FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 15, ISSUE 1 ■ JANUARY–FEBRUARY 2007

Shedding Light on Deep Tissue

BIOPHOTONICS: THE NEW "AGE OF ENLIGHTENMENT"

by Fran Pollner

Fran Pollner

Amir Gandjbakhche leans into his lab's home for mice in the mouse-imaging area in Building 9

Sometimes Dr. McCoy's handheld device rendered an instant diagnosis as he waved it over a fallen crew member of the Star Ship *Enterprise*; sometimes it achieved an instant cure.

Such rapid, noninvasive bedside management is not as much a fantasy as one might think.

Amir Gandjbakhche calls it his "dream," but it's a dream that gets closer to reality, he says, with each advance in optical-imaging research.

"They say the 18th century was the 'Age of Enlightenment.' But, really, it's the 21st century. It's optical imaging that's enlightening us, moving us from subjective to quantitative diagnosis," says Gandjbakhche, chief of the Section on Biomedical Stochastic Physics in the Laboratory of Integrative & Medical Biophysics, NICHD.

Gandjbakhche and his team collaborate with other NIH investigators in animal studies and on clinical protocols that involve noninvasive in vivo optical imaging to characterize the physiologic and metabolic environment of diseased tissues.

continued on page 4

THREE YEARS AND GROWING: STEM CELL FACILITY EAGER TO SHARE ITS EXPERTISE

by Christopher Wanjek

Pam Robey describes it as an art, coaxing stem cells in a culture to behave the way nature would have them behave in the body.

"A lot is in the way it feels," she says. "There are nuances you can't quite put into words."

Robey, chief of the Craniofacial and Skeletal Diseases Branch, NIDCR, is conducting a comparative stem-cell study to understand the genes involved in pluripotency and their effect on disease.

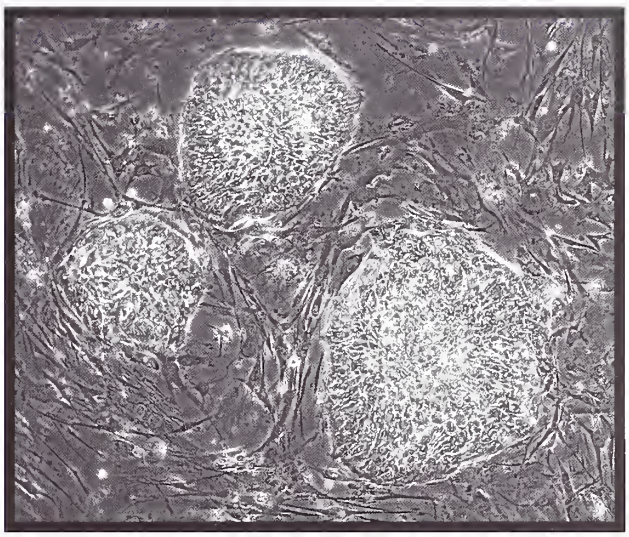
With expertise primarily in postnatal cells, she and her staff three years ago turned to what was then the newly established NIH Stem Cell Unit for a headlong plunge into embryonic stem cells. It was the best way she knew to get to the root of the problem.

The end goal—years away, she readily acknowledges—is to grow tissue to cure diseases such as fibrous dysplasia, a bone development abnormality. But along the way, she is learning the essence of how disease develops.

Not that the path is so straightforward. As anyone who has wrestled with embryonic stem cells would testify, they need more than a dash of Miracle-Gro.

To her frustration, Robey found early on that cultures would be growing fine one day, then die the next for no apparent reason. Or they would spontaneously differentiate. "These cells are just plain hard," she says. "There's nothing easy about them."

And so she knocked on the door of what is now known as the Stem Cell Characterization Facility, still one of the best-kept secrets on the NIH campus. Through hands-on training both in their own lab and at the facility, Robey's colleagues, biologist Joanne Shi and staff



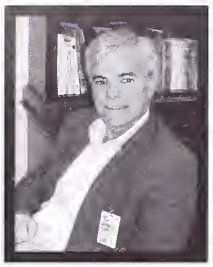
Phase-contrast image courtesy of Tom Cimato

Three colonies of GFP-labeled undifferentiated human embryonic stem cells (cell line UC06) grown by NHLBI's Tom Cimato, with technician Jeanette Beers of NHGRI and Kye-Yoon Park of the NIH Stem Cell Characterization Facility, who introduced the green protein into the cells; the cells surrounding the colonies are murine embryonic fibroblasts, or feeder cells. [Readers, please note: The green fluorescence is vividly visible in the online edition of the Catalyst *at* **<http://www.nih.gov/catalyst/2007/07.01.01/page1.html>**.*]*

continued on page 6

CONTENTS

Building Bridges to Scientific Independence



Michael Gottesman

There are 3,850 fellows at NIH—900 IRTAs and CRTAs, 1,800 visiting fellows, 50 special volunteers with private funding, 800 research fellows, and 300 clinical fellows. All are at NIH to get advanced training in biomedical research that will enable them to make important contributions when they leave, many as independent researchers.

Although our "Guide to Mentoring and Training in the Intramural Research Program" clearly states that "As the fellow matures and prepares to define a scientific niche, a good mentor knows when to step back and allow more independence," precisely how to encourage and support that independence and the career transition that follows is not always obvious.

Two recent programs—one called the "Pathway to Independence Award" that is just underway, and the other a new career track for clinical investigators that is a work in progress—provide concrete ways for fellows to build bridges to independent research careers.

Pathway to Independence

The Pathway to Independence Award is intended to support one to two years of independent research activities within a mentored postdoctoral program and provides up to $249,000 total support per award for three years in an independent research program.

Also known as the K99/R00, this award is both a career development award (K series) and an R award to the grantee academic site (including complete negotiated overhead) when the scientist takes up an academic position. It is therefore quite attractive to both the scientist recipients and the awardee institutions and should serve the purpose of supporting outstanding independent research and encouraging faculty appointments.

The Pathway to Independence award is also meant to accelerate the rate at which postdocs achieve independence. Because the intramural program has a limit of five years total postdoctoral experience, applicants who are intramural postdocs may not have exceeded four years of postdoctoral training. They can, however, extend the postdoc for up to two years if an award is received.

The first review round for this award has been completed, and the awardees have been announced. Our intramural postdocs are clearly competitive for these awards: 4 of 44 intramural applicants and 60 of 962 extramural applicants received awards.

The new application deadlines this year are February 12, June 12, and October 12. All intramural postdocs who have had four years or less of postdoc experience are strongly urged to apply as described at the website:

<http://grants1.nih.gov/grants/new_investigators/pathway_independence.htm>.

The application process is supported by mentoring from appropriate supervisors and/or formal training within each institute and center, so that all fellows who apply, whether or not they are successful, will have the valuable experience of writing a research proposal that is critically reviewed. The award is open to all of our fellows, including visiting fellows and clinical fellows, but not to contractors who, by definition, are not supervised by NIH employees.

Clinical Investigator Career Track

We are well aware that many clinical fellows are not ready for full independence within five years of starting their fellowships and that additional opportunities for graduated independence in a career track for physician-scientists are needed at NIH.

Clinical and translational researchers at extramural sites can apply for various K awards, including K08 (mentored laboratory research) and K23 (mentored clinical research) awards and receive status at their institutions based on receipt of these awards. There has been no equivalent recognition at NIH.

Based on recommendations of the 2004 Blue Ribbon Panel on Clinical Research at NIH, the Advisory Board for Clinical Research has endorsed a proposal to create an associate clinical investigator position at NIH for senior clinical fellows who, by virtue of a research plan and outstanding performance, compete successfully for this position.

It is expected that they will pursue clinical research of their own design in a mentored environment, frequently as part of a clinical research team. An equivalent position for physician-scientists interested in more laboratory-based or translational research is also under consideration. Graduates of this career track will be able to compete effectively for tenure-track positions in clinical research at NIH and elsewhere.

Although more vetting and discussion of the details of this proposal are needed, we hope to get such a pathway underway before the end of the calendar year.

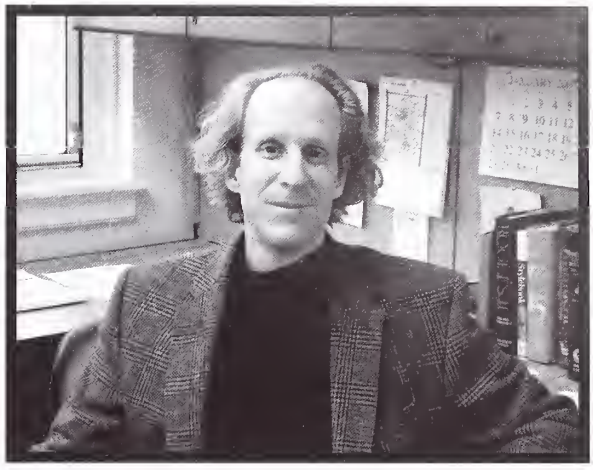
More Bridges

In addition to these new programs, let me remind you of several other bridge awards available for NIH fellows, including K22 awards, the American Heart Association Scientist Development Grant, and the Burroughs Wellcome Fund Career Awards Program. For additional information, see **<http://www1.od.nih.gov/oir/sourcebook/ir-communictns/awardscovermemo.htm>**.

These awards are quite competitive, and NIH postdocs win them on a regular basis; again, the application process itself is a useful training experience.

To improve training and accelerate the path to independence for our fellows are basic and ongoing NIH priorities. Our efforts in this arena will continue. ■

This Guy Might Come Knocking



Fran Pollner

Christopher Wanjek

As the new director of communications for the Office of Intramural Research, I need to hear from you and learn about your research. Seeing how there are only a few thousand intramural scientists working at NIH, this shouldn't take too long.

Hopefully I can wrap this up by the spring, although I won't venture as to which year this would be.

I see my role here as enabling you to share information with each other. Perhaps this will be through newsletter articles relaying your research or through electronic bulletin-board postings with Michael Gottesman about day-to-day issues and concerns that affect your work. I'm open to new ideas, and one of my first tasks is to assess the OIR's communications needs.

This is a new challenge for me, although I'm not entirely unfamiliar with your work. Most of my career has been spent relaying scientific information to the public. I have an undergraduate degree in journalism and a master's degree in health. For the past nine years I wrote primarily about astrophysics for NASA.

During this time, however, I freelanced considerably. My output included two health books, a weekly health column on LiveScience.com, and about 100 health and science articles for the *Washington Post* and other publications. The most recent book, *Food at Work: Workplace Solutions for Malnutrition, Obesity and Chronic Diseases*, was written for the U.N.'s International Labor Organization.

Please call or e-mail me with news about your research or ideas about improving communications in the OIR. Ultimately I would like to meet face to face. It has been a thrill so far, in my first month here, to visit a few labs and to see how the work is actually done. Meeting scientists in their work environment enables me to bring some of the research excitement to life.

I'm at 301-402-4274 or <**wanjek@od.nih.gov**>.

—Christopher Wanjek
Director of Communications, OIR

She Hopes to See Thousands of You

Fran Pollner

Sharon Milgram

Sharon Milgram is in the process of moving her lab from the University of North Carolina School of Medicine, Chapel Hill, to the Bethesda campus of NIH. She's also packing her two well-worn hats for the trip.

A professor of cell and developmental biology at UNC, Milgram has also been an administrator there for more than a decade, serving as a postdoc advisor, the director of various graduate programs, and the director of the Interdisciplinary Biomedical Sciences Program.

With a joint NHLBI-NHGRI appointment that will enable her to continue her cystic fibrosis research here, she is poised to become the first director of the recently reconstituted Office of Intramural Training and Education (OITE). Her first priority in that position is to replace "hit or miss" career counseling that varies with the resources of the individual institutes and centers with a formal science-focused career center on the Bethesda campus that will help trainees in transition at all levels. She'd like to see it up and running before year's end.

The career center will reach out to trainees at all the NIH facilities, which means that staff will need to travel occasionally to outposts such as Frederick and North Carolina. "Breeze and iChat," Milgram observes, "are certainly improvements, but sometimes you need to be able to sit opposite someone and talk."

Nationally, she notes, career counseling and professional development have been identified as inadequately met needs in the field of biomedical research. According to the Sigma Xi postdoc survey (see *The NIH Catalyst*, November-December 2006, page 3), participation in career-development activities is a major component of success. "It's not just getting papers published in high-visibility journals—that's the wrong message to send to trainees."

Milgram has been commuting between Chapel Hill and Bethesda, but will take up permanent residence here in April. She expects to split her time between her laboratory and OITE much the way she has for the past decade at UNC. "I think being an active scientist—active in the lab training students, postdocs, and postbacs—is value-added to the job at OITE. It's a huge benefit to have a scientist's perspective."

Milgram will be bringing a cohort of grad students, postbacs, and postdocs with her—between five and seven people—to continue her lab's cell-signaling and cystic fibrosis research. "Several years ago, we embarked on developing proteomic approaches to identify novel proteins associated with cystic fibrosis. We've identified three clear, interesting proteins and characterized the function of two of them; we want to do the same for the third and explore more exhaustively the first two—how they regulate the movement of CFTR through the cell as well as their ability to function on the surface of the cell."

The new director will also be offering *The NIH Catalyst* a continuing OITE column starting with the May-June issue. "I'll have some time for this," she says, smiling, "since I won't be spending time any more writing grants."

—Fran Pollner

To learn more about the work of the Milgram lab, see <**http://www.med.unc.edu/cba/milgramlab/welcome.html**>.

Biophotonics

continued from page 1

Modeling Stochastic Processes

The group devises quantitative theories and designs instrumentation for optical spectroscopy and tomographic imaging of tissues.

"We are looking at biological systems with randomness in time and space—that's a stochastic process," says Gandjbakhche.

Using mathematical models to localize lesions and track their changing functional status requires analyzing different optical sources of contrast such as fluorescent labels, absorption, and/or scattering.

Gandjbakhche also builds countertop prototypes of the instruments that might be used at the bedside to characterize the tissues under scrutiny and to monitor response to therapy, instruments that involve neither ionizing radiation nor surgical biopsy, just light.

When light enters biological tissue, it doesn't go straight in but scatters in many directions, requiring sophisticated methods such as "random-walk theory" to explain the path the light takes through the tissue. This stochastic method, developed at NIH, takes into account the absorption and scattering properties of tissue, which are wavelength dependent, Gandjbakhche explains, noting that a wealth of information can be obtained by spectroscopic methods.

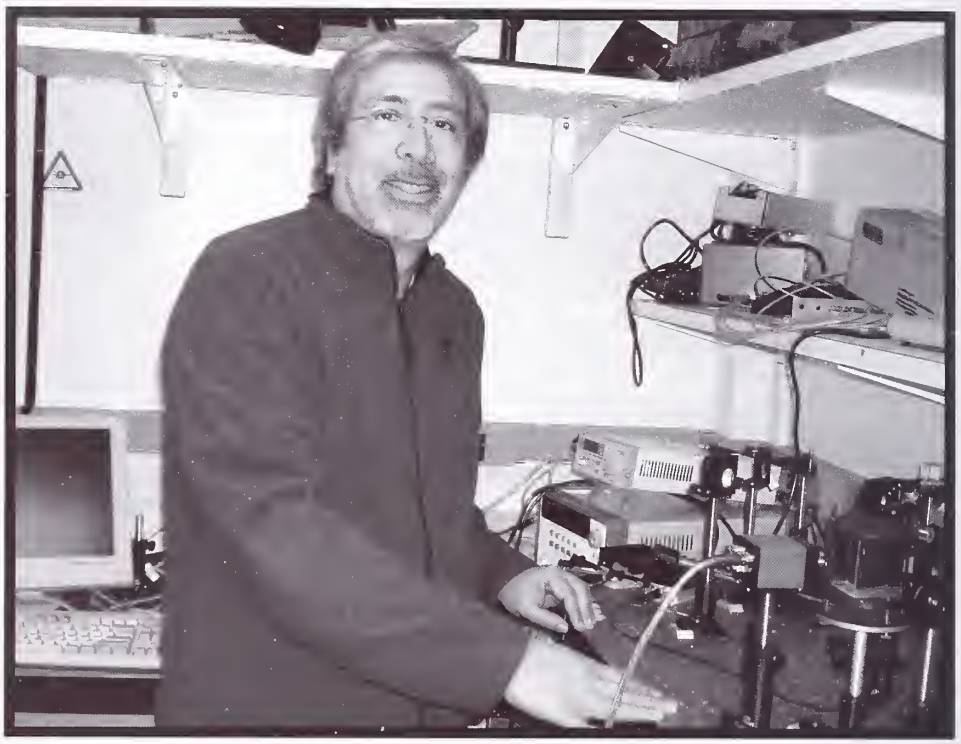
"Light traveling through tissue is a stochastic process. The photons are going everywhere. It's beautiful," says Gandjbakhche, pointing to a display on his computer screen.

The creation of the vascular network is also stochastic. Fellows Franck Amyot and Alex Small are modeling the stochastic process of tumor-induced angiogenesis in collaboration with NCI's Kevin Camphausen.

Some Ongoing Studies: Tracking Vasculature Responses In Kaposi's Sarcoma Patients

Graduate student Abby Vogel and postdoc Moinuddin Hassan, with NIBIB's Paul Smith, are monitoring the effect of experimental drugs to counter angiogenesis.

For the past five years, Gandjbakhche's team has collaborated with Robert Yarchoan, chief of the HIV and AIDS Malignancy Branch, NCI, and Richard Little, senior oncologist, on four clinical protocols involving drug regimens for patients with Kaposi's sarcoma (KS), a highly vascular tumor.



Fran Pollner

Master builder: *In addition to constructing equations in his mind, Amir Gandjbakhche builds countertop prototypes of potential bedside instruments. He notes that once an experimental technology reaches a point of clinical commercial potential, his lab moves on to other basic explorations.*

"We created quantitative methods to assess the vascularity of these tumors using three noninvasive imaging modalities," Gandjbakhche says. "We monitor the results of the drugs being tested and discuss them with the physicians."

"All three imaging modalities," he adds, "take less than five minutes."

Each of these modalities—laser Doppler imaging (LDI), infrared thermal imaging (thermography), and near-infrared multispectral imaging—provides specific, complementary information.

LDI measures blood flux, a combination of red blood cell velocity and concentration; spectral imaging measures changes in blood volume and oxygenated and deoxygenated hemoglobin; and thermography measures temperature as a reflection of blood flow, providing confirmation that changes in the vasculature are related to blood flow.

The spectral-imaging component came about through collaboration with Stavros Demos of the Lawrence Livermore National Laboratory, Livermore, Calif., in designing a portable spectral-imaging system, Gandjbakhche notes.

Overall, the KS studies have thus far demonstrated that the lesions—in addition to being hotter than normal tissue—also register higher blood volume, deoxyhemoglobin, and blood velocity. The cytotoxic/anti-angiogenic combination of liposomal doxorubicin and interleukin-12 is among the agents that have been tested. These imaging techniques can easily be adapted to a variety of skin diseases.

Fluorescence Lifetime Imaging In Mouse Tumor Studies

The applications of fluorescence imaging are limitless, constrained only by the development of fluorophores sensitive to the biological targets of interest.

A fluorophore, Gandjbakhche explains, "is a molecule that has the property to be excited in one wavelength and emits light in a longer wavelength after a delay called lifetime."

"Any condition in which receptors on the cell surface play a role, for instance, is a candidate for fluorescence imaging," says Gandjbakhche. "We need only create antibodies tagged with fluorophores that bind to a specific receptor of interest."

For the past two years, the Gandjbakhche lab has assisted in the mouse tumor studies conducted by Jacek Capala, an investigator in the Radiation Oncology Branch, NCI.

Postdocs Jason Riley and Hassan and staff scientist Victor Chernomordik, along with NICHD colleagues Hacene Boukari and Dan Sackett in the lab of Ralph Nossal, have developed quantitative methods to characterize the molecular and functional status of deeply embedded tumors—breast cancer cells expressing high levels of the HER2 protein.

Gandjbakhche's team measures fluorophore lifetime because it varies, for instance, with degree of oxygenation or pH value.

"The instrinsic optical properties of the tissues under investigation," he explains, "will yield different functional properties." Fluorophores are chosen for their specific sensitivity to the tissue environment.

Localization of the tumor and quantification of pH was achieved with the use of a fluorophore-Herceptin (a HER2-specific monoclonal antibody) conjugate. The fluorophore was a pH-sensitive near-infrared dye called Alexa Fluor 750. The team intends to continue these studies to investigate an affibody-based molecular probe for imaging HER2 receptors.

"We know exactly what kinds of antibodies to use; we know that tumor cells tend to be hypoxic and have lower pHs than the surrounding tissues."

The IPDC Connection

Gandjbakhche sits on the steering committee of the Imaging Probe Development Center, a new NIH core resource, directed by NHLBI's Gary Griffiths, for the production of imaging probes, both known but not commercially available and novel (see *The NIH Catalyst,* January-February 2006, p. 1).

Gandjbakhche's proposal to the IPDC that it manufacture a near-infrared dye for optical-imaging research (the review of which he did not participate in) was recently approved. "This dye uses metal chelates to modulate the fluorescence lifetime," Gandjbakhche says, "and it will increase our ability to detect smaller fluorophore concentrations."

Acknowledging that familiarity with the concepts, language, and calculations of his research is not widespread, he observes that the "most important part of this work is its multidisciplinary nature—it takes physicists, engineers, biologists, physicians, chemists. . . ." ■

Optical Imaging Advantages at a Glance

The attributes that make optical imaging a choice diagnostic and monitoring modality, says Amir Gandjbakhche, are these:

- Optical imaging uses nonionizing visible and near-infrared light and is therefore safer than such techniques as X-ray, CT, and PET imaging to gather information about what's going on beneath the skin's surface.
- Optical-imaging instruments are portable and can be brought to the patient, not large and stationary like MRI machinery.
- Optical imaging provides functional information and penetrates far deeper than the 1–2 mm accessible by two-photon or confocal microscopy.
- Other kinds of imaging equipment, such as MRI and PET, are between 10 and 20 times as expensive as the tools of optical imaging.

In short, it's "portable, safe, cheap, fast," and provides functional information, says Gandjbakhche. ■

For an in-depth look at the Gandjbakhche lab and its collaborative work, visit **<http://www.sbsp-limb.nichd.nih.gov/index.html>**.

On the Right Wavelengths: *Science* and *Discover* Cite Achievements by NIH Researchers

Good Vibrations

The discovery that the cochlea's spiral form actually serves a specific hearing function landed a place among *Discover* magazine's 100 top stories of 2006—a tribute to the work of Richard Chadwick, chief of the Section on Auditory Mechanics, Laboratory of Cellular Biology, NIDCD, and his colleagues, Daphne Manoussaki, formerly a visiting fellow in that lab and now at Vanderbilt University in Nashville, Tenn., and Emilios Dimitriadis, formerly a senior research associate in the lab and currently an ORS staff scientist in the Division of Engineering and Physical Science.

The team used a mathematical model to determine that the cochlea's tight central coil steers low-frequency sound waves in a way that amplifies the ability to hear the deepest vibrations.

The work corrects the previously held impression that the spiral shape has no effect on hearing. Instead, the investigators found that increasing curvature redistributes wave energy toward the cochlea's outer wall, affecting the shape of waves especially in the regions where low-frequency sounds are processed. ■

PALM Pilots

Researchers in the NICHD Cell Biology and Metabolism Branch contributed to a molecular imaging advance that was ranked among *Science* magazine's top 10 breakthroughs of 2006.

The team includes George Patterson, Rachid Sougrat, O. Wolf Lindwasser, Juan Bonifacino, and Jennifer Lippincott-Schwartz.

The technique, called photoactivated localization microscopy (PALM), enabled them to beat the diffraction limit that otherwise prohibits resolving images smaller than half the wavelength of the light used to illuminate the object, about 200 nanometers for optical light.

With colleagues Eric Betzig and Harald Hess at Howard Hughes Medical Institute in Ashburn, Va., and Michael Davidson at Florida State University in Tallahassee, the team imaged target proteins in thin sections of lysosomes and mitochondria with nanometer resolution. They accomplished this by using fluorescent tags that could be turned on one tagged molecule at a time to create a composite image. ■

The NIH Stem Cell Characterization Facility

continued from page 1

Christopher Wanjek

***It's all in the wrist**: Exercising what NIDCR lab chief Pam Robey calls the "art form" of pipetting when it comes to growing embryonic stem cells, staff scientist Sergei Kuznetsov adds growth fluid to stem cells*

***The face behind the wrist**: Sergei Kuznetsov*

scientist Sergei Kuznetsov, became adept cell cultivators.

Employing a mix of scientific rigor and artful pipetting, they can now grow the embryonic stem-cell line known as HSF6 and ward off spontaneous or otherwise unwanted differentiation with near regularity. And the group is now off and running on its research project.

Learning Curves

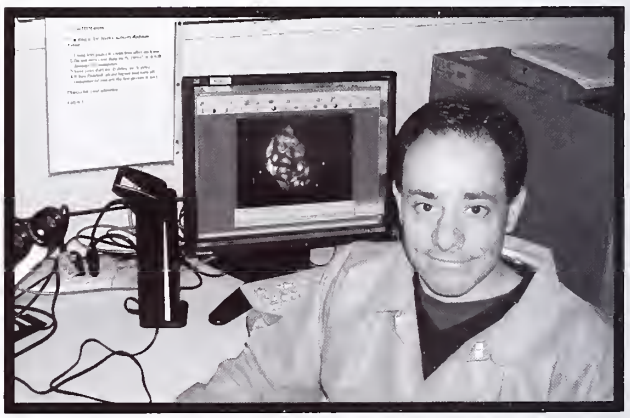
Nearly three years into the stem-cell-growing business, the facility is renewing its effort to reach out. Having cultured 17 of the 21 federally approved human embryonic stem-cell lines—with great success in karyotyping them and establishing growth protocols—the facility wants to place more emphasis on training intramural scientists.

"We are developing a world-class facility at NIH," says Ron McKay, the NINDS investigator who runs it. He deems the facility one of the best laboratories in the world to develop expertise in human embryonic stem-cell science. "The whole technology is on the move. We are inviting people to participate."

Along the lines of the adage about teaching a person to fish, McKay sees the facility as teaching the skills of how to grow embryonic stem cells, and he is planning a series of special seminars and training sessions this year. Without some training, he notes, one can waste a lot of time trying to grow undifferentiated embryonic stem cells.

In the stem-cell facility, all the cells are grown under identical conditions, and they are mostly normal, McKay says. His group has crafted a relatively reliable experimental system—essential to accomplishing anything with the cells.

***"Checking his garden"** is the way NHLBI clinical fellow Tom Cimato describes how he monitors the progress of his stem-cell colonies*



Christopher Wanjek

***In full view:** Tom Cimato, with the picture behind him of the 21-cell stem-cell colony he viewed under the microscope*

Growth Curves

Barbara Mallon, a scientist at the facility since its incarnation as the NIH Stem Cell Unit in 2004, speaks of stem cells as invigorating the very core of basic cellular research.

Yes, the science can be intimidating, and the methods daunting, Mallon observes, but the reward will be unprecedented advances in biomedical science. The facility has much to offer in terms of handholding and lesson sharing, she says, but sometimes scientists unfamiliar with its purpose come with false expectations.

"We want people to understand that we're here but also what we do, and what we can and can't do," Mallon said. "Some people think we'll be able to supply them with cells. That's not really our role."

That is, her group cannot make copies of undifferentiated cell lines for scientists to take back to their own labs. Doing so would be analogous to bootlegging a DVD and is forbidden by the Material Transfer Agreement set by the suppliers. NIH possesses the stem-cell lines but does not own them. The owners, companies such as WiCell Research Institute in Madison, Wisc., stipulate what can be done with the cell line.

What the Stem Cell Characterization Facility can do is offer quality training and support simply not available elsewhere. For visiting NIH scientists there is the opportunity to use the facility's cells and equipment, housed in Building 37. Mallon and her colleagues—Kye-Yoon Park, Kevin Chen, and Becky Hamilton—also visit other NIH labs to assist.

There are other places around the country that offer training, for a steep

price; but these are short, one-shot deals, Mallon said, and scientists returning to their labs often must face myriad culturing problems on their own.

For example, Mallon says, "people can grow cells, but they can be growing junk and not realize it. The markers that are used may not be sensitive [enough] to detect the fact that your cells are going downhill."

Her group has been there and done that with trial and error and a little more error. They have established standards for growth, continue to make growth easier and better, and know how to monitor the health of the cultures. These were hard lessons to learn that they can now pass on.

Biology 101

Stem cells, says McKay, are at the root of diseases such as cancers and neurological disorders. "How do you figure out the relationship between mutation and altered phenotype," McKay asks. "That's why this is important."

"Stem-cell biology is part of the continuum of understanding human development, growth, and differentiation," says NHLBI Director Elizabeth Nabel, who is working with NHLBI's Tom Cimato, a clinical fellow, to grow cardiac tissue.

Catalyst photo

NHLBI's Elizabeth Nabel and NINDS' Ron McKay at a panel discussion on adult stem cell research at NIH in 2004

Catalyst photo

NIDCR's Pam Robey discussing fibrous dysplasia in 2002

Nabel is among the IC leaders who encourage their staff to become more active in and stay abreast of this type of research. "I think stem cells provide the perfect niche of applying many of those principals of basic biological processes to clinical medicine. We're very keen in supporting that translational work," she says.

Indeed, Cimato, who occupies one of the facility's two visiting scientist spots, was attracted by the "Biology 101" quality of stem-cell research. He says that heart disease is, in essence, a stem-cell disease. Some toxin, perhaps nicotine or a fatty acid, he muses, disrupts the routine process of replacing endothelium and vascular cells.

Cimato is tracking down the cardiac stem cell, asking, "how does it grow, how does it differentiate, how many steps does it go through in creating new endothelium?"

And he's closing in on some answers. He has successfully nursed an embryonic stem cell through the stages of differentiating into endothelium, one step away from cardiovascular tissue. To do so, he has had to learn how to inhibit all other kinds of differentiation while simultaneously enticing the the cells down the path to endothelium, a two-year laboratory effort.

Christopher Wanjek

***The stem cell facility crew**: (left to right): Kye-Yoon Park, Barbara Mallon, Becky Hamilton, and Kevin Chen*

Like others, he calls upon a certain intuition to grow these cells, an intuition, he observes, that would have been difficult to develop without the help of the stem-cell facility.

Cimato sees embryonic stem-cell science as enabling unambiguous insight into disease development.

Robey, taking a different path of differentiation, has arrived at a similar conclusion. She calls fibrous dysplasia a skeletal stem-cell disease.

"Any change in a skeletal stem cell's metabolism, either by mutation or change in the microevent, will result in a skeletal disorder," she says. Yet how that disorder manifests depends on the stem cell. She stumbled upon this in her work on fibrous dysplasia, she recalls. The same mutation caused lytic bone growth below the neck yet sclerotic growth above the neck, a phenomenon that traces back to the cell origin, either mesoderm or neuroectoderm.

Robey has traced the three features of fibrous dysplasia—patches of *café au lait* skin, precocious puberty, and dysplasia—to the three germ layers.

Embryonic Technology

The Stem Cell Characterization's Facility's services are different from, say, DNA sequencing, where the technology is mature enough to set a price for a given product to be produced in a given time period. "We're not there yet," McKay says. In other words, fees vary.

He anticipates that interactions with the NIH community will increase as the power of the technology grows.

Nabel notes that the field is young: "We are still in our early years in terms of understanding the biology of stem cells—understanding how stem cells grow and differentiate, what markers are expressed at different points of differentiation. A lot of that is simply descriptive discovery work that needs to be done. Once we have a better handle on the cells per se, then I think the opportunities for application will be enormous." ■

For more information on stem cell facility services and fees, contact Barbara Mallon at ***<malloub@mail.nih.gov>***.
For more information on stem cells, visit ***<http://stemcells.nih.gov/index.aspm>***.

NIEHS Launching an Outpatient Clinical Research Unit —With Visions of an Inpatient Facility on the Horizon

by Eddy Ball
NIEHS

Architectural design has begun on what will be the first Clinical Research Unit (CRU) at the NIEHS campus in Research Triangle Park, N.C.

Groundbreaking is anticipated in early spring, and researchers expect to begin working in the new 11,500-square-foot facility this summer.

drawing courtesy of Williams-Scotsman

Architect's rendering of the NIEHS Clinical Research Unit

It was at first thought that CRU studies would focus on environmental lung diseases such as asthma for which there is abundant evidence on the role of exposure to airborne materials. But investigators have been proposing an expanded research portfolio that includes epigenetics, cardiovascular disease risk, and reproductive health. (Studies already planned or proposed—and awaiting IRB approval and/or funding—are listed on the next page.)

Projects and People

The CRU will provide the institute with unprecedented resources for clinical research: Previously, NIEHS research involving human tissue sampling or functional assessment could not be performed on the main campus. The new facility is situated adjacent to the main laboratory and will permit physician-scientists to conduct studies that involve on-site sample collection, pulmonary function assessment, and laboratory analysis.

The CRU will accommodate outpatient research only and will offer routine patient evaluation, fluoroscopy, X-ray and ultrasound imaging, and sample collection and processing. It will also feature specialized diagnostic and analytical capabilities, such as inhalation-exposure measurement and bronchoscopy with bronchial sampling.

The new facility, notes Perry Blackshear, director of clinical research, "overcomes a geographical obstacle for investigators who have wanted to engage in clinical research, [and] it will allow our clinical research to expand in new directions."

Blackshear, an endocrinologist and PI in the Polypeptide Hormone Action Group in the Laboratory of Neurobiology, was a key figure in the development of the CRU and will direct its operations and future expansion. He is also one of the NIEHS investigators eager to stroll between his lab and the CRU.

"We've established a large DNA registry, the Environmental Polymorphism Registry, for which we have subject identifiers. With the new unit," Blackshear says, "we can bring subjects in for blood cell collection to investigate possible physiological or biochemical changes related to the polymorphisms."

Michael Fessler, head of the Host Defense Group, and Darryl Zeldin, a senior scientist—both of whom are pulmonary and critical-care specialists in the Laboratory of Respiratory Biology—express similar enthusiasm.

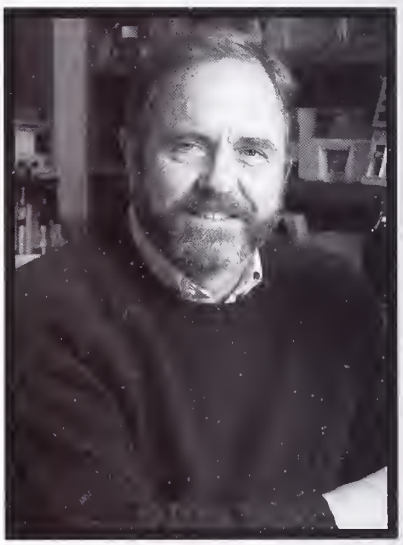
"Our group's initial studies at the CRU," says Zeldin, "will be a direct outgrowth of prior epidemiological and laboratory studies and will involve assessment of human subjects that [before] would not have been possible."

Zeldin and his colleagues will use the CRU to collect blood, saliva, and DNA samples and to conduct periodic assessments of asthma symptoms and lung function, as well as noninvasive assessment of vascular function. They will use their laboratory to examine oral bacteria and other samples.

"The overall strategy," Fessler says of his group's intended use of CRU resources, "will be to partner cell, laboratory animal, and human models of lung disease to discover and validate new aspects of disease induction."

Both Zeldin and Fessler will take a disease-oriented translational approach to develop potential clinical applications out of their group's work on the mechanisms of infection, inflammation, and induction of the lung's preprogrammed immune response to the environment.

For NIEHS researchers, the CRU expands the traditional concept of what a laboratory is. The unit is a laboratory in its own right, a controlled setting for asking a focused question, as well as an extension, both physically and conceptually, of the institute's existing lab resources. It provides what Fessler calls "an extra tier in the bench-to-bedside spectrum" and could even one day serve as a repository of preserved human specimens for future studies.



Steve McCaw

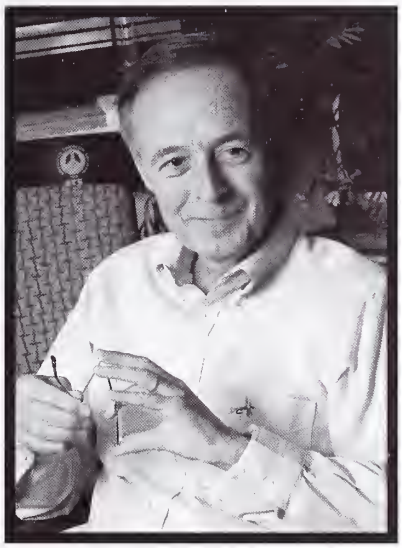
Perry Blacksbear, director of clinical research

Steve McCaw

Darryl Zeldin, senior scientist

Steve McCaw

Michael Fessler, head of the Host Defense Group



Steve McCaw

William Martin, director of translational research

Bethesda Presence And Collaborations

Despite having relatively few researchers based in Bethesda and the difficulties of long-distance research, NIEHS has maintained a research agenda at the NIH Clinical Center oriented primarily toward autoimmune diseases. NIEHS investigators anticipate continuing these activities, at least for the foreseeable future, as North Carolina-based investigators expand clinical research on-site.

Although NIEHS has a long history of collaboration with sister NIH institutes, initial studies at the CRU will follow the more routine collaboration patterns involving universities and private partners.

William Martin, director of translational research, emphasizes, however, that interinstitute collaboration at the CRU will also become a more attractive option as the facility expands.

"One of our overarching goals is to integrate our science with the work of other institutes and other federal agencies," he says.

In addition to expanding multisite studies with sister institutes to include clinical research, Martin anticipates the development of "sophisticated technologies and unique resources that others at NIH would be willing to travel [to North Carolina] to use."

A Reflection of NIEHS' Future

According to Martin, the CRU will encourage NIEHS scientists and grantees to develop protocols that promote interdisciplinary research at various levels. He sees the CRU as a tangible and evocative "metaphor for the changes that are happening within the institute in terms of the Strategic Plan."

"There's nothing quite like a building to say that we are going in a new direction," Martin observes.

The CRU is expected to influence scientific culture at the institute and to advance several of the goals set in the NIEHS 2006 Strategic Plan:

- Enhancing the impact of intramural research on understanding human health and disease in a "bidirectional" feedback process between basic science research and clinical research
- Providing a model for applying basic science to problems in clinical research in environmental health
- Serving as a role model to help extramural scientists develop similar programs at NIEHS-supported research centers nationwide

The CRU is also viewed as a prelude to building a larger clinical facility with inpatient capabilities, advanced imaging, and other features.

As the outpatient unit progresses, the NIEHS National Advisory Environmental Health Sciences Council will advise and monitor clinical research efforts and help address questions about the future direction of the inpatient facility, which is planned for some time around 2012 if construction funding becomes available.

In addition to Martin and Fessler, who were recruited in 2006 to join Blackshear and Zeldin, there will be a staff clinician to oversee day-to-day CRU operations and management and two to three additional physician-scientists to complement the clinical team.

The team expects that increasing numbers of the institute's scientists will propose research at the facility. "If this really works, we'll see other people coming in with new ideas, and we'll see the portfolio evolve even more dramatically than it has already. Five years from now, our research may be going in directions we never envisioned initially," Martin suggests. ■

CRU Research On the Drawing Boards

- Screening samples from the Environmental Polymorphism Registry for polymorphisms believed to be associated with physical or behavioral phenotypes (Perry Blackshear, Darryl Zeldin, et al., in collaboration with researchers from the University of North Carolina at Chapel Hill [UNC-CH])
- An epigenetic study of polycystic ovary syndrome using data from twins identified by the Mid-Atlantic Twin Registry to determine disease-related environmental components (Blackshear and Patricia Chulada, in collaboration with researchers from Duke University, Durham, N.C., and Virginia Commonwealth University, Richmond)
- A series of in vitro signal-transduction studies to elucidate the pathways by which primary human immune cells recognize and communicate with external stimuli such as microbial molecules; isolated neutrophils and macrophages, collected from as many as 350 healthy volunteers a year, provided under an IRB-approved protocol (Michael Fessler)
- A secondary prevention trial to examine the effects on asthma morbidity in high-risk children of removing cockroach allergen from inner-city homes (Zeldin, in collaboration with researchers from the Johns Hopkins University in Baltimore and Mt. Sinai Hospital in New York)
- A respiratory study examining the relationship between the presence of specific strains of oral bacteria and asthma and allergy in children to help determine whether colonization of the oral cavity by bacteria and other microbes may play a protective role in the etiology of allergic diseases such as asthma (Zeldin, in collaboration with researchers from the UNC-CH School of Dentistry)
- A collaborative study to examine whether specific polymorphisms in fatty acid metabolism genes are involved in regulating endothelial function as assessed by flow-mediated vasodilation, a noninvasive indicator of cardiovascular disease risk (Zeldin, in collaboration with UNC-CH researchers) ■

Another Step in the Strategic Plan for NIH Obesity Research: CC's Metabolic Clinical Research Unit Makes Its Debut

by Christopher Wanjek

NIDDK Scientific Director Marvin Gershengorn, spearheaded the unit's development

Monica Skarulis, chief of the Clinical Endocrine Section, Clinical Endocrinology Branch, NIDDK

Jack Yanovski, head of the Unit on Growth and Obesity, NICHD

Not one calorie escapes from the Metabolic Clinical Research Unit, a roomy new facility tucked into two floors in the Clinical Center's southwest wing.

The state-of-the-art facility opened its doors on January 25 and is expecting its first round of patients and volunteers by winter's end.

The unit is a contrast of extremes—with extra-wide gurneys and wheelchairs, reinforced toilets, and special scales to accommodate morbidly obese patients, countered with advanced devices to keep track of every morsel eaten and calorie burned, measuring metabolic changes down to the molecular level.

***In a corner of the exercise room**, Megan Rothney, predoctoral fellow in the Clinical Endocrinology Branch, NIDDK, simultaneously monitors the pulmonary, cardiac, and metabolic parameters of a volunteer treadmill runner, overseen by Kong Chen, director of the new unit's Metabolic Research Core*

Although the focus is on obesity, the unit intends to recruit subjects of all body designs to understand the basic science of human metabolism and the physical and behavioral processes involved in weight gain and weight loss. It is expected that the unit will attract researchers across NIH who are variously interested in such issues as the metabolic changes seen in organ transplants, cancer, HIV, and many other diseases.

The unit is greater than the sum of its parts. Much of its equipment exists elsewhere, but not all in one place. Consider the Bod Pod and DXA to measure body composition, the physical activity monitors, sleep-monitoring equipment, ten inpatient rooms, and three metabolic suites to measure oxygen–carbon dioxide exchange minute by minute—precisely enough to determine calories burned from fats versus carbohydrates. Added up, the unit is intended to give NIH researchers "an unfair advantage" in understanding metabolism, says Monica Skarulis, an NIDDK senior clinical investigator.

"We are set up to study human metabolism in a way that we were never able to do before," says Marvin Gershengorn, NIDDK scientific director, who describes the unit as a melding of the fields of metabolism, endocrinology, nutrition, cardiovascular biology, gastroenterology, hepatology, genetics, and behavioral sciences.

Kong Chen of the NIDDK, an expert in metabolism and nutrition recently recruited from Vanderbilt University in Nashville, Tenn., speaks of the basic and translational science that he hopes to accomplish as director of the Metabolic Research Core at the unit. "As an extramural scientist three months ago, I couldn't do this," Chen says.

About a dozen clinical protocols have worked their way through the IRB and are ready to be implemented, says Gershengorn, a principal architect of the unit's concept and champion of its development. Most are headed by NIDDK investigators; some of the others, which originate in NICHD, are moving over from the CC pediatric unit.

Many more protocols are in the pipeline. Gershengorn has heard of interested parties in NCI, NHLBI, NIDA, NIMH, and NIAAA, exactly the kind of cross-campus interest the unit was established to accommodate.

"This is really the most modern and cutting-edge place to do research," says John Gallin, CC director. "We can provide really precise phenotyping of patients with any type of disease. . . . We want people to know the resource is there." ■

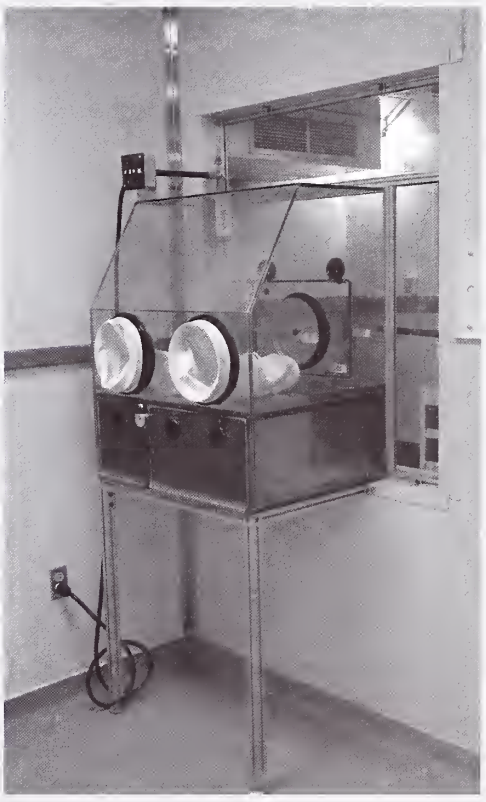
The NIH Metabolic Clinical Research Unit, developed by NIDDK with the CC, is a component of the Strategic Plan for NIH Obesity Research. To see the Strategic Plan for NIH Obesity Research, visit **<http://www.obesityresearch.nih.gov/About/strategic-plan.htm>**.

Merel Kozlosky, supervisory metabolic dietitian

***The Bod Pod**: A person sits inside, with a view of the outside world, and his or her total body density is determined within a couple of minutes*

***The seat inside the Bod Pod**: Kong Chen, director of the Metabolic Research Core at the Metabolic Clinical Research Unit, reveals the pod's inner sanctum; behind him is the unit's DXA (dual-energy X-ray absorptiometry) table. "Using the [DXA's] total-body mode," Kong says, "we can measure total fat, lean, and bone contents, as well as distributions, with only a low dose of radiation exposure (about one day of background exposure). The unique feature of our system is its expanded capacity to measure patients up to 450 pounds."*

***The bed of choice**: The above 460-pound capacity bed can measure the weight of a person in bed and can readily convert into the chair position; it is the bed that will be used throughout the metabolic unit.*



***Airtight**: To the left is one of the unit's three rapid-response respiratory suites, where people can be sealed in for 24 hours, food delivered and removed via sleeves, for a precise determination of metabolic rate and calorie dynamics*

Photos by Bill Branson

People

Recently Tenured

John (Jay) Chiorini *received his Ph.D. in genetics from the George Washington University in Washington, D.C., in 1993. He completed a postdoctoral fellowship at NHLBI and a PRAT fellowship awarded by NIGMS before joining NHLBI as a senior research fellow in the Molecular Hematology Branch. In 2000, he joined the Gene Therapy and Therapeutics Branch, NIDCR, where he is now a senior investigator.*

John Chiorini

The development of improved vectors for gene transfer is a critical element for the advancement of the field of gene therapy.

Vectors based on adeno-associated virus (AAV) offer several advantages as a platform for gene transfer—such as stable long-term transfer in vivo into several different cell types—but our understanding of the biology of this virus is limited. My overall research goals are to define the interactions of AAV with its target cell and to develop improved vectors for gene transfer. Our underlying hypothesis is that by understanding these interactions as they apply to the biology of the virus, we can contribute to the development and use of AAV vectors for gene therapy.

AAV is a human parvovirus with a single-stranded DNA genome that contains only two open reading frames that encode either the nonstructural (Rep) proteins or the capsid (VP) proteins.

Our early work focused on the role of the Rep proteins in virus production and regulation of cellular activity; much of our recent work focuses on the capsid genes and the use of recombinant AAV vectors for gene transfer.

The interactions between the capsid of an AAV particle and its host cells that result in transduction are complex and largely not understood.

Our initial work in this area demonstrated that not all AAV serotypes have the same cell tropism. This observation led to the search for and identification of new AAV serotypes and has resulted in improved gene transfer to several target cell types.

However, this process of matching new isolates to those targets where they would be most effective was largely empirical because we lacked understanding of the cellular components necessary for transduction with these new vectors.

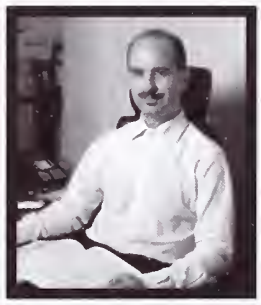
We therefore worked to develop a novel bioinformatics-based screening assay that has allowed us to identify the cellular genes responsible for the distinct cell tropism of the different AAV isolates.

The identification of genes critical for transduction with AAV vectors has yielded better matches and, in addition, has enabled us to increase transduction in poorly permissive cells by upregulating the expression of these critical genes.

For example, we have identified the platelet-derived growth factor receptors (PDGFR) as critical for efficient transduction of AAV type 5 (AAV5).

Our further characterization of PDGFR as a receptor for AAV5 has allowed us not only to target the use of the vector to specific cell types in which PDGFR is highly expressed, but also to manipulate the expression level of PDGF and improve transduction.

Currently, natural viral isolates serve as a rich source of vectors for gene transfer. But we envision being able to build on our understanding of the biology of these natural isolates and develop engineered vectors with specific and defined tropisms.



Philipp Kaklis

Toward this goal, we have worked to understand gene transfer from the perspective of the virus by developing structural models for different AAV vectors and identifying key regions involved in receptor binding and transduction.

In this way, we can rationally design vectors that incorporate targeting epitopes into the vector capsid. These targeting epitopes will fill the same role as the natural targeting domain in current vector but bind receptors of our design.

Philipp Kaldis *received his Ph.D. in 1994 from the Swiss Federal Institute of Technology in Zürich and did postdoctoral work at Yale University in New Haven, Conn. He joined the NCI-Frederick in 2000 and is currently a senior investigator in the Mouse Cancer Genetics Program.*

I am interested in how cells divide and multiply. Misregulation of cell growth and proliferation plays an important role in many diseases, including cancer. Detailed understanding of the regulation of cell growth and proliferation is therefore a prerequisite for designing strategies to treat such diseases. Cyclin-dependent kinases (Cdks) are among the central regulators of cell growth and proliferation.

Originally, I was trained as a "hard-core" biochemist and spent lots of time purifying proteins and analyzing enzyme kinetics. But a growing desire to relate my results to in vivo situations inspired an interest in genetics, especially mouse genetics to model cell cycle and cancer as they relate to humans.

Right now, I am trying to dissect the genetic pathways of cell-cycle regulation in the mouse. It will take many years to analyze all the essential pathways that regulate the cell cycle.

Mouse models to investigate in vivo functions of Cdks. Studies of Cdk2 in vitro and in cell lines suggest that Cdk2 controls the transition from G1 (interphase) to S phase, where DNA replication takes place. Inactivation of Cdk2 in mammalian cell lines results in growth arrest in the G1 phase.

To explore Cdk2 functions in the context of a living animal, we have generated Cdk2 knockout mouse models. We have found that these $Cdk2^{-/-}$ mice are viable but sterile and therefore that Cdk2 seems to be essential for meiosis but not mitosis. These results suggested that other genes can compensate for Cdk2 functions in the mitotic cell cycle.

Among the best candidates were the family members Cdk1, Cdk3, Cdk4, and Cdk6. Cdk3 could be excluded because it is not expressed in the mouse. To investigate the functional overlap, we generated $Cdk2^{-/-}Cdk4^{-/-}$ and $Cdk2^{-/-}p27^{-/-}$ double-knockout mice. Our results indicate that $Cdk2^{-/-}Cdk4^{-/-}$ knockouts die in utero, suggesting that Cdk2 and Cdk4 have overlapping essential functions in controlling the retinoblastoma protein.

The Cdk inhibitor $p27^{Kip1}$ has been thought to act primarily by controlling its major target, Cdk2. Interestingly, we observed all phenotypes of the single

knockouts in the Cdk2$^{-/-}$p27$^{-/-}$ double-knockout mice. This result suggested that p27 has other targets in addition to Cdk2, one of which we identified as Cdk1.

We used to think that Cdk2 functions in the G1/S phase and Cdk1 only in mitosis, but our results opened the possibility that Cdk1 could also regulate the G1/S transition in addition to its mitosis function. We found that cyclin E (usually a partner of Cdk2) binds to and activates Cdk1 and that this is essential for S phase entry in the absence of Cdk2. Therefore, Cdk1 compensates for the functions of Cdk2 in G1/S.

Our results indicate that Cdk2 and Cdk1 bind the same cyclins and are functioning during the same cell-cycle phases.

These findings raise questions as to why there are two Cdks fulfilling the same functions and why Cdk2 cannot compensate for Cdk1 in mitosis. (Cdk1 seems to be an essential gene in the mouse.) To answer these questions, we will focus our future studies on Cdk1.

In summary, my goal is to investigate the in vivo functions of Cdks during normal development and tumor formation. To achieve our goal, we are generating mouse models that cover pathways that impinge on the functions of Cdks. Our work enhances the basic understanding of cell-cycle regulation and also provides information essential to the design of cancer therapies.

Mouse genetics requires patience and careful long-term planning. I am very grateful to the past and present members of my lab for their contributions. We welcome continuing collaboration with other scientists at NCI, NIH, and elsewhere.

Carole Parent *received her Ph.D. from the University of Illinois at Chicago in 1992. She had her postdoctoral training in the laboratory of Peter Devreotes in the Department of Biological Chemistry of the Johns Hopkins University School of Medicine in Baltimore and in 1996 became an instructor in the same department. In May 2000, she joined the Laboratory of Cellular and Molecular Biology, NCI, where she is now a senior investigator.*

Research in my group focuses on uncovering the mechanisms by which chemotactic signals are integrated at the cellular and multicellular levels to regulate directed cell migration.

Christopher Wanjek

Carole Parent

We use genetic, biochemical, and cell biological approaches to study this clinically relevant topic in two related and complementary model systems, the social amoeba Dictyostelium and human neutrophils.

We are particularly interested in exploring two fundamental questions in chemotaxis:

- How do external signals establish and maintain signaling and cellular polarity?
- How are chemotactic signals relayed to neighboring cells—that is, how do cells transition from single to group migration?

Dictyostelium cells, which are amenable to genetic study, track down and phagocytose bacteria during normal growth. When starved, they enter a developmental program that culminates in the formation of a multicellular organism composed of spores atop a stalk of dead cells.

This impressive transition is driven by chemotaxis. Dictyostelium cells migrate toward secreted adenosine 3', 5' cyclic monophosphate (cAMP), which is recognized by a family of G protein–coupled seven-transmembrane receptors.

Thus, in this system, cAMP acts as both an intracellular second messenger and a chemotactic cue. Surface-receptor activation leads to dissociation of G protein into α- and $\beta\gamma$-subunits, which then activate a variety of effectors that control signaling cascades leading to cell polarity, a prerequisite for migration.

As Dictyostelium cells chemotax, they align in a head-to-tail fashion and migrate in chains. Our quest to understand how chemoattractants regulate cell polarity and migration led us to discover—thanks in large part to live cell imaging—a novel mechanism of chain migration: Polarized and migrating Dictyostelium cells concentrate their adenylyl cyclase protein at their back, generating and secreting cAMP, which in turn attracts neighboring cells.

Cells lacking adenylyl cyclase activity, or in which the enzyme is active but mislocalized, do not exhibit this remarkable chain migration behavior.

We proposed that this asymmetric distribution provides a compartment from which cAMP is secreted to act locally as a chemoattractant, thereby allowing cells to follow each other and greatly amplifying the chemotactic response.

Indeed, the relaying of chemotactic signals that is exhibited by Dictyostelium cells leads to the recruitment of significantly more cells to aggregates, a potential mechanism of survival of this species.

Using highly sensitive fluorescent microscopy analyses, we also established that adenylyl cyclase is not only highly enriched at the back of chemotaxing Dictyostelium cells but also found on very dynamic vesicles that coalesce at the back of cells.

Photo-bleaching experiments showed that these vesicles are involved in replenishing the plasma membrane, suggesting that they are actively engaged in membrane trafficking and perhaps are part of a regulatory mechanism that controls adenylyl cyclase activity.

This unique chemoattractant amplification mechanism may well be conserved in higher eukaryotes. Migration of cells as groups is common during development and wound repair, as well as in metastatic processes. Yet the mechanism by which this occurs remains obscure.

We are currently expanding our studies to include neutrophils, which share striking behavioral and mechanistic similarities with Dictyostelium cells, and to metastatic cancer cells, which have been shown to migrate in files as they leave tumors and invade other tissues.

Such studies should continue to exert a profound impact on our general understanding of the mechanisms used by various types of cells to attain a specific destination in the context of both normal physiology and disease.

Shyamal Peddada *received his Ph.D. in 1983 from the University of Pittsburgh, under the supervision of C. R. Rao. He held various academic positions and was a tenured full professor in the Department of Statistics at the University of Virginia in Charlottesville before joining the Biostatistics Branch, NIEHS, in 2000. He is currently a senior investigator and director of the Statistical Consulting Service in that branch. He is an elected fellow of the American Statistical Association and an elected member of the International Statistical Institute, as well*

Recently Tenured

as a recipient of the American Statistical Association's Outstanding Statistical Application Award.

Shyamal Peddada

I have worked in several different areas of statistical theory, methodology, and applications; one of my major contributions is in the area of statistical inference under order restrictions.

In many applications, a research hypothesis of interest can be formulated in terms of mathematical inequalities (known as order restrictions) among the unknown parameters.

For instance, when conducting dose-response studies, a researcher may hypothesize a particular shape or pattern of mean response with respect to dose, which can be expressed in terms of mathematical inequalities.

As an example, consider a dose-response study with a control, a low-dose, and a high-dose group. The experimenter hypothesizes that the mean response has an increasing pattern (or shape) with dose.

The hypothesis can then be restated in this manner: The mean of the control group is at most as large as that of the low-dose group, which is at most as large as that of the high-dose group. By incorporating the shape of the curve into the statistical analysis, one can greatly enhance the power of a test.

The most commonly used statistical procedures (such as ANOVA and the pairwise t-tests) do not incorporate such information and hence are not powerful in the context of dose-response and time-course experiments, especially when the sample sizes are small.

On the other hand, the order-restricted statistical procedures, such as those developed by my colleagues and me, enjoy substantially greater power over the standard procedures.

Further, results obtained from such analyses have biological implications because they describe the pattern of biological response to treatment over dose and/or time.

An important application of my research program is the analysis of microarray gene-expression data from time-course and/or dose-response experiments.

We have developed user- friendly and freely downloadable JAVA-based computer software called ORIOGEN (order-restricted inference for ordered gene expression) for analyzing microarray gene expression data. This software is available at

<http://dir.niehs.nih.gov/dirbb/peddada/peddadamain.htm>.

Though it was developed specifically for analyzing microarray gene-expression data, ORIOGEN can be applied to data from any time-course or dose-response experiment.

We are now developing new methodologies, including Bayesian techniques, to allow for more complex dependence structures.

These methodologies would be useful for many different applications, such as analyzing gene-expression microarray data obtained from dose-response studies with repeated measures, analyzing data on multiple tissues obtained from the same subject, and associating different types of correlated data, such as correlating phenotype with genotypes. ■

NCI Symposium on Chromosome Biology

An NCI Symposium on Chromosome Biology, exploring the current status of chromosome and chromatin biology research, will be held **April 26–27** at the Natcher Conference Center. Topics include transcriptional regulation, chromatin structure, epigenetics, DNA replication and repair, and nuclear architecture.

Speakers from outside NIH include:

Genevieve Almouzni, Institut Curie in Paris; Frederick Alt, Children's Hospital Boston; Carlo Croce,Ohio State University in Columbus; Titia de Lange, The Rockefeller University in New York; Mark Groudine, Fred Hutchinson Cancer Research Center in Seattle; Stephen Jackson, University of Cambridge in England; Jeannie Lee, Massachusetts General Hospital in Boston; David Pellman, Dana-Farber Cancer Institute in Boston; Robert Roeder, The Rockefeller University; David Spector, Cold Spring Harbor Laboratory in Cold Spring Harbor, N.Y.; Thea Tlsty, University of California, San Francisco; Robert Tijan, University of California, Berkeley; Carl Wu, NCI; Yi Zhang, University of North Carolina at Chapel Hill; and others.

Among NCI speakers are Michael Bustin, Shiv Grewal, Gordon Hager, Michael Lichten, James McNally, and Tom Misteli.

There is no registration fee, but space is limited.

For information, registration, and poster abstract submission, visit

<http://www.palladianpartners.com/cecb2007>.

Reminder: NIH-Duke Application Deadline

Applications are being accepted for the 2007–2008 NIH-Duke Training Program in Clinical Research. The deadline for applying is **March 1, 2007**.

Designed primarily for physicians and dentists who desire formal training in the quantitative and methodological principles of clinical research, the program calls for part-time study, allowing students to integrate their academic with their clinical training.

Courses are offered at the NIH Clinical Center via videoconference. Credit earned may be applied toward satisfying the degree requirement for a Master of Health Sciences in Clinical Research from Duke University School of Medicine in Durham, N.C.

Applications are available in the Office of Clinical Research Training and Medical Education, Building 10, Room B1L403. Additional information on coursework and tuition costs can be found at

<http://tpcr.mc.duke.edu>.

Interested individuals should check with their institute or center regarding funding for participation in this program. Successful applicants will be notified by July 2, 2007. ■

Pain, Opioids, and Addiction

To find out about the NIDA-sponsored conference **March 5–6** at Natcher, visit

<http://conferences.masimax.com/opioid/index.cfm>.

Calling All FARE Fellows: Abstracts and Awards

The 14th annual Fellows Award for Research Excellence (FARE) 2008 competition will again provide recognition for outstanding scientific research performed by intramural postdoctoral fellows. FARE winners will each receive a $1,000 travel award to use for attending and presenting their work at a scientific meeting. Twenty-five percent of the fellows who apply will win an award.

FARE applicants must submit an abstract of their research, which will be evaluated anonymously on scientific merit, originality, experimental design, and overall quality/presentation. The travel award must be used between Oct. 1, 2007, and Sept. 30, 2008.

The FARE 2008 competition is open to postdoctoral IRTAs, visiting fellows, and other fellows with fewer than five years total postdoctoral experience in the NIH intramural research program. Pre-IRTAs performing their doctoral dissertation research at NIH are also eligible to compete. Visiting fellows and scientists must not have been tenured at their home institute. Questions about eligibility should be addressed to your institute's scientific director.

Fellows are asked to submit their application, including abstract, electronically from **March 12 through April 16, 2007,** via **<http://felcom.nih.gov/FARE>**.

Winners will be announced by the end of September 2007. More information is available on the above-mentioned web site. Questions may be addressed to your institute's Fellows Committee representative. ■

The Catalyst Needs . . .

The NIH Catalyst is seeking the following:

- One or two additional Editorial Advisory Board members (see current roster on back page) from the ranks of staff scientists and/or fellows
- Volunteer writers
- Someone to revive and oversee the once very popular "Hot Methods" series. To see examples, go to **<http://www/nih.gov/catalyst>**. Hit "Search The NIH Catalyst" and type "Hot Methods" in the search box.

Interested parties, please contact Fran Pollner at **<catalyst@nih.gov>**.

Kids' Catalyst

Viscosity Ferocity: Flow in the Kitchen

You want some ketchup with your fries, and there you are pounding the end of the bottle and nothing is happening. You want some honey in your tea, and it's a lot harder to squeeze the bottle now than it was during the summer.

What we do for food!

When you've finally achieved the small victory of sweetening your tea, don't just sip away. There's science here—specifically, there's viscosity, which basically means how something flows.

Try putting that bottle of honey in warm water for a few minutes to change the viscosity. It will be a lot easier to pour, but once it cools again, it will return to its hard-to-squeeze state.

It's the same for gold and rock: Heat them enough and they will flow to sometimes beautiful (and sometimes disastrous) consequences.

One wonderful exception to this is something you can demonstrate in your kitchen without too much mess (or a mess that is very easily taken care of).

Today we're serving a marvelous mixture of cornstarch and water that I affectionately refer to as "morph." I've also heard "glop" and "ooze," which doesn't quite get it for me. You could try "non-Newtonian liquid," but that's a bit long and may make classmates either look at you funny or designate you as the homework helper. I stick with morph.

For this experiment you'll need

- 1 cup of cornstarch
- 1/2 cup (approximately) of water
- Large bowl
- Thick straw (a casing from a ballpoint pen works perfectly)
- Plastic water bottle
- Time (I played with this mixture for longer than I care to admit)

Put the cornstarch in the bowl and play with it a little while. If you squeeze the powder, it will sound like crunching snow. So now that your fingers are coated in powder, start adding water a little at a time (a tablespoon will do if you are being exact) and mixing with your hands. (Of course, you could mix with a spoon, but how much fun is that?)

You will notice immediately that this is not mixing like pancake batter and milk—it doesn't taste like it either—but that is the consistency you want to achieve for perfect morph. You will also experience something that is not at all common in liquids: If you stir slowly, there's no particular problem. If you stir quickly, it suddenly feels as if you are stirring sand.

Try to pick up some morph. After you finally manage to get some of it in your hands, try to shape it into a ball. Trust me, it can be done. Then hold it in the palm of your hand and watch it liquidate. . . all of your hard work morphing back to its original form! But it felt solid.

After you've perfected the ball, try a cube. It starts caving in before you know it, but just for a moment it felt as if you could roll for your next turn.

When you finally tire of doing this, take the thick straw and poke it into the surface of the morph. What do you think will happen?

If you go slowly, the straw will go right to the bottom of the bowl. Go quickly, and you'll never hit it. Let your hand sink to the bottom of the bowl and then pull it out. Again, going slowly is no problem. Go quickly, and you'll pick up the bowl with one finger!

So when you're finally told it's time to do "real" homework instead of playing with the stuff, don't throw it away! Discover yet another interesting property of morph by pouring it into a plastic bottle. The morph will form ribbons on its journey from bowl to bottle.

When you're done with the experiment, just throw away the bottle, and don't put the mixture down the drain. (Clumps would not be good for the pipes.)

Morph changes its viscosity when you apply pressure. But not honey, which is changed by temperature. Of course you could try applying pressure to honey— it will taste better in your tea, but it won't become morph!

—Jennifer White

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...

- What's New In Biodefense?
- Common Diseases And the Genome
- Research Roundup

We hear this band hasn't had a gig since October 2005, when it played the Wilson Hall at a send-off for Dushanka Kleinman, who had been detailed to the OD to get the NIH Roadmap up and running. After her success, she was returning to her home at NIDCR, where she is deputy director, and NIH wanted to thank her not only with commendations and food but with a rip-roaring musical tribute as well.

So this group that called itself "The Directors"—three guitarists and one keyboardist—was called in. They did a great job. They filled the hall with music both raucous and harmonic; invented lyrics that fit the occasion to songs like "Do the Locomotion," "Homeward Bound," and "On the Road Again"; and generally rocked out like there was no tomorrow. But we always thought there would surely be a tomorrow for so talented a crew. And we have waited these many months for their return to the stage. But, alas, they seem to have disappeared.

So we are running this picture just because it gives us pleasure simply to look at it and we've been wanting to for over a year now—and because Kids' Catalyst ran long this issue (see p.15) we had this fitting space . . . .

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>

Publisher
Michael Gottesman
Deputy Director
for Intramural Research, OD

Editors
John I. Gallin
Director, NIH Clinical Center

Henry Metzger
Scientist Emeritus

Managing Editor
Fran Pollner

Writer-Editor
Christopher Wanjek
Director of Communications, OIR

Copy Editor
Shauna Roberts

Contributor
Jennifer White

U.S. Department of Health and Human Services
National Institutes of Health
Building 2, Room 2E26
MSC 0235
Bethesda Maryland 20892

FIRST-CLASS MAIL
POSTAGE & FEES PAID
DHHS/NIH
Permit No. G-802

Official Business
Penalty for Private Use $300

Printed on 50% recycled content paper and can be recycled as office white paper.