FOSTERING COMMUNICATION AND COLLABORATION

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Hot Methods

RNAi Catching On As Gene Silencer

by Masashi Rotte

Running interference—that is, interfering with the expression of targeted genes—has become a popular sport in some scientific circles.

Called RNA interference (RNAi), this approach to intervening in biological processes is gathering momentum as researchers refine their techniques and envision widening applications in human health.

RNAi has been the subject of an increasing number of research articles and in February was the theme of two NIH seminars—as well as an e-mail posted on the NIH fellows' ListServe by an NIMH researcher asking, "Is anybody out there synthesizing siRNAs? We need a considerable amount to study functional roles of a gene possibly involved in mood regulation." [siRNAs are short interfering RNAs used in RNAi in mammals, discussed later.]

from the Therapeutics Oligonucleotides Interest Group web site

Background

RNAi is a naturally occurring form of post-transcriptional gene silencing (PTGS) mediated by double-stranded RNA (dsRNA).

By taking advantage of RNAi mechanisms, researchers have been

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The Greening of NHGRI

by Celia Hooper

Befitting its domain—one of the most dynamic and rapidly evolving fields of biomedical research—10-year-old NHGRI has been led by the youthful and energetic. Its second scientific director is no exception.

The new SD, Eric Green, was selected from the intramural program after a worldwide search and following the departure of Jeff Trent late last year. Green, 43, also continues to maintain his laboratory research program in the Genome Technology Branch and to serve as the director of the NIH Intramural Sequencing Center.

On the Move

Under Green's leadership, the more-than-400-person, approximately $90-million intramural program is churning: New programs are sprouting, cores are evolving and teaming up with scientists in and out of the institute, and both intramural and extramural NHGRI constituencies are planning celebrations and zooming off with a new research roadmap, drawn up through a year-long exercise.

Green spoke to *The NIH Catalyst* in January and said he doesn't think his youthfulness accounts for his success. But he says it also hasn't hurt. "A decade ago, this institute was led by two people [Director Francis Collins and SD Trent] who were then roughly my age. I would argue it has done quite well."

The institute-wide planning process gave NHGRI a chance to study its progress and look ahead. The resulting new vision for genomics research will be a prominent feature of NHGRI's joint celebration of the completion of the human genome sequence and the 50th anniversary of the discovery of the double-helical structure of DNA in April (see box of April events, page 5).

"If I were to name one theme of the intramural program in the first decade," Green says, "it has been figuring out what are the best ways to use the fruits of the Human Genome Project [HGP] for doing research into human genetic diseases—medical genetics, cancer genetics, gene therapy, genetic medicine, . . . what's the best way to do such research in this new exciting era?"

Maggie Bartlett, NHGRI

Eric Green

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The NIH Intramural Program: Vive la Difference!

by Joram Piatigorsky Chief Laboratory of Molecular and Developmental Biology, NEI

Joram Piatigorsky

I have been an intramural research scientist at NIH since 1967 and am deeply grateful for the extensive support and opportunities I've had to pursue what I believed were exciting scientific directions. As Michael Gottesman pointed out in a recent column ("What Is Special About the Intramural Research Program?" *The NIH Catalyst*, September-October 2002, page 2), NIH's unique research environment—long-term funding, unparalleled material and human resources, and a premier clinical research facility—encourages innovative science.

This article gives voice to my concern that a changing role for the Boards of Scientific Counselors (BSCs) may threaten the most important features of the intramural research program (IRP). This fear may be unfounded, but I would rather express what turns out to be an unnecessary concern than remain silent about something that means a great deal to me.

Let me make clear that I understand the need for critical review of the research of principal investigators and the usefulness of a scientific advisory board for an institution; indeed, I have benefited from reviews and have served on numerous advisory boards that were often (not always) helpful. After all, limited resources cannot be given out strictly on the basis of trust and longevity. But neither should advisory boards govern institutions or make final decisions, particularly on the thorny topic of future directions. And, certainly, no advisory board should have such decision-making power without an appropriate and effective court of appeals. I fear that reliance on the BSCs for sage advice is growing into acquiescence in BSC determination of the direction of NIH intramural research.

> **I fear that reliance on the BSCs [Boards of Scientific Counselors] for sage advice is growing into acquiescence in BSC determination of the direction of NIH intramural research . . . . There ought to be no blurring of the distinctions between the NIH intramural and extramural programs.**

One argument that I hear for turning to the BSCs for leadership is accountability. Certainly NIH intramural scientists must be accountable to the scientific community for the quality and impact of their work. In the past, BSC reviews have been essentially retrospective; today we hear rumors that a major part (even 50 percent in some institutes) will be prospective. Retrospective reviews were never meant to eliminate considerations of future directions or clinical relevance, nor have they done so. But the notion that BSC reviews become as prospective as they are retrospective suggests that they are coming to be seen as substitutes for NIH R01 grants. If this is the case, it presages the elimination, not the improvement, of the IRP.

There ought to be no blurring of the distinctions between the NIH intramural and extramural programs. But I believe that's what started to happen as R01 grants became difficult to obtain ("triaged" or "approved but not funded") at the same time academic institutions were having increasing financial problems. The IRP response to growing extramural resentment was to try to minimize the perceived advantage of intramural researchers. NIH tried its best to level the playing field of the intramural and extramural programs, as it were. But philosophical differences cannot be modified without cost, and maybe it isn't wise to change philosophy without understanding the cost.

Clearly, academic institutions cannot survive without funding from the outside; they need to seek grants and produce "marketable" material. This says nothing about their high standards of excellence and valuable contributions, which are self-evident; it speaks only about the reality of their circumstances. By contrast, the NIH IRP has a more stable money supply, as well as a responsibility to use it well.

The mission of NIH is to alleviate human suffering from disease, and this requires synthesis of research results from basic, clinical, and applied research. Imagination and rigor are the greatest challenges for intramural scientists—not necessarily the suggestive "preliminary data" that so often inform the successful bid for NIH funding of extramural scientists. Intramural scientists co-exist and collaborate with extramural scientists; they are not and need not appear to be extramural scientists. It must be underlined that intramural scientists need not solicit funds, nor should they gear their thinking and research agenda to that end. They can take risks, fail, and try again.

They are the fortunate benefactors of a generous public's investment of approximately 10 percent of the NIH budget. And the return on that investment has been extraordinary (see "Reflections and Projections: Taking Stock of the Intramural Program,"

The NIH Catalyst, May-June 2001, for a brief review).

At best (why aim for less?), intramural scientists provide new ideas, new ways of thinking about old problems and disabling diseases, and new opportunities for medicine. They can excel in such endeavors because doing research in the NIH IRP means having the time to develop ideas without the need for short-term proof of their value; it means being able to change directions when it is wise to do so.

I believe it is shortsighted to make support within the IRP depend on the BSCs to the same degree that extramural support depends on the NIH study section. Rigorous BSC review serves to improve the performance and maintain the quality of intramural research. It has great value. But to rely on the BSCs for dictating directions undermines the rationale upon which the IRP is based—and threatens to curtail the kinds of advances fostered by that rationale for more than 50 years.

The intramural program is not static, and I am not advocating that it become so. But it must be recognized that we are not the extramural program, and to move the IRP in the direction of the extramural program will not improve it; it will end it. ■

Michael Gottesman

Response from the DDIR:

Dr. Piatigorsky makes many important points about the differences between the intramural program and the extramural program in his commentary. These issues are a continuing subject of discussion by the chairs of the Boards of Scientific Counselors (with whom I meet annually) and the scientific directors. Dr. Piatigorsky and I are in agreement on most of these points, especially the need for rigorous, regular review of intramural science to encourage and sustain the highest quality, most innovative science possible.

To continue this discussion, I would like to summarize the current philosophy underlying the intramural review process.

The purpose of evaluation by outside reviewers is to make sure that our standards of excellence and sense of scientific direction are not parochial, but reflect the best advice from experts in our fields. We are fortunate to have members on our BSCs who are themselves first-class scientists; they are carefully vetted within the institutes and must all be appointed by me prior to service on a BSC. I look for evidence of leadership and ongoing contributions to their fields.

Three institutes (NHGRI, NICHD, and NCI) use site-visit teams led by at least two BSC members. This approach means that the initial review is mostly by subject matter experts, but the entire BSC weighs in at the end with a balanced perspective. PIs under review may challenge a reviewer in writing based on a real conflict of interest, but being "expert," and thus a potential competitor in their subject matter, does not constitute a basis for challenge.

The BSC and site-visit members are told that their function is to provide advice to the scientific directors on the scientific work of their PIs. Although this advice is almost always taken, a scientific director may choose, after discussion with me and the institute director, to follow his or her own instincts about particularly risky work or innovative investigators. There is a pamphlet given to BSC and site visitors that defines the criteria for review: **<http://www1.od.nih.gov/oir/sourcebook/sci-review/review-criteria.htm#Criteria>**.

This document makes clear that the review is primarily retrospective, but that approximately one-third of the site-visit report and the oral presentation should focus on Future Plans. The purpose of this prospective component of review is to reassure the reviewers that the PI is able to formulate good questions and develop strategies that use current (and future) technology to answer these questions.

For tenure-track investigators, who have little or no track record, such prospective review is critical. For more established investigators who have repeatedly solved difficult scientific problems in the past, Future Plans are a chance to obtain support for new investments that might be needed to advance their work. The BSC members are encouraged to use the site visit as an opportunity to evaluate the state of science in the lab at the present time, as reflected in both past accomplishments and future directions. This is a subtle point—and a strong distinction from the extramural prospective review process. This emphasis on past performance and current work is an important distinction that must be made to sustain the creativity and productivity of the intramural research program.

I hope this discussion will reassure PIs who, like Dr. Piatigorsky, are worried about the drift of the intramural review process to a more extramural review style. There is general agreement among NIH leaders that the distinction between extramural and intramural must be maintained if the intramural program is going to continue to make unique contributions to biomedical research. ■

The Greening of NHGRI
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Celia Hooper

Carrying the NHGRI Torch:
New SD Eric Green

Adieu Dichotomy

By contrast, the extramural program was intensely focused on obtaining the sequence of the human genome. "So for a long time, there was this huge dichotomy: Extramural did the HGP; intramural did applications of genomics and genetics," Green says. But as the new vision emerges, he predicts, the extramural program "is going to start looking a lot more like what the intramural program has been building—with a major emphasis on applying genomics to research into health and disease."

Green says the vanishing dichotomy reflects visionary planning by NHGRI's leadership. "They were saying 'this is where the puck is going to be in the future. . . . We're going to start going there now'"—and that's why, Green says, the intramural program is ideally positioned and equipped.

"There will be fabulous new opportunities for interfacing genomics and genetics with clinical research," he says. "The most distinguishing feature of the intramural program at this campus . . . is the great infrastructure for doing clinical research. . . . Now let's figure out how best to harness it for answering challenging questions in genetics and genomics."

Shades of Green

NHGRI's new scientific director isn't exactly green, even if he is Eric Green. Six years of building the NIH Intramural Sequencing Center (NISC) from the ground up have given him experience and perspective in team-building, science management, and coping with the special challenges of getting science done in government labs.

The number-one lesson thus far, Green says, is that "you always benefit by doing things in a collegial way." This approach was a key to the success of NISC, a 35-person, state-of-the-art DNA sequencing facility that has defied expectations, almost from the outset.

"Nobody ever thought we could build a 35-person group—never thought we'd be able to hire that many people, acquire and spend that kind of money, get the equipment, get the space." In fact, Green says, he was a little surprised himself. "Nine and a half years ago, when I left Washington University [St. Louis, Mo.], I consciously thought I was making a decision never to do large-scale genomics again. I was leaving a large genome center and Francis [Collins] was recruiting me. I knew I was doing the right thing, but I thought I'd never be able to have a large genome group—greater than, say, 15 or 20 people. I shouldn't have thought that."

The trait that built the lab and that he takes with him to his job as SD was boldness. "That's another theme of our institute—be bold. Don't say 'it will never work in the government.' We proved them wrong with NISC. . . . If you have a good idea, be bold about it."

Also important to Green was having creative ideas and being able to rely on the people around him as he juggled responsibilities. "When you tackle a pet project that involves this many people and a lot of money and a lot of space, you have to learn how to multitask" and surround yourself with "good people who can take charge and get things done."

"What I think we've accomplished at NISC is getting good people and giving them a chance to run the place—that's what we want to continue to do for the whole intramural program," Green says.

—CH

The Luxury of Risk Taking

Green observes that "it's not entirely clear which aspects of genetics, especially when applied to clinical problems, are going to be best performed on a large scale vs. a smaller scale. I think that's something we all have to learn and almost handle on a case-by-case basis," he says. But he expects NHGRI will be able to tackle any size project, large, small, and in-between.

Intramural research, he says, "will always take advantage of the luxury we have of secure funding and, coming with that, the opportunity to do higher-risk research." NHGRI's intramural emphasis on technology development—for example, in the areas of genome mapping and functional genomics—has reflected this, he observes. "Part of the reason we were able to do that effectively, I believe, is because we could move quickly on something, even if it was risky."

One high-risk proposition would be tackling rare diseases—"beyond just knowing what the gene is, being able to develop therapies or at least explore therapeutic options." Green notes that rare diseases are a particular interest for his new clinical director, Bill Gahl.

Another high-risk research path still being pursued in NHGRI is clinical gene-therapy trials. The program's basic research into vector biology and design could help advance the problem-plagued gene-therapy field and presage a new generation of clinical trials down the road, Green says.

Core Strengths

One cultural feature of NHGRI that has and will continue to serve the intramural program well is its penchant for interaction and its collegiality. Green notes that much of the work by NHGRI

investigators in identifying disease genes was carried out in collaboration with intramural investigators in other institutes who were experts in the diseases or physiological system in which the genes play a role.

Green says these partnerships "led to some fabulous sets of experiments," and he cites as an example his own lab's work with NIDCD in the identification and characterization of a gene responsible for a common form of inherited deafness. This sort of teamwork, he says, "leads to first-rate studies and first-rate publications."

NHGRI's technical cores and centers provide other examples of fruitful collegiality. The Center for Inherited Disease Research on the Bayview campus in Baltimore does large-scale genotyping for intramural and extramural investigators. Green's own NIH Intramural Sequencing Center (see "Bringing Up 'Baby,'" page 7) was established by 14 cooperating institutes and has cranked out scores of large-scale sequencing projects for intramural investigators on a fee-for-service basis.

Two keys to successful technical cores and centers, Green says, are ongoing evaluation of the size and need for the core, and being ready to discard the old in favor of better new approaches. "If you are going to develop cutting-edge techniques, you've got to be ready to retire old ones."

Intramural scientists in other institutes have also tapped into NHGRI's expertise in microarray development and ap-

'DNA Day' and 'Human Genome Month' Give April 2003 a New Persona

Variously regarded as the "cruelest month," the month of showers, and the bane of taxpayers, April in the year 2003 takes on new luster. It is during that month, says NHGRI director Francis Collins, that "we [will] declare the sequence of the human genome essentially finished, and we will also celebrate the 50th anniversary of the Watson and Crick publication [describing the structure of DNA] that appeared in *Nature* on April 25, 1953."

These two causes for celebration have inspired the federal government to proclaim April as "Human Genome Month" and April 25 as "DNA Day."

A series of commemorative events will take place in the Washington area April 13 through 16th, including:

- A preview **April 13** of a **genomic exhibit,** "Genome: How Life Works," at the Smithsonian Institution's Arts and Industries Building. Produced by Clear Channel Communications with support from Pfizer, Inc., the full exhibit will open at the Smithsonian in June 2003.
- A half-day **scientific symposium**, "Linking the Double Helix with Health: Genetics in Nursing," the afternoon of **April 13**, 2:00 p.m–6:30 p.m., at Georgetown University School of Nursing, St. Mary's hall, sponsored in part by NINR.
- A two-day scientific symposium, "From Double Helix to Human Sequence—and Beyond," April 14–15 at NIH's Natcher Conference Center, which will be webcast within NIH and to institutions around the world. Participants—including James Watson and members of the International Human Genome Sequencing Consortium—will describe the science and history of the Human Genome Project. The symposium will explore the future of science and medicine made possible by breakthroughs in genomic science and will include the unveiling of NHGRI's plan for the future of genomics and the institute. For more information about this event, contact Allison Peck at (301) 451-8323 or by e-mail:
  <pecka@mail.nih.gov>.
- A half-day **public symposium**, "Bringing the Genome to You," will be held the morning of **April 15** at the Smithsonian's National Museum of Natural History. The talks are designed to convey how genomics influences health and society. For more information, contact Mini Nair at (301) 402-0955 or by e-mail: **<nairm@mail.nih.gov>**. If you are interested in receiving this videocast, please contact Maggie Bartlett at (301) 594-0632 or by e-mail:
  <m4b@mail.nih.gov>.
- A half-day **scientific symposium**, "Genetic and Gene-Environment Interaction in Human Health and Disease," **April 16**, 8:30 a.m.–12:30 p.m., at Masur Auditorium, Building 10, sponsored by NIEHS, in collaboration with NHGRI and NIAAA. For more info and to register, visit
  <http://www-apps.niehs.nih.gov/odconfer/gxe/home.htm>
- An all-day **scientific symposium**, "Genes, Brain, Behavior: Before and Beyond Genomics," **April 16**, sponsored by NIMH and other NIH institutes, 8:30 a.m.–5:45 p.m. (breakfast from 7:30), Wilson Hall, Building 1. No advance registration required.
- A national **"DNA Day," April 25**, on which high schools throughout the country celebrate the 50th anniversary of the description of the DNA double helix. NHGRI scientists, many of whom are part of the mentors network of the American Society of Human Genetics, are being encouraged to consider speaking at a local school to mark this day. For more information, contact Susan Vasquez at (301) 402-2205 or by e-mail:
  <vasquezs@mail.nih.gov>.

In addition to these specific events, NHGRI is also planning a long series of scientific, educational, cultural, and celebratory events across the United States, including:

- **Activities at science museums** across the country. Items available to museums include a program guide of genomics-related events, a training workshop for museum staff, a kit of materials and equipment, and advertising graphics to call attention to the events. For more information, contact Kris Wetterstrand at (301) 435-5543 or by e-mail:
  <wettersk@mail.nih.gov>.
- **Public outreach** through television, radio, and print features on genetics and genomics.
- **Classroom outreach**—Lesson plans, challenging activities, and curriculum supplements regarding the Human Genome Project, genomic science, and the basics of human genetics will be developed and made available online. More information is available on the NHGRI website at **<www.genome.gov/Education>** or contact Susan Vasquez at (301) 402-2205; e-mail.
  <vasquezs@mail.nih.gov>.

For more details about the NHGRI-sponsored events, visit
<http://www.genome.gov/About/April2003/>.

plication under Trent's leadership, and others have benefited from NHGRI's developments in computational genomics—including new tools available on the web.

Still others have kept up with the fierce pace of genetics discovery through a lecture series called "Current Topics in Genome Analysis," which Green, along with Deputy SD Andy Baxevanis, has organized and run about every 18 months. "Close to 2,000 people have sat through those lectures," Green says. "Nobody asked us to do it—we just did it."

Looking ahead, Green is excited about a joint venture with NICHD to create "what's going to be one of the largest zebrafish facilities in the world. It's going to be built on this campus because we recognize that zebrafish, as a genetic model, is incredibly important"—or will be very soon, with the imminent completion of the sequencing of the zebrafish genome by the Sanger Institute in England.

In addition to providing state-of-the-art facilities for NHGRI's three zebrafish investigators—and no doubt many collaborators—Green says NHGRI is also in early pilot stages of developing a resource, potentially for the world, of zebrafish mutants.

The Genome Culture—And the Future of Genomics

"We have a culture in our institute—it's the genome culture—that if you do good work and it's good for you, that's wonderful; if you do good work and it's good for more than just your lab, it's even better." This mindset, says Green, is heavily rewarded and highly regarded—and it's been gratifying to him personally to see junior investigators embracing it as they rise through the ranks.

The institute also continues to build new schemes for creating an even more robust academic and intellectual environment, including its Associate Investigators Program (see "Fresh Fields" at right) and a Visiting Investigators Program; the latter brings to NHGRI roughly one scientist a year from outside NIH—typically on sabbatical—and pays partial salary and research support.

Green says the visitors often come to acquire expertise in genetics or genomics or to use the help of NHGRI's technical cores to get over the next hurdle in a research project.

From new schemes to a culture of collegiality, NHGRI's intramural program is beautifully suited for contemporary biomedical research, Green thinks. And it's in a great position, he says, to find answers to the key questions of the newly entered genomic era: "How do you take a conventional laboratory, say a half-dozen or a dozen people, and grapple with massive datasets, even at a computational level? How do you study thousands of genes all at once using technologies like microarrays? How do you mine this information efficiently to find genes and show those that are implicated in human disease? How do you grapple with the complexities of diseases that have multiple genetic components?"

These questions—these "hard, hard problems"—are what the future is all about, Green says, and "they're the ones that our program wants to tackle." ■

Fresh Fields: NHGRI's Associate Investigator Position

One organizational experiment underway at NHGRI is a perhaps trendy new category of scientific staff called "associate investigators." NHGRI's scientific director, Eric Green, says his institute launched the program about 18 months ago and now has 16 associate investigators. "This is very novel," he says. "Other institutes have been talking to me about this."

Neither a hiring mechanism nor an official government designation, the title instead describes a group of researchers who provide vital scientific leadership in the institute—people who must be at the table when it comes to faculty meetings or critical research planning but who are not investigators with their own independent research funding. The associate investigators are all assigned to particular branches within the institute. Their work is considered part of the total program of the branch during its quadrennial review.

Comparing associate investigators to research-track faculty at universities, Green says, "We regard them as faculty members, and we wanted to acknowledge their role as leaders within the institute." In terms of professional designation, most of the associate investigators are staff scientists, but not all. "And there are some staff scientists who are not associate investigators," Green adds.

Green says there also is a mixture of fields represented by associate investigators, including informatics staff, core directors, genetics counselors, a bioethicist, the associate clinical director, and key leaders in production genomics facilities. He's happy with the institute's current mix of senior investigators, investigators, and associate investigators.

In Green's view, the concept of the associate investigator is very much in keeping with the need in the genome era for "more interdisciplinary, multidisciplinary, and larger consortium-like efforts." He says this has actually been a hallmark of the 10-year-old NHGRI since its inception, which included supportive cores specializing in particular technical areas.

"We've developed a culture of recognizing that to do contemporary genetics and genomics research, it's not just about individual people working in individual labs doing individual projects," Green says. "The foundation of our institute was built to include . . . infrastructure that investigators could tap into." With technology evolving rapidly, high-level expertise is required. "If you want to have state-of-the-art sequencing, genotyping, microarrays, or computational biology, you have to have state-of-the-art good people who are running those facilities" and "deserve recognition as faculty."

The Associate Investigator Program "may be one of the reasons we have really good people at our institute," Green brags, "not just at the very top, but throughout our ranks."

—CH

Bringing Up 'Baby'

In addition to its fee-for-service sequencing services, Eric Green's "baby," as he calls the NIH Intramural Sequencing Center (NISC), has exciting new projects underway.

"The coolest thing we are doing now is a major effort on behalf of the Human Genome Project to investigate which additional animal species to sequence in the future," Green says.

In conjunction with Green's laboratory in the Genome Technology Branch, NISC is doing some reconnaissance work—taking carefully selected regions of the genome and then sequencing them in about two dozen animal species. The resulting sequences are compared and contrasted to see what and how much is to be learned from each genome's tale. "Did you learn a lot? Did you learn a little? Would it be worth getting the whole genome?" The main focus will be on vertebrates, Green says, because the ultimate goal is to get the most help in interpreting the human genome.

Sampling the same region from each genome, NISC has sequenced DNA from baboon, chimpanzee, macaque, lemur, gorilla, dog, cat, cow, pig, rabbit, opossum, platypus, chicken, zebrafish, and pufferfish, for starters. "It's a real Noah's ark project, with many more species to come," Green says—a perfect-sized project for NISC as a mid-sized sequencing center.

The reconnaissance will include a few more distantly related species, Green says, because "we actually don't know the right points on the evolutionary tree to sample to maximally understand genome function and evolution."

Sampling small bits of sequence—maybe 1 percent of each animal's genome—before committing to an entire genome will help assure that the most informative genomes get the full work-up, maximizing the benefit-cost ratio.

In addition to giving scientists "a sneak preview of the future," when as many as a dozen or more vertebrate genomes will have been sequenced, the reconnaissance project will also give computational biologists some datasets, "to allow them to start building tools to better compare and analyze genomes," Green says.

—CH

Double-B Roundup: Congress Puts Its Stamp on the NIH Agenda

Five months into FY 2003, Congress finally passed an omnibus appropriations bill for the fiscal year beginning October 1, 2002. The bill accords NIH $27.2 billion—thereby completing the promised doubling of the NIH budget five years ago.

The measure was signed into law February 21 by President Bush, who earlier that month submitted a presidential 2004 budget request that validated the cautionary words of NIH Director Elias Zerhouni late last year: NIH could be in for a bumpy landing after its five high-flying years (see "Roadblocks, Road Maps, and 'The Perfect Storm,'" *The NIH Catalyst*, January-February 2003, page 5).

The president's 2004 request would increase the NIH budget for fiscal 2004 by 2 percent, from $27.2 billion to $27.9 billion, well below the usual levels of inflation. On the heels of the president's proposal, however, Sens. Arlen Specter (R-Pa.) and Tom Harkin (D-Iowa), longtime champions of NIH, announced plans to introduce a bill that would triple the NIH budget between 1998 and 2008. (For some details of the president's proposed budget, see *The NIH Record*, March 4, 2003.)

Meanwhile, Congress has some definite ideas about how NIH should spend the money appropriated for the current fiscal year. Among the suggestions offered in the congressional "conference agreement," issued along with the omnibus bill, are the following:

- NCI ought to create multi-institutional, multidisciplinary lung cancer consortia dedicated to overcoming a "pervasive sense of 'therapeutic nihilism.'"
- NHLBI, in collaboration with NINDS, ought to develop a diagnostic test for transmissible spongiform encephalopathies for screening the blood supply; NHLBI also ought to support intramural and extramural clinical trials aimed at finding a cure for lymphangioleiomyomatosis, a rare lung disorder.
- NIDDK ought to launch new training initiatives to stave off an anticipated shortage of nephrology specialists.
- NINDS, in collaboration with NIAID, ought to suport more controlled clinical trials on the effect of neutralizing antibodies on current multiple sclerosis therapies, as well as amass better clinical data on the effectiveness of current combination therapies; scientific workshops should be held on these issues.
- NIAID is permitted to transfer $100 million of its funds to the Global Fund to Fight HIV/AIDS, Tuberculosis, and Malaria. (The conferees also state that they "intend to provide NIAID with flexibility to determine the appropriate share of the Institute's funds directed to bioterrorism research versus infrastructure.")
- NICHD ought to expand research on "stem cells in the most clinically relevant models" using approved stem cell lines to study adult and embryonic stem cells in vitro and in nonhuman primates; NICHD also ought to address standards of care and rehabilitation for persons who have lost limbs, as well as sponsor a prosthetic outcomes research consensus conference.
- NEI is directed to "be prepared to report on advances in research in ocular albinism."
- NIEHS is commended for research initiatives on environmental influences on breast cancer and urged to establish an advisory group to advise the director in this area—and to report on progress toward creating the group in time for the 2004 appropriations hearings.
- NIAMS ought to expand research to identify causes of and develop pediatric treatment options for vitiligo.
- NIMH ought to study the effects of events such as the September 11, 2001, terrorist attack on survivors, emergency workers, and the general public.
- NCCAM is allocated "sufficient funds to increase support for the chiropractic research center."
- The NIH Director's Discretionary Fund is doubled from the previously earmarked $10 million to $20 million so the director may pursue his "roadmap" activities.

The conferees also call for a timetable for building a new NLM facility, a comprehensive assessment of the state of autism research, an answer to any remaining questions regarding ephedra products, and a line-item accounting of research funding for temporomandibular disorders.

They invite NIA and NINDS to support more research on Pick's disease and other τ-protein–related dementias, and they ask NICHD, NINDS, NIDCD, and NIGMS to study the neurological disorder Rett syndrome, especially the effects of the newly discovered putative gene *MECP2*.

—Fran Pollner

RNAi: GENE SILENCER

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able to reduce the expression of specific target genes on an individual cell to organism-wide scale.

History: Plants

In the early 1990s, researchers were attempting to enhance the purple color of petunias by introducing a pigment-producing transgene under the control of strong promoter.

Instead of overexpressing the pigment gene and enriching the color of the plants, the flowers appeared nearly white—the expression of both the transgene and the endogenous pigment gene was "co-suppressed." This co-suppression refers to silencing of gene expression principally at a post-transcriptional level.

Masashi Rotte

Tips on the RNAiceberg: *NHGRI's Natasha Caplen discussed the "Characterization and Application of RNAi in Mammalian Cells"*

Worms and Flies

The phenomenon of PTGS was observed in other plants and fungi during the 1990s, but not until work done with the nematode *Caenorhabditis elegans* by Andrew Fire and his colleagues was a similar effect seen in animals. It was shown that the introduction into embryos of antisense (but, unexpectedly, also sense) dsRNA induced a sequence-specific gene silencing at a post-transcriptional level, an effect that was termed RNAi.

Subsequent studies—one of the first carried out by Leonie Misquitta and Bruce Paterson, NCI—showed a similar response in Drosophila ("Targeted disruption of gene function in *Drosophila* by RNA interference [RNA-i]: A role for *nautilus* in embryonic somatic muscle formation," *Proc. Natl. Acad. Sci. U S A* **96**:1451–1456, 1999).

Mammals

Having found RNAi gene silencing in worms and flies, investigators now turned their attention to determining whether RNAi could be induced in mammalian cells. It was known, however, that in somatic mammalian cells, the introduction of long dsRNAs (~70+ nucleotides) led to nonspecific suppression of gene expression instead of the sequence-specific gene silencing seen in RNAi. Studies revealed that long dsRNAs activated either the protein kinase PKR, leading to the repression of translation, or RNaseL, leading to nonspecific RNA degradation. The subsequent global changes in gene expression usually resulted in cell death.

The development of a strategy to overcome this problem was aided by work in plants and subsequently in Drosophila embryos. This work showed that dsRNA added to the cells was processed by an RNase III enzyme called Dicer to nucleotides 21–23 base pairs in length.

Size Matters

These RNAs were termed short interfering RNAs (siRNAs). Their importance in RNAi emerged with the finding that homologous Drosophila mRNA is cleaved at a site corresponding to the approximate middle of an siRNA sequence.

This knowledge, coupled with previous data showing that RNA duplexes of less than 60–70 nucleotides do not trigger PKR, led researchers to test chemically synthesized siRNAs, 21–27 nucleotides long, in mammalian cells.

These landmark studies, including one by NHGRI's Natasha Caplen, showed that synthetic siRNAs of 21-23 nucleotides in length introduced by transient transfection effectively induce RNAi in mammalian cultured cells in a sequence-specific manner (N. Caplen, S. Parrish, F. Imani, A. Fire, and R.A. Morgan, "Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems," *Proc. Natl. Acad. Sci. U S A* **98**: 9742–9747, 2001).

Mechanism

The working model that has now emerged for RNAi is as follows:

- siRNAs are incorporated into a multiprotein RNA-induced silencing complex, or RISC.
- The siRNA within the RISC unwinds, and the antisense strand acts to guide the complex to homologous mRNA transcript by base pairing.
- The target mRNA is then catalytically cleaved by an undefined component or components of RISC approximately 12 nucleotides from the 3' terminus of the siRNA.

Recently, another RNA species has also been found to use the enzyme Dicer. First identified in *C. elegans,* small temporal RNAs (stRNAs) regulate developmental timing through translational repression of target transcripts using Dicer.

stRNAs have now been found to be members of a large family of noncoding RNAs called microRNAs (miRNA), which have been identified in plants, Drosophila, *C. elegans*, mouse, rat, and human cells. miRNAs require Dicer for their processing and are now believed to play a critical role in regulation of gene expression.

These and other links to endogenous cellular pathways have led to speculation that PTGS evolved as a defense mechanism against transposons and RNA viruses or as a means for the cell to rid itself of unnecessary transcription products.

Implementation

There are several key considerations when designing siRNAs and implementing RNAi. Two recent seminars on campus—one by Caplen, a staff scientist in the Medical Genetics Branch, NHGRI, and the other by Stephen Scaringe, chief scientific officer of Dharmacon, Inc., Lafayette, Ohio—addressed some of those considerations, as well as recent advances in RNAi.

Taking the Measure of Silence

The efficacy of gene silencing by siRNAs can be evaluated by the drop in mRNA concentration of the gene of interest, by the drop in protein expression, or by functional changes in cell phenotype.

During her talk at the Therapeutic Oligonucleotides Interest Group seminar on February 27, Caplen described the use of GFP-proteins to test silencing efficiency of siRNA. A drop in cell fluorescence measured by flow cytometry corresponds to an RNAi-mediated silencing of gene expression, she said.

The most effective siRNAs can reduce mRNA levels and expression of protein by greater than 90 percent. A primary factor that determines the efficacy of silencing is the selection of optimal siRNA sequences to the target mRNA: siRNAs that target different sequences within the same mRNA can show varying degrees of efficacy.

Designer Basics

Caplen gave some of the basic criteria for design of siRNAs:

- They must be 21–23 nucleotides long.
- They should have sequence-specific homology to the target mRNA.
- They should have as close to a 50 percent GC content as possible.
- They should have two nucleotide 3' overhangs.

Researchers are advised to synthesize siRNAs to several sequences in the target mRNA (usually two or three). Having identified a successful siRNA, researchers may then want to make base pairs iterations upstream or downstream of the initial sequence as a way to optimize their siRNA.

Masashi Rotte

Maximizing Beginners' Luck: *Stephen Scaringe, of Dharmacon, Inc., offered best practices in siRNA design, target selection for optimal knockdown, and pooling to maximize the chance of success the "first time out"*

siRNA Pools

In his talk on February 28 on "Developments in siRNA-based Gene Silencing," Scaringe described software that Dharmacon developed to select the most favorable targets in mRNA sequences based on 34 criteria. (A free software module on his company's website selects target sequences but uses only four criteria.)

In addition, Dharmacon uses another five criteria to "pool" the four or five most effective siRNAs. Scaringe claims that his company's algorithms can design siRNA pools that will knock down mRNA levels at least 50 percent. Synthetic siRNA can show RNAi effects six hours post-introduction and the effects can last as long as 10 days, although the degree of silencing will be diminished by that time.

Currently, three main companies offer ready-made, chemically synthesized siRNAs: Dharmacon, Qiagen (Valencia, Calif.), and Ambion (Austin, Texas). Dharmacon has ready-made siRNA kits for several human and mouse genes and also offers custom siRNA synthesis. Qiagen offers a cancer siRNA Oligo set with siRNAs to 139 human cancer genes. Qiagen's website has a database to help select siRNA targets for mRNA of several species in the GenBank database.

Transfection Techniques

A variety of methods have been used to introduce siRNA into cells and organisms ranging from microinjection to soaking cells in dsRNA to feeding *C. elegans* bacteria engineered to express dsRNA. The most common means to introduce siRNA is a lipid-based transfection reagent, but this method is transient at best.

Many researchers are now using plasmids or viral vectors to continually express siRNAs in transiently and stably transfected mammalian cells (mainly from RNA polymerase III promoters using a short-hairpin structure that is processed intracellularly by Dicer to generate an siRNA).

To test transfection efficacy, Caplen has labeled siRNAs with a fluorescence tag. This allows the researcher to monitor uptake of the siRNA and to enrich for cells that have been successfully transfected with fluorescence-activated cell sorting.

Horizons

Caplen notes that among recent developments in the field is the generation of transgenic mice from embryonic stem cells harboring an siRNA expressed from a viral vector. The investigators successfully recapitulated features of animals produced by traditional homologous recombination knockout technologies.

Scanning the recent scientific literature provides a glimpse of some of the targets of RNAi research. Some investigators have determined the specific effects of protein inhibition arising from silencing their gene of interest by using cDNA microarray analysis. Others have used RNAi to silence gene expression in vivo in mice, including a study in which the silencing of *Fas* gene expression blocked hepatocyte cell death. And still others have investigated siRNAs as a therapy to inhibit expression of oncogenes or to downregulate expression of CD4 cells to block HIV entry.

Once optimal siRNAs have been designed and introduced into a cell, the experimental possibilities are limitless. Caplen says that her main research goals are adapting RNAi for high-throughput functional analysis and the development of RNAi as a therapeutic. ■

Disclaimer: *Mention of specific products in this article does not constitute an endorsement of those products, nor does it signify that other similar products are less desirable.*

Catalytic Reactions

Some Suggestions For the Back-Page Space

How about using the back page as an "IC Employee Recognition" page? It would be a nice way to show appreciation to those who may not necessarily be discovering the cure for cancer but who make the day a little brighter by kind words or deeds—someone on staff or who services a building, or IC offices.

Just a thought.

—Chanee Jackson, OD

A suggestion for the back page of the *Catalyst*: You often feature PhDs or top people in their field in issues (which is good), but what about the average employee that makes a significant contribution to their job? Perhaps you could ask for nominations from division heads, select a candidate, and run a short piece on the person and their idea or performance that rated special mention.

—Gail McMullen
Veterinary Associate, NCI

—The Catalyst *would be pleased from time to time to publish back-page photos and a few words about individuals selected for such recognition. Please send us your nominations!—ed.*

Fogarty Scholars

Happy Returns: Tadashi Yamamoto

A visiting fellow from Japan does postdoctoral work from 1977 to 1980 in Ira Pastan's Laboratory of Molecular Biology, NCI, where he studies the mechanisms of replication of retroviruses and the transformation by Rous sarcoma virus. He and LMB colleagues collaborate on eight scientific papers that elucidate the structure and expression of the collagen gene as well as the role of the cellular matrix in cell growth. In 1981, he returns to his home—to the Institute of Medical Science at the University of Tokyo. And then?

If his name is Tadashi Yamamoto—which it is—he spends the next 20-plus years uncovering the molecular basis of cancer development and becoming an international leader in the cell-signaling and oncogene field. And in 2003, he comes back to Pastan's lab, this time as a Fogarty Scholar. Yamamoto's appointment runs from July 1, 2003, through October 31, 2006; he will divide his stay into four three-month visits.

Tadashi Yamamoto

Yamamoto's achievements include:

- Determining the nucleotide sequence of the v-*erb*B gene of avian erythroblastosis virus and its role in the induction of sarcoma and erythroleukemia and establishing that the gene encodes a receptor-type protein kinase—v-ErbB protein, a part of the EGF receptor—shaping our understanding of the relation of normal and aberrant cell growth regulation
- Identifying a homologue of the EGF receptor gene—called c-*erb*B2—and establishing that this gene is amplified and overexpressed in various human tumors and contributes to tumor progression
- Identifying Lyn and Fyn, novel Src family members, and their various roles in B-cell activation, autoimmune disease, and central nervous system function
- Elucidating the nature and function of a variety of kinases, phosphatases, and signaling molecules

His current research focuses on cell cycle regulation and mechanisms of malignant transformation, as well as the roles of protein tyrosine kinases and phosphatases in brain development, especially axon guidance and synaptic plasticity.

As a Fogarty Scholar, Yamamoto will participate in the LMB gene discovery program. Among his projects will be analyzing the phenotypic expression of two newly discovered genes—*NGEP* and *GDEP*—in prostate cancer cells. He will also interact especially with the Cell Cycle and Immunology Interest Groups. ■

World-Class Research Physician: David Weatherall

David Weatherall, regius professor of medicine emeritus, University of Oxford, and fellow emeritus, Magdalen College, Oxford, England, has been appointed a Fogarty Scholar for four months in 2003—in April and May and for another two months in the fall.

NHGRI and NIDDK are co-sponsoring Weatherall's appointment, and the Fogarty International Center (FIC) is providing his base of operations at the Stone House.

The author of 13 books, published between 1967 and 1997, as well as hundreds of chapters and articles, Weatherall is credited by NHGRI director Francis Collins as being "the recognized authority in the world on the molecular genetics of hemoglobinopathies," whose elaboration of the clinical and molecular features of these disorders was the "model for the general molecular understanding of genetic disease."

David Weatherall

Weatherall will speak at the NIH symposium marking the 50th anniversary of the double helix (April 14–15, see page 5), at the FIC 35th anniversary symposium on global health in May, and at the bimonthly meetings of the IC International Representatives Committee.

His involvement with NIDDK, according to NIDDK director Allen Spiegel, will include many speaking engagements on clinical and basic research topics, attending rounds on the clinical hematology services, participating in laboratory projects related to the pathophysiology and treatment of people with sickle cell anemia and thalassemia, and advising the institute on genetic studies of hematologic and nonhematologic disease.

Weatherall, says Spiegel, is "one of the most knowledgeable, productive, and important research physicians in the world." ■

Preventing Breast Cancer

Preventing breast cancer will be the theme of the **March 26** meeting of the Women's Health Special Interest Group.

JoAnne Zujewski, medical director of clinical research operations, NCI, and head of the breast cancer clinical research section, will discuss the effects of raloxifene in premenopausal women, as well as future directions for research.

The meeting will be held in Wilson Hall (3rd floor), Building 1, from 11:30 to 1:00. ■

Recently Tenured

David B. Dunson *received his doctorate in biostatistics from Emory University in 1997 and then began his career as a research fellow at NIEHS working with colleagues Clarice Weinberg on methods for adjusting for measurement error in fertility studies and Joseph Haseman on methods for analysis of tumor data from transgenic mouse bioassays. He is currently an adjunct associate professor of biostatistics at the University of North Carolina at Chapel Hill and an adjunct associate professor of statistics and decision sciences at Duke University, in Durham, N.C., as well as a senior investigator in the Biostatistics Branch, Environmental Diseases and Medicine Program, NIEHS.*

David Dunson

My research focuses on developing and applying improved statistical methods for the analysis of data from biomedical studies. Such data have become increasingly complex and multidimensional as researchers attempt to characterize the time-varying relationships among exposures and different health outcomes.

There is a pressing need for innovative statistical approaches that allow for missing, mismeasured, and censored observations, and for joint analyses of correlated outcomes having a variety of measurement scales (such as binary, categorical, or continuous). We have made substantial progress in addressing such problems using Bayesian hierarchical models and Markov chain Monte Carlo computation.

My work with latent variables and order restrictions has been particularly innovative and has resulted in major advances in the fields of fertility and reproductive epidemiology, developmental toxicology, and tumorigenesis. Parameter or shape constraints can be incorporated to limit the loss of efficiency typically associated with the use of nonparametric methods (for instance, for assessing a dose-response trend). However, novel methods of model fitting and inference need to be developed for analyses under such constraints.

One of the primary focuses of my research has been the development of general Bayesian methodology for order-restricted inference. Such methods are particularly useful in toxicology and epidemiology studies in which investigators are interested in the association between a health outcome and an exposure that can have multiple levels.

In addition to conducting theoretical research in statistics, I am also actively involved in answering several intriguing medical and public health questions. Recent studies carried out in collaboration with colleagues Donna Baird of NIEHS and Bernardo Colombo of the University of Padua in Italy showed that female fertility begins a noticeable decline in the mid to late 20s, whereas male fertility begins to decline in the mid 30s. Another finding—that more than half of couples diagnosed as infertile can conceive naturally in the second year of attempting pregnancy—has challenged the common practice of referring couples to assisted reproductive therapy after one year of unsuccessful attempts. Other interesting work in reproductive epidemiology includes collaboration with Baird on the NIEHS Uterine Fibroid Study, as well as work with NIEHS colleagues Weinberg, Baird, and Allen Wilcox on analyses of the North Carolina Early Pregnancy Study.

Ongoing research focuses on developing improved methods for factor analysis when outcome variables have a variety of measurement scales—including categorical, count, and event times—and when there is uncertainty in the correlation structure. Such methodology would greatly benefit studies of complex predictors of pregnancy outcomes, such as stress, bleeding, and exercise.

Mark Fortini

Mark Fortini *received his Ph.D. from the University of California at Berkeley in 1990 and did postdoctoral work at Yale University in New Haven, Conn., from 1991 to 1996. Before moving to NIH, he was an associate professor of genetics at the University of Pennsylvania School of Medicine in Philadelphia. At NIH, he joined the Regulation of Cell Growth Laboratory at NCI-Frederick, where he is now a senior investigator.*

My laboratory studies developmental signal transduction and cell-fate specification using the fruit fly Drosophila. We are most interested in the signaling pathway controlled by the Notch receptor, a cell-surface protein that receives signals from neighboring cells, thereby allowing cells to adopt the correct differentiation program in response to their local environment.

Notch was first identified in the early 1900s through genetic studies in Drosophila. Embryological studies in the 1930s revealed that it plays a key role in sorting undifferentiated progenitor cells into neuronal and epidermal lineages. Subsequent work by many groups has shown that the Notch pathway is responsible for transmitting cell-fate instructions between cells during the development of many organisms, ranging from sea urchins and nematodes to humans.

In humans, Notch signaling is known to be important for organogenesis, for epithelial patterning, and for the proper differentiation of B- and T-cell lineages during immune system development. Altered forms of human Notch cause T-cell acute lymphoblastic leukemia and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), an adult-onset syndrome that includes stroke and dementia.

Genetic lesions in the genes that encode ligands for Notch result in clinical developmental disorders such as Alagille syndrome (abnormalities in bile ducts, liver, and sometimes cardiovascular system, spinal column, eye, and kidneys of children) and spondylocostal dysostosis (multiple deformities of the ribs and vertebrae). Owing to the central role that Notch plays in the determination of many different cell types, this pathway is likely to be involved in the genesis and progression of many types of human cancer.

In earlier work, my colleagues and I focused on defining the molecules that act downstream of Notch to transmit the signal into the cell. In recent years, we have become more interested in complex proteolytic processing events that alter the Notch receptor during its synthesis and cell-surface activation. In particular, we have been studying a multimeric complex, called γ-secretase, which was initially identified through

Recently Tenured

human studies of families affected by a severe, early-onset form of inherited Alzheimer's disease.

Remarkably, the γ-secretase complex appears to play an analogous biochemical role in signaling by Notch and in Alzheimer's disease pathogenesis. In the case of Notch, γ-secretase cleaves the receptor at the cell surface in response to ligand binding, releasing a signaling fragment to the inside of the cell. This event is a normal part of the signaling pathway operation.

In Alzheimer's disease, a different cell-surface protein, the amyloid precursor protein (APP)—which is likely to function normally as a cell-surface receptor like Notch—is cleaved by γ-secretase. However, in this case, the proteolysis of APP leads to secretion of a small leftover "sticky peptide," β-amyloid, which accumulates in neurotoxic plaques in brain tissues of affected individuals.

Many researchers believe that the production of β-amyloid is the event that triggers the development of Alzheimer's disease, eventually snowballing into the inflammatory responses and neurodegeneration that characterize the later stages of the illness.

Our goal is now to understand the properties of γ-secretase and its involvement in Notch signaling in greater detail, using the powerful genetic and molecular approaches available in Drosophila.

One of the most unusual features of γ-secretase is that it apparently cleaves its substrate proteins, such as Notch and APP, within their membrane-spanning segments, implying that γ-secretase is able to promote the hydrolysis of peptide bonds despite the hydrophobic environment of cell membranes.

The large γ-secretase complex has four major identified proteins (Presenilin, Nicastrin, Aph-1, and Pen-2), and we have identified mutations in the genes for most of them in Drosophila. We have shown that they are all essential for Notch signaling, and that they affect the ability of Notch to be cleaved in response to developmental signaling cues. We have also performed stepwise addition studies to investigate the individual functions of each protein in the complex.

Our goal is to understand the proteolytic function of γ-secretase and its relationship to the intracellular trafficking of Notch during synthesis of the receptor. We are also exploring other approaches to identify additional proteins that are involved in developmental signaling, neuronal cell-fate specification, and neurodegeneration.

Brian Oliver *received his Ph.D. from Case Western Reserve University School of Medicine in Cleveland, Ohio, in 1988 and did postdoctoral work at Stanford University, Stanford, Calif., before taking a group leader position at the University of Marseille in France in 1991. In 1995 he joined NIDDK's Laboratory of Cellular and Developmental Biology, where he is now senior investigator.*

The humble fruit fly, *Drosophila melanogaster*, is an important model system, boasting facile genetics, complex organ systems, complex behaviors, and a sequenced genome. Fruit flies are easy to propagate and require little lab space. We and our collaborators at NHGRI, NIA, and Incyte Genomics have been involved in adding high-throughput genomics techniques, such as Expressed Sequence Tags (ESTs) and DNA microarrays, to our Drosophila toolkit and in making reagents for array production widely available to the worldwide Drosophila community.

Fran Pollner

Brian Oliver

While we have been active in developing technology, mostly we use fruit flies to address basic biological problems. Our long-term interest has been in determining how the sex of the germ line is established—in other words, how a germ line stem cell gives rise to either sperm or eggs. Stem cell development occurs in a defined niche. Our work suggests that when a male stem cell develops in a female niche, a tumor results; when a female stem cell develops in a male environment, stem cell proliferation or survival is poor. We are using microarray technology to identify diagnostic markers for germ line stem cells of the two sexes.

We have also expended considerable effort on the detailed analysis of the *ovo* gene, which is required for the viability of female germ line stem cells regardless of niche environment. The regulatory circuit controlling *ovo* expression is devilishly complex. The locus encodes both positively and negatively acting transcription factors from alternative promoters. These alternative promoters are cross-regulated by the antagonistic *ovo* transcription factors. Additionally, the mechanism of *ovo* biochemical function is unusual, in that *ovo* proteins bind and function directly at transcription start sites of target genes (locations normally occupied only by basal transcription factors). It will be some time before we fully understand the function and regulation of this fascinating gene.

One of the advantages of working on a model organism at NIH is exposure to scientists interested in a range of topics, from basic research to insect disease vectors to translational and clinical studies. In addition to work on our core subject of germ line development, we have also enjoyed deploying fruit flies to assist NIDDK colleagues Dean Londos and Allan Kimmel in their study of lipid droplet function. We have also participated in the NIH multiple endocrine neoplasia type 1 consortium led by Francis Collins, Steve Marx, and Allen Spiegel.

It is our hope that fruit fly studies will provide an (exo-)skeletal view of gene regulation pathways to be fleshed out by research in mammalian systems.

Kanta Subbarao *received her medical training at the Christian Medical College in Vellore, India, in 1982 and completed a residency in pediatrics at St. Louis University, St. Louis, in 1985, followed by a fellowship in pediatric infectious diseases and an MPH in epidemiology at the University of Oklahoma Health Sciences Center in Oklahoma City. From 1990 to 1995, she worked on the development of influenza vaccines as a senior staff fellow in the Laboratory of Infections Diseases, NIAID, and then served as chief of the Molecular Genetics Section of the Influenza Branch of the Centers for Disease Control and Prevention before returning to NIAID in 2002 as a senior investigator in the Respiratory Viruses Section, Laboratory of Infectious Diseases, NIAID.*

Three pandemics of influenza were recorded in the last century. In each instance, novel strains of influenza A were introduced into and spread among a susceptible human population. Aquatic birds serve as a reservoir from which

novel influenza hemagglutinin (HA) and neuraminidase (NA) genes are introduced into the human population. In waterfowl, these infections are largely asymptomatic. However, when avian influenza viruses are introduced into the human population and acquire the ability to spread efficiently from person to person, they cause pandemics associated with significant morbidity and mortality.

Fran Pollner

Kanta Subbarao

In 1997, an outbreak of H5N1 influenza occurred in Hong Kong, and the viruses isolated from humans were sent to the CDC for characterization. My laboratory was responsible for the genetic characterization of the viruses; we established that the Hong Kong H5N1 viruses were avian influenza viruses that had not reassorted with human influenza A viruses. Before this observation, scientists generally believed that avian influenza A viruses were restricted in replication in humans and required passage through an intermediate animal host to adapt to replication in humans or to acquire one or more gene segments from human influenza A viruses through reassortment.

My laboratory also identified the likely source of the HA gene of the Hong Kong H5N1 viruses (influenza A/Goose/Guangdong/1/96). Progenitors of the Hong Kong H5N1 viruses continue to circulate in waterfowl in Southern China and have periodically re-emerged in poultry in Hong Kong. In 1999, avian H9N2 viruses were isolated from two children in Hong Kong, again demonstrating that avian viruses can directly infect humans; these events underscore the need for development of specific vaccine candidates.

In the absence of effective interventions, a mathematical model estimates that the first year of a pandemic by a novel influenza virus would result in 89,000 to 207,000 deaths and 314,000 to 734,000 hospitalizations in the United States, with an economic impact of $71 to $166 billion.

Vaccines are the best option for preventing severe morbidity and mortality associated with influenza. Preparation for a future influenza pandemic requires that candidate vaccines against avian HA and NA subtypes be generated and characterized well before a pandemic virus emerges in nature, because it is unlikely that there would be sufficient time to generate a vaccine before the pandemic spread to the United States.

My laboratory will focus on the development of live attenuated influenza vaccines that could be used in a pandemic. These will be administered intranasally and are satisfactorily attenuated by being restricted in replication to the upper respiratory tract, yet sufficiently immunogenic to elicit a protective immune response.

We will apply two methods for the generation of vaccine candidates: the classical method of genetic reassortment and a technique called plasmid-based reverse genetics, whereby infectious virus is recovered from cells that are co-transfected with 8 to 12 plasmids encoding the virion RNA and messenger RNA of the virus under the control of polI and polII promoters, respectively.

Each candidate vaccine will be tested extensively in preclinical tests and animal models. The development of animal models for the evaluation of the replication and virulence of avian influenza viruses will be a significant effort because data in this area are sparse. We will study the molecular correlates of virulence and relate specific amino acid changes in virus proteins to changes in immune reactivity, growth phenotypes, and host range.

Based on preclinical safety data, candidate vaccines will then be evaluated for safety, immunogenicity, and infectivity in clinical trials. We will learn whether the rules regarding safety, infectivity, and immunogenicity of the vaccines established with one subtype extend to other subtypes. If the vaccine viruses are satisfactorily attenuated but able to replicate in humans, they can be used as challenge viruses to assess the efficacy of candidate vaccines against pandemic viruses.

In summary, this systematic approach to the preclinical and clinical evaluation of pandemic influenza vaccines will result in the generation of seed viruses for use in the event of a pandemic, as well as enhanced understanding of the optimal regimens to induce protective immunity against avian influenza A viruses. ■

Conference Calls

Proteome Exploring

A symposium on recent developments in proteomics technology and applications—"Exploring the Proteome II"—will be held in the main Natcher auditorium **May 2, 2003**. Invited lectures will be delivered by eight scientists from leading proteome research groups in the United States and Europe. The symposium is sponsored by 15 NIH institutes and centers and organized through the Proteomics Interest Group.

NIH pre- and postdoctoral fellows are invited to submit posters for display in the Natcher lobby on May 1 and 2. Posters should describe either a significant application of proteomics methods to a biomedical research problem or a specific technical or methodological advance in proteomics. Space is limiterd, and priority will be assigned to fellows from sponsoring institutes and centers. Abstracts may be submitted online through links at

<http://proteome.nih.gov>

beginning March 1 and ending March 22. Authors will be notified of acceptance by April 1. ■

AIDS Malignancies

The 7th International Conference on Malignancies in AIDS and Other Immmunodeficiencies: Basic, Epidemiologic and Clinical Research" will be held **April 28–29, 2003**, in the main Natcher auditorium. To register online, go to

<http://www3.cancer.gov/dctd/registration.html>

Stem Cells

This year's General Motors Cancer Research Foundation annual scientific conference and awards ceremony will be devoted to a cutting-edge exploration of stem cell research and will be held **June 10–11, 2003**, in the Masur Auditorium.

For more information, contact:
<ruemk001@surgerytrials.duke.edu>
Telephone: (919) 668-8018 ■

Altered Perception

Another in NCCAM's Distinguished Lectures series will be held **May 6, 2003,** 12:00 p.m.–1:00 p.m., in the Masur Auditorium, Building 10.

David Spiegel, of the Stanford University School of Medicine, Stanford, Calif., will discuss "Hypnosis and Group Support in Medical Care: Altering Perception and Reality." ■

NIH Hangs Varmus

by Celia Hooper

A corridor on the first floor of Building 1—just outside the door of the director's office—became the home for the official portrait of former NIH Director Harold Varmus on January 15. Art aficionados, history-of-science buffs, Varmus fans, and curiosity seekers may find the picture as unusual as its subjects.

At an unveiling ceremony in Wilson Hall, Varmus described some of the references that went into the painting by New York artist Jon R. Friedman. Shown behind Varmus in the painting is a famous portrait of French scientist Antoine-Laurent Lavoisier and his wife, Marie-Anne-Pierrette Paulze, painted by Jacques-Louis David in 1788. This painting-within-the-painting is a good background, Varmus said, because of the many parallels and connections to his life and term as head of NIH.

The first connection is the interplay of science and art, Varmus said. Lavoisier's connection to art is demonstrated by his commissioning the portrait by David—one of France's leading artists of the day. Lavoisier's wife was an artist, and a book of her sketches is shown in the background of David's painting. The depictions of science in the David painting abound, with Lavoisier's equipment and notebooks clearly shown in both the David painting and Friedman's depiction of it. The Metropolitan Museum of Art, where the David painting hangs, notes in its guidebook that "Lavoisier is best known for his pioneering studies of oxygen, gunpowder, and the chemical composition of water. In 1789 his theories were published in the 'Traite élémentaire de chimie.' The illustrations in this book were prepared by his wife, who is believed to have studied with David."

Varmus' choice of Friedman to paint the picture seems to be as deliberate as Lavoisier's choice of David. Friedman proves that his drafting skills are on a par with David's in the amazing verisimilitude of his representation of Varmus and his wrinkled blue shirt—so realistic that a viewer wants to touch it to see whether it's cloth or paint. Varmus' love of art is also reflected in his choice of the David painting as the backdrop for his portrait—a completely novel choice, as you will see if you inspect the other portraits of NIH directors that line the corridor. Other directors include small pictures of family or the buildings constructed at NIH during their tenure, for example. Varmus' complex choice of the David painting connects him more symbolically to his passions—science, art, and family—and yet creates a familiar setting for the former director: A poster bearing the Lavoisier portrait is a Varmus favorite and hung for years on his office wall at NIH and, before that, at the University of California at San Francisco. The poster advertises a David exhibit in Paris some years ago.

John Crawford

Framed in Building 1: *The Varmus portrait by Jon R. Friedman now hangs outside the office Harold Varmus occupied from mid-1993 through 1999*

Other areas of comparisons that Varmus mentioned are connections to family and the crossing paths of the David painting and Varmus himself. The presence of Lavoisier's wife in the background of Varmus' portrait could be viewed as a symbolic representation of his connection to his wife, Constance Casey. Whereas Lavoisier's wife played a role in illustrating his science, Casey, a journalist, has served as a key reader and editor for Varmus, he said. The provenance of the David painting conveyed it from Lavoisier's descendants to John D. Rockefeller Jr. and the Rockefeller Institute for Medical Research (now the Rockefeller University in New York), whence it was sold to New York's Metropolitan Museum of Art. The latter two homes to the painting are in the neighborhood where Varmus now lives and works.

Beyond these connections is the interplay of science and politics. Lavoisier was caught up in the politics of the French Revolution, not so much by his science as by other jobs he held, including commissioner of gunpowder and tax collector. Lavoisier's controversial status attending the gunpowder commission led David to withdraw the portrait from the French Academy's Salon exhibition of 1789. Lavoisier's work as a tax collector led to his death on the guillotine in 1794, even though he had supported the Revolution.

Varmus' encounters with the political world were happier, and many observers of his interactions with politicians cite his remarkable ability to explain to nonscientists the compelling advances and pressing needs of biomedical research. But, as has been true for all directors of NIH, Varmus' work was surrounded by political storms—from stem cells to electronic publishing and convincing Congress to support new construction and a doubled budget. Varmus' fate, so far, is his hanging—on the first floor corridor—yet keeping both his head and his life. Varmus is now the president of the Memorial Sloan-Kettering Cancer Center in New York. ■

Still at large: *At a December 1997 "celebration of leadership," at NIH, which happened to coincide with his birthday, Harold Varmus received many irreverent tributes from people in high places—like the cartoon above (a product of NCI creative genius), depicting how the NIH director integrates art and science after hours (from* The NIH Catalyst, *Jan.-Feb. 1997)*

Pictures at an Exhibition

A leap off the canvas: *Harold Varmus and Constance Casey —21st century counterparts to the Lavoisiers of the late 18th?*

Hanging out: *Editor Constance Casey (left) and pediatrician Nadia Azza (right) flank the former and current NIH directors—their respective husbands, Harold Varmus and Elias Zerhouni.*

Photos by John Crawford

Faces in the crowd . . .

Who's that Hanging in Wilson Hall?

Mutual Regard *Former NIH director Harold Varmus (right) shakes the hand of portraitist Jon R. Friedman. Current NIH director Elias Zerhouni is in the background, and facing them all is the solemn likeness of Luke Ingalls Wilson, after whom Wilson Hall is named and whose visage reflects the more somber mode of traditional portraiture.*

Luke Wilson (1872–1937) was a wealthy man who owned the property on which NIH now sits. He inherited his wealth from a Chicago family that made men's clothes (particularly, men's underclothes). He did not do anything related to science.

In the 1930s, he and his wife, Helen Woodward Wilson (of the Woodward & Lothrop department store family), whose photo also hangs in Wilson Hall, owned property with other wealthy Washingtonians along Rockville Pike, when it was a rural area. These estates were used primarily as summer homes. In 1935, Wilson offered his estate, called "Treetops," to the federal government, hoping that it would be used as a place for an institute to promote peace (he was active in left-wing peace movements between World Wars I and II; he also no doubt appreciated the tax relief he would get by donating this land).

No one in the State Department was interested in the offer, so it was circulated around the government. The NIH, then located at 25th and E Sts., N.W., in the District, was experiencing a shortage of space for housing research animals. Then–Assistant Surgeon General Lewis Ryers Thompson, soon to become NIH director, followed up on the Wilsons' offer and obtained the land for this purpose. In 1936, however, the more conservative Surgeon General Hugh Cumming was replaced by a Roosevelt New Dealer, Thomas Parran, who promptly appointed Thompson, another liberal Democrat, to head NIH, and the two of them seized the opportunity afforded by the Wilson offer to rebuild the entire NIH in Bethesda.

In 1937, as construction began, Luke Wilson died of cancer at about the same time the National Cancer Institute was created. Helen Wilson was then motivated to donate extra land to build Building 6, the original Cancer Institute Building, along with the five buildings and the PHS officers' quarters (along Cedar Lane) included in the first wave of construction.

The Stetten Museum has a website in the early stages of development called "Seventy Acres of Science" about this story—stay tuned.

—Victoria Harden
NIH historian and director of the Stetten Museum

Call for 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 2W23.**

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

In Future Issues...

- The Graying Of NIH?
- What's My Line?: NIH Professional Designations
- Science by Contract?

John Crawford

NIH Director Elias Zerhouni and his predecessor Harold Varmus share a pleasant moment in a picture too good not to appear in the Catalyst

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