-L Jan Doe. Alternatively, visit the ListServ website for this Bulletin Board at
<http://list.nih.gov/cgi-bin/wa?SUBED1=ddirbb-l&A=1>
and type in your name and e-mail address. ■



CALL FOR CATALYTIC REACTIONS

In this issue, we are asking for your reactions in four areas: translational research, meeting travel, ATLAS small-animal PET studies, and giving credit within "Big Science" projects.

Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2W23.

In Future Issues...

- IRP Accomplishments and GPRA
- New Building Views and Blues
- NCI's New Director

1) In addition to a competitive awards program, how else can the intramural program encourage bench-to-bedside translational research?

2) What is your reaction to the elimination of the "Rule of Five" for meeting travel? How is the new system playing out in your institute?

3) How might the ATLAS small-animal PET scanner be useful to you in your studies?

4) With reference to the Alex Dent cartoon (page 15), do you have any suggestions on how to reduce the anxieties and best recognize the contributions of team members involved in "Big Science" projects?

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 2, Room 2W23, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

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