FOSTERING COMMUNICATION AND COLLABORATION

# The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 7, ISSUE 5 ■ SEPTEMBER–OCTOBER 1999

*Letter to Basic Lab Scientists*

## Y2K: READY OR NOT

*by Garrott Christoph, Ph.D.*
*Laboratory of Chemical Physics, NIDDK*

The task put to me by the NIH Center for Information Technology (CIT) was daunting and even necessary. I was asked to help evaluate Y2K risk priorities for laboratory equipment and to help inform working scientists about their Y2k responsibilities. So, how do NIH scientists respond to the Y2K challenge?

A random survey of some friends and colleagues elicited the following responses:

- *"Next January I will decide what is important and I will fix what is broken then. I am not looking for problems now."*
- *"Just turn the damn computer dates back!"*
- *"Scientists are in the business of solving problems; they are not in the business of looking for hypothetical problems."*
- *"We are not clinical . . . don't bother us."*
- And my particular favorite, *"So file dates are wrong—so what? Go work on a real problem."*

Add to this a matching tone of voice, an exaggerated eye rolling, and my own not unsympathetic preconceptions—well, you get the picture.

But this is the reality. After an enormous effort by the NIH clearinghouse people, there remain 3,200 devices (about 25 percent of the NIH clearinghouse database) whose compliance status is either "NC" (not compliant), "CC" (conditionally compliant), or "NT" (not tested, meaning that their Y2K certification status is problematic or unknown). Equipment is old, some companies are unresponsive, others have gone out of business, new equipment is found,

*continued on page 6*

## BATTLE'S LINES DRAWN WHERE APPROPRIATIONS FAIL TO TREAD

*by Fran Pollner*

"We're not just going to sit here in this little corner of the Cloister on the NIH campus," says Constance Battle, the new executive director of The Foundation for the National Institutes of Health. She contemplates making the rounds—beginning in the fall—to meet with all the NIH institute and scientific directors.

Fran Pollner

*Constance Battle and Ted Colburn*

Battle wants everyone to know what the Foundation is all about—that it's an entity created by Congress solely to further the mission of NIH through private-public partnerships.

This mandate could be met in many ways: aiding an individual scientist, an institute program, a trans-NIH initiative, a national consortium that includes an NIH component, or an international project in which NIH participates. In fact, the Foundation is involved in all of the above.

> "WE WANT NIH STAFF TO COME AND EXPLORE WITH US WHAT THEIR VISION IS FOR PROJECTS THAT ARE NOT FUNDED—BECAUSE ALMOST ALL THE ARRANGEMENTS IN WHICH WE'VE BEEN SUCCESSFUL OR ARE CURRENTLY INVOLVED ARE QUITE IDIOSYNCRATIC. PERHAPS IN THE FUTURE WE'LL DISCERN PATTERNS, BUT WE HAVEN'T YET. SO WE ARE READY TO BRAINSTORM . . . ."
>
> *—Constance Battle*
> *Executive Director*
> *The Foundation for the NIH, Inc.*

As Battle and Foundation Scientific Director Ted Colburn, former NIAAA deputy SD, explain it, Foundation support for NIH steps in where congressional appropriations leave off.

Certain activities or projects are not embraced in federal NIH funding: "For example, building a residence for fellows is not something for which NIH receives government appropriations, nor could NIH ask for such money," Battle said. The Foundation, however, has two housing projects on its agenda: a guest house for the families of adult patients on protocols at the Clinical Center and a campus residence to house students enrolled in the Foundation-supported and Pfizer-funded Clinical Research Training Program (CRTP).

Meeting other sorts of funding needs through congressional appropriations may be challenging, Battle added, citing bureaucratic delays related to procurement and travel and "timing problems" when congressional monies must be spent within the year for which the money was appropriated. Money that resides within the Foundation, on the other hand, may be used

*continued on page 4*

CONTENTS

# From the Picnic Table to the Lab Bench: Fostering Community at NIH

Michael Gottesman

As summer draws to a close, many of us are returning from relaxing and perhaps even productive vacations. From this agreeable state of mind, I ask you to consider a pleasant aspect of life at NIH: community, camaraderie, fellowship. Call it what you will, we are all part of a vital, interactive community of people, and this can make our work especially rewarding. Elements of this community begin in the laboratories and clinics of NIH and extend throughout our campus and outward to our neighbors and scientific colleagues.

At the laboratory level, we create small communities by encouraging group meetings to discuss science and other matters related to the functioning of a laboratory. Socially, individuals within labs have interactions (lunches in the cafeteria; dinners in Bethesda; pick-up games of soccer or group runs; help with housing and recruiting), and labs themselves have picnics and other get-togethers to welcome new lab members, to recognize achievements, and to say farewell to friends and colleagues whose careers are taking them elsewhere.

All of these activities build esprit de corps and strong ties of friendship and should be encouraged. It is sometimes easy to overlook these kinds of social occasions in the excitement of scientific discovery, but they are an important component of building the teamwork needed to succeed in any enterprise.

The community that is NIH itself has created several venues to encourage social, cultural, and scientific interactions. Following this year's Wednesday Afternoon Lectures (see page 3 and <**http://www1.od.nih.gov/wals/schedule.htm**>), NIH's component institutes will again be sponsoring informal receptions featuring poster displays by the recipients of this year's Fellows Awards for Research Excellence.

Students and postdocs at NIH have worked hard to develop their own venues for socializing and helping their peers get the most out of their NIH experience. Their groups include the Fellows' Committee and a postbaccalaureate gathering. Activities of these groups are generally announced via electronic bulletin boards. We especially hope to see improving connections among graduate students over the next few years as we focus on how we can better coordinate and improve what NIH has to offer predoctoral students.

But students are not the only ones who can benefit from social, cultural, and scientific interactions. For example, Recreation and Welfare (R & W)–sponsored clubs and activities—including the NIH choral groups, the lunchtime concert series, sailing and photography clubs, and our musical theater group—welcome all NIHers. The Foundation for Advanced Education in the Sciences (FAES) sponsors a bookstore, continuing education courses, and a chamber music concert series—among the best in the Washington, D.C., area.

We also have some very active interinstitute scientific interest groups that support scientific meetings and seminars where valuable networking takes place before or after formal presentations (see the Interest Group Directory in the July-August 1999 issue of *The NIH Catalyst* for a complete list). In addition, the Bethesda Chapter of the Association for Women in Science (see page 5, "AWIS in Action"), the Black Scientists Association, NIH Hispanic Employee Organization, the NIH-FDA Chinese American Association, and other similar groups provide additional forums for mutual support, assistance, and fellowship.

In the last several years, the NIH community has extended its hand to the wider Washington, D.C., area by hosting events designed to attract friends and neighbors. These include two movie series (Science in the Cinema and the very popular outdoor summer movie series on the lawns of NIH), Medicine for the Layman, and our Mini-Med School. Each of these events allows us to share special expertise and the excitement of the science we do with our neighbors in a way that encourages creation of a larger community and informs people about the work that we do. Our community liaison, Janyce Hedetniemi, highlights many of these opportunities in her newsletter to our Bethesda neighbors.

I know only too well how easy it is to become lost in one's own research, working through lunch and dinner on an absorbing project, forgetting that there is anyone else in the lab, much less the fascinating lectures, interest groups, and concert series out there. Sometimes such intense focus is exactly what is necessary to move research past a critical hurdle. But I also encourage all of you at NIH to look up occasionally from the desk or lab bench and reach out to your colleagues. It wouldn't hurt if we all brought back a bit of the relaxed and sociable spirit of our summer vacations to the NIH campus. Building community is one vital way to improve morale and support the mission of NIH. ■

I know only too well how easy it is to become lost in one's own research, working through lunch and dinner on an absorbing project, forgetting that there is anyone else in the lab, much less the fascinating lectures, interest groups, and concert series out there. . . . . Building community is one vital way to improve morale and support the mission of NIH.

## Catalytic Reactions

### On Graduate Student Needs

I have one suggestion for the needs of graduate students at NIH. I am a graduate student who is finishing up a summer internship at NIH, and I have noticed one common desire among myself and the other summer interns I have met—centralized housing.

Centralized housing facilities (dorm, apartments, hotel, etc.) would facilitate interaction among students and allow for more frequent exchange of ideas. It would also provide an opportunity for weekly discussion groups, journal clubs, and lectures and seminars to be held. Many of the problems associated with renting in this area (lack of housing, high prices, transportation, and bad landlord-tenant relationships) would be eliminated, allowing the students to concentrate more on their research and learning experiences.

*—Michelle White, NIMH*

*—Centralized housing for students at NIH is one of the projects of the Foundation for the NIH. See story, page 5.—Ed.*

### On Interest Group Gaps

A cross-disciplinary suicide interest group would be useful.

*—Michelle White, NIMH*

## *Clarification*

He may be "retired" from NIH, but former NHLBI Cardiology Branch Chief Stephen Epstein is still working about 60 hours a week as the director of vascular biology research at the Cardiovascular Research Foundation in Washington, D.C.

Brian Greer

*Stephen Epstein*

In the July–August issue of *The NIH Catalyst*, we reported that *Science Watch* had listed "retired NHLBI investigator Stephen Epstein" among the ten most-cited clinical investigators in their fields between the years 1980 and 1998.

"Most cited," he is. "Retired," he is not.—*Ed.* ■

## *Back to the WALS*

From September 15 on, the Masur Auditorium is the place to be Wednesdays at 3 pm. The Wednesday Afternoon Lectures are back. Here's the fall schedule. For more info, go to the web: **<http://www1.od.nih.gov/wals/schedule.htm>**.

**—Sept. 15:** Wolfram Schultz, "Reward Processing in Primate Basal Ganglia and Frontal Cortex"

**—Sept. 22:** Vilayanur Ramachandran, "What Neurology Can Tell Us about Human Nature"

**—Sept. 29:** Robert Tjian, "The Biochemistry of Eukaryotic Transcription: More Surprises and Complexities"

**—Oct. 6:** Research Fest: No Lecture

**—Oct. 13:** Virginia Zakian, "A Tale of Two Helicases: The Saccharomyces Pif1p and Rrm3p Helicases Have Antagonostic Effects on Replication of Both Telomeric and Ribosomal DNA"

**—Oct. 20:** James Spudich, "Single-Molecule Biomechanics and the Myosin Family of Molecular Motors"

**—Special Thursday Lecture, Oct. 28:** Tim Mitchison, "Biochemical and Small-Molecule Approaches to Dissecting Mitosis"

**—Special Tuesday Lecture, Nov. 2:** Leland Hartwell, "Studying the Fundamentals of Cancer in Yeast"

**—Nov. 3:** Allen Steere, Jr., "The Elucidation of Lyme Arthritis"

**—Special Starting Time: 2:45, Nov. 10:** Franklyn Prendergast, "Elegant Photosynthesis in the Green Fluorescent Protein"; Bernard Witkop, "Introduction to the Life of Percy Julian"

**—Nov. 17:** Purnell Choppin, "A Role fort Private Support of Biomedical Research"

**—Nov. 24:** Thanksgiving Break

**—Dec. 1:** Shirley Tilghman, "The Mechanism and Function of Genomic Imprinting in Mammals"

**—Dec. 8:** Patrick Brown, "The Living Genome"

**—Dec. 15:** Stephen Harrison, "Viruses as Molecular Machines"

**—Dec. 22–Jan. 5:** Winter Break

## *Say What?*

Need a special cell line? Or a line on a special technique? Want to know who's working on a particular disease or gene? [The *full* web address appears below; only half made it into the last *Catalyst* issue.]

Search rapidly through 2,603 1998 Annual Reports online to get this info. Any search combination will do: last names, institutes, a word in the title, other keywords. The website is

**<http://tango01.cit.nih.gov/phantom/1998reports.html>**.

## Rodbell Memorial: Symposium and Exhibit

*Marty Rodbell*

NIDDK will celebrate its 50th anniversary with a memorial symposium honoring Marty Rodbell, former NIDDK and NIEHS intramural scientist and 1994 Nobel laureate. The symposium—"G Proteins and Transmembrane Signalling"—will be held Friday, **November 5, from 1:00 to 5:00 pm** in the Masur Auditorium.

Speakers include Al Gilman, with whom Rodbell shared the Nobel, and Dean Londos, a former Rodbell postdoc and current NIDDK investigator, who will close the symposium with reflections on Rodbell, including portions of a video done at NIEHS and shown at the memorial service held there this past February.

A reception in the ACRF lobby will follow, with refreshments and a brief ceremony to open a Rodbell memorial exhibit. Sponsored by NIDDK and the DeWitt Stetten, Jr., Museum of Medical Research, the exhibit, "Martin Rodbell: Discovering How Cells Respond to Signals," will run indefinitely in the ACRF portion of the NIH Clinical Center.

The exhibit traces Rodbell's early work on lipid metabolism in isolated fat cells through pioneering experiments that transformed understanding of how cells respond to signals. He demonstrated that there must be an intermediary—a "transducer"—that carries the external signal (one of many hormones) from its receptor on a cell to the molecule that activates the hormones. The discovery of the transducer molecules, called "G proteins," provided the basis for explaining not only how hormones function, but also how light and odors are perceived, how signals travel between neurons in the brain, and how some diseases affect the body.

*—Victoria Harden, Ph.D.*
*NIH Historian*
*Director, DeWitt Stetten, Jr., Museum of Medical Research*

## Battle's Lines

*continued from page 1*

and distributed as needed.

Colburn noted that the Foundation can more smoothly handle cross-NIH training programs, such as the CRTP and the new Biomedical Engineering Summer Internship Program (BESIP), another Foundation-supported program begun this June in collaboration with the Whitaker Foundation (for a glimpse at the research of one of this summer's BESIP students, see "Bounty . . ., page 8). In such programs, he said, students are selected on the basis of academic credentials and research interest and then select for themselves the institute in which they would like to work.

Fran Poliner

*Constance Battle*

He pointed to the biomedical engineering internship as an "interesting example of a program initiated by a donor." The Whitaker Foundation suggested to the NIH Foundation that NIH establish a summer program for undergraduate biomedical engineering students who had completed their junior year. The Whitaker grant and interested NIH staff combined to provide housing and training for 10 weeks for the 12 top candidates in an eligible field of 2,000. Battle and Colburn fully anticipate that the program will continue.

The need for a training program for veterinary students, brought to the Foundation's attention by an academic institution, suggested another potential project. Also in the contemplation stage is a role in the Multilateral Initative on Malaria, an international project to which NIH Director Harold Varmus has committed the NIH. Such involvement is "prospective" at the moment—an idea that might materialize in the form of the Foundation's serving as a fiscal agent for the initiative, a neutral repository for and disperser of funds from disparate organizations with a common mission. The Foundation plays a similar role in the National Coalition for Health Professional Education in Genetics, a coalition of more than 100 organizations that is co-chaired by NHGRI Director Francis Collins.

Colburn emphasized that unlike many foundations, The Foundation for the NIH does not have a "pot of money" to distribute, but, rather, works to set up partnerships in which "particular donors can be targeted to particular projects."

"Our primary task," Battle said, "is to identify projects, programs, buildings, other needs at NIH that are not funded—and obtain funds for them. Our purpose, quite simply, is to help scientists with whatever it would take to enable them to further their work. They have to know about us so they can approach us," Battle said, "and we have to be flexible."

Battle succeeded the Foundation's first head, Anne Alexander, in May. According to Colburn, her application for the position had sailed upon arrival to the top of a rather tall stack—she was the only seeker of the position who not only had major organizational and fundraising experience but was also a physician with an impressive clinical and academic background, a penchant for mentoring, and a prior connection with NIH.

A professor of pediatrics at George Washington University in Washington, D.C., Battle says she hopes to continue to spend several evenings a week teaching first- and second-year medical students the art of patient interview and physical examination.

The Foundation may be reached at <**foundation@fnih.org**> or 301-402-5311. Its web site is <**http://www.fnih.org**>. ■

*The Foundation for the NIH participates in the Combined Federal Campaign (CFC) and, according to Battle, last year received "generous gifts" from NIH employees. The CFC number for those interested in supporting the Foundation's mission is 7109.*

## *Virology Award*

The Foundation for the NIH will award the first annual Norman P. Salzman Memorial Award in Virology at the November 18 meeting of the Virology Interest Group, to be held at the Cloister. The winner—a postdoc fellow or other intramural research trainee in the virology field at an NIH or SAIC lab—will deliver a lecture based on the award-winning abstract. The winner will receive a plaque and an unrestricted $2,500 gift; the mentor will receive a plaque. A reception will follow.

The award honors the 40-year career of NIH virologist Norman Salzman and is funded by donations to the Foundation for this purpose. ■

## Research Challenges In the New Millennium

Anticipating the millennial divide, fellows in the NCI Division of Clinical Sciences hosted their first symposium, in July, on "Research in the 21st Century: Challenges for the Next Generation of Biomedical Investigators."

Some of the research challenges of the next millennium, as remarked upon by NCI speakers Philippe Bishop, Ed Liu, and Susan Lord, sounded familiar: weathering the effects of public policies and politics on the conduct of research; and vectoring ideas between the clinic and the lab, a two-way street for both basic and clinical scientists, to conquer diseases.

Research fellows lucky enough to enter the millennium at NIH, however, will have access to "big science," like vaccine development, gene therapy, genome mapping, transgenic modeling, and bioinformatics, Liu pointed out.

And NCI's Michael Gottesman presented a David Letterman-like "top 10 list of biomedical research challenges 'til the year 2009 (see below)" His list, he said, was not meant to be complete—omitting such broad areas as infectious diseases and vaccine development, for instance—but rather to "appeal to DCS fellows" and also to reflect his "own personal research agenda."

*—Cynthia Delgado, NCI*

**Top Ten Research Challenges**

**—10.** Accurately predict a protein's tertiary structure from its amino acid sequence.

**—9.** Use the knowledge of a protein's structure to predict its interactions with other proteins.

**—8.** Describe how transcription factors and post-transcriptional regulation affect gene expression for each human gene.

**—7.** Provide the complete directory of genes with altered expression or function in each of the common cancers.

**—6.** Write a complete time course for all gene products expressed at each stage of mammalian development.

**—5.** Develop highly effective and specific anti-cancer drugs based on the knowledge of altered gene products in cancer.

**—4.** Stimulate stem cells to differentiate into specific tissues for use in tissue remodeling and organ transplantation; develop biomimetics for the same purpose.

**—3.** Introduce genes specifically into cells to correct genetic defects.

**—2.** Apply information about cell and molecular biology to treat degenerative diseases of the central nervous system and the major psychoses.

*And: the top biomedical challenge for 2009 [add a drum roll and a little levity, please]:*

**—1.** Devise a molecular/integrative description of the brain functions involved in thinking about the Top Ten Biomedical Challenges for 2009.

*—Michael Gottesman*

## AWIS in Action

The Bethesda chapter of the Association for Women in Science has scheduled five meetings for its 1999–2000 season—four at the chapel at the Cloister (Building 60):
**—Thurs. Sept. 16:** "Science in Forensic Medicine," Jerry Spencer, medical examiner, and Jeannie Willard, DNA specialist, Armed Forces Institute of Pathology
**—Tues. Jan. 18:** "Careers in Science Writing and Editing," Alison Davis, science writer, NIGMS, and Laura Garwin, North American editor of *Nature*
**—Tues.March 14:** "Patents and Intellectual Property," Susan Cullen, consultant, and Prema Mertz, primary examiner, U.S. Patent and Trademarks Office
**—Tues. April 25:** "Reflections on a Scientific Career," Janet Rowley, University of Chicago

And one will be held at the FAES Social Center, 9109 Old Georgetown Road, Bethesda:
**—Thurs. Nov. 18:** "Women's Health Research in the 21st Century," Vivian Pinn, Director, Office of Research on Women's Health, NIH, and Jill Panetta, research manager, Lilly Center for Women's Health, Eli Lilly. ■

## 25 Candles for NIDA

NIDA's 25th anniversary will be celebrated Monday, September 27, in a day-long event that includes a scientific symposium from 1–5 pm in the Masur Auditorium, morning and evening poster sessions, and a program for the public beginning at 7 pm.

The afternoon session is geared to clinicians and researchers and addresses addiction vulnerability, treatment research, neuroimaging, and HIV. The evening talks cover drug-abuse research in general and adolescent issues in particular. For more info, call 301-443-1124 or check NIDA's web site at
**<http://ww.nida.nih.gov>**.

## Day Care Board

The NIH Day Care Board needs volunteers for a three-year term starting September 1999. Contact Lori Thompson at 496-1967 or Deborah Henken at 496-5541. ■

## Research Festivities

From the cutting edge of research to tomorrow's jobs to music that defies description, this year's NIH Research Festival has it all. Block out **October 5 through 8** on your calendar and head over to the Natcher Conference Center, with a half-day side trip to the Masur Auditorium, and take in the following (all at Natcher except as indicated):
**—Tues. Oct. 5, 10–3:** Postdoc Job Fair
**—Wed Oct. 6, 8:30–10** and **10:30–12,** Masur: **Plenary Sessions** on **Advances in Medical Imaging** and **Advances in Transplantation Research**; **12:30–2:** poster session; **2-4:** six simultaneous mini-symposia; **4:30–6:30,** picnic tent behind Natcher: picnic dinner and **The Battle of the Bands/Dance, featuring "The NIH Directors" vs "Wild Type"** (ever hear of Francis Collins, Rick Klausner, Steve Katz, Bert Vogelstein, Bruce Springsteen? At least four of these will be rockin' at Natcher)
**—Thurs. Oct. 7, 8:30–10, 12:30–2:** poster sessions; **10:30–12: Plenary Session** on **Advances in Gene Therapy**; **2–4:** six simultaneous mini-symposia
**—Thurs. Oct. 7, 9:30–3:30,** and **Fri. Oct. 8, 9:30–2:30:** tent in Natcher visitor parking lot: Technical Sales Association Exhibits

This year's organizing committee was chaired by Jeffrey Trent, NHGRI SD, with NINDS SD Story Landis and Clinical Center Director John Gallin; Job Fair organizers were OE's Brenda Hanning and Shirley Forehand. More on the web:
**<http://www.nhgri.nih.gov/festival99>**.

## Phage-Tech on Deck

The new Phage-Tech Interest group (PhTIG) was launched in September and will meet regularly (see PhTIG web site—**<http://www.nih.gov/sigs/phtig/>**).

The PhTIG looks at novel uses of bacteriophage, such as phage therapy of multidrug-resistant infectious organisms and phage-display technology to "dissect" single cells or a single mitochondrion.

Contact Steve Zullo at 435-3576
**<zullo@helix.nih.gov>**
or Carl Merril at 435-3583
**<merrilc@helix.nih.gov>**.

## Phases of Life

The NIH Work and Family Life Center (WFLC) and the NIH Employee Assistance Program are presenting the following seminars.
**—Sept. 2, 2–3,** 1/Wilson Hall: "Stress Wars: The Workplace Menace"
**—Sept. 22, 12–1,** 31/6C6: "Aging: The Unfinished Business of Living"
**—Sept. 30, 1–3,** 31/6C10: "Jumpstarting Your Career"
**—Oct. 5, 12–1:30,** 31/6C6: "Home Alone: Helping Your School-Age Child Be Safe"
**—Oct. 6, 1–3,** Masur Auditorium: "Enough is Enough! Practical Tools for Regaining Control of Your Life in Today's Fast-Paced World"
**—Oct. 13, 11:30–1,** 31/2C19: Trinity College: "Graduate Program Options"
**—Oct. 21, 2–3:30 ,** 1/Wilson Hall: "Legal Issues Concerning Older Relatives"
**—Nov. 2, 12–1:30,** 31/6C6: "Dual Career Relationships: Coping Strategies for Couples Who Work"
**—Nov. 10, 12–1:30,** 31/6C10: "How to Help Your Child Do Better in School"
**—Nov. 17, 12–1:30,** 31/6C10: "Where Will My Older Relative Live?"
**—Nov. 30, 11–1 ,** 31/6: **"**Navigating the Course of Your Career: Setting Career Goals"
**—Dec. 1, 12–1:30,** 31/6C6: "Survival Tactics for Managing the Holidays"

Sign language interpretation and televideo at most sites are available. Call WFLC at 301-435-1619 (TTY: 301-480-0690). More info on each talk is at
**<http://wflc.od.nih.gov/wflc/news/events/seminars2.html>**.

## Retirement Fair

Retirement has a place in most people's lives—and is center stage at the Quality of Work Life Retirement Fair, Wednesday, **October 27, 10 am–3 pm** at the Natcher Conference Center. There will be exhibits, video presentations, and the following talks:
**—11:00–11:45:** General retirement
**—12:00–12:45**: Medicare and Social Security
**—1:00–1:45:** Thrift Savings Plan
**—2:00–2:45:** Financial planning
For more info, contact Sandy Jones, 301-496-7700, ext. 285, or Wendy Leech, 301-402-8676. ■

Y2K: Ready or Not
*continued from page 1*

and complicated issues arise.

My assignment began by looking at the NIH Biomedical Clearinghouse with an eye toward composing a list of the most critical, most commonly used, and highest-impact equipment. CIT wanted a "Top Ten List of Vulnerable Equipment." It became clear that such a list was of limited value. Individual laboratories are quite different from one another; they are not rubber-stamped production centers. They have different equipment, different priorities, and different critical elements.

I searched the web for scare stories to help illustrate the potential seriousness of Y2K noncompliance and found examples of Y2K glitches in software and instrumentation control mechanisms that caused problems and sometimes even precipitated life-threatening crises. I found them in large integrated systems and stand-alone devices in clinical settings and in other disciplines. But I could not find them centered on scientific instrumentation. This does not necessarily mean that they do not happen—and it might mean that scientists just don't post horror stories on the web.

**The take-home lesson** for me, after thinking about the issue and certifying the Y2K compliance in our own laboratories, was that there are surprisingly few Y2K scientific instrumentation questions in the basic science laboratory. This paucity makes it all the more easy and sensible to identify now those few pieces of critical or expensive equipment in your lab that might be adversely affected by Y2K and to avert the problems that otherwise will be encountered later.

### The Y2K and Scientific Equipment

There seem to be three main prospective Y2K biomedical instrumentation concerns.

## A Process To Evaluate Y2K Compliance

While web searching for Y2K instrumentation problems, I found a useful "algorithmic prescription" at <http://www.y2kjournal.com/issues/issue_9bettinger.htm>. It is used to identify instrumentation with Y2K issues. With it, you can cull a large laboratory-wide list of instrumentation to a short, manageable list of critical and problematic equipment. I used it in my laboratory. It worked, it was fast, and it strongly affirmed that I was asking the right questions. I modified it to include equipment examples found in many scientific laboratories.

To identify embedded chip problems in stand-alone (noncomputer) electronic devices, answer these six questions. You might consider #1, and perhaps #2, gratuitous, but questions about such devices have arisen.

| Question | Examples of Low-Risk Items |
|---|---|
| 1. **Does it operate with electricity? If yes, look further** | Wind-up clocks, beam balances, optical components, old microscopes, optical tables, cameras, and old column chromatography apparatus. |
| 2. **Does it have a battery or power supply? If yes, look further** | Lamps, hair dryers and hot-air guns, electric pencil sharpeners, analog clocks, benchtop centrifuges, magnetic lab stirrers, refractometers, osmometers, microtomes, conductivity meters, thermal block heaters, freeze-drying apparatus, ultraviolet lamps, ultrasonic cleaners, variacs, machine tools, ultralow temperature freezers, incubator stirrers, and low-power lasers. |
| 3. **Does it have a display? If yes, look further** | Paper shredders, power supplies, refrigerators, older microwaves, older circulator baths, older floor centrifuges, older ultracentrifuges, stop-flow pump mechanisms, high-performance liquid chromatography (HPLC) pumps, peristaltic pumps, HPLC systems, fast-performance liquid chromatography (FPLC) systems, old pH meters, anaerobic chambers, power supplies, laser-power meters, some microscopes, and frequency synthesizers. |
| 4. **Does it have a microprocessor? If yes, look further** | Television sets, stereo equipment, computer monitors, VCRs with clocks but no calendars, old oscilloscopes, lab timers, voltmeter/multimeters, calorimeters, some ultracentrifuges, chillers, some peptide and nucleotide synthesizers, new pH meters, older fluorescence instruments, and piezoelectric controllers. |
| 5. **Does it have a calendar? If yes, look further** | Microwave ovens, coffeepots, printers, most copier machines, most modern circulator baths, modern Mettler balances, high end powerful lasers, A/D converters, most spectrometers, CD instruments, old NMR spectrometers, stop-flow instruments, some modern ultra centrifuges, sterilizers, and water purification systems. |
| 6. **Does the device use the calendar to schedule events?** | Digital clocks or calendars that don't schedule anything, cameras, watches, etc. These are low risk because operation of the device is not dependent upon an accurate calendar. The device doesn't care what date is shown, it simply shows a date. Find other low-risk examples in category 5. |

| Examples of High-Risk Items | |
|---|---|
| **Smaller Stand-Alone Systems** | Fax machines, irrigation systems, energy management systems that use time and date to control such things as light and heat, uninterruptible power supplies, desktop PCs, Unix workstations and databases with date calculations. Any device with date-scheduled maintenance or operation functions like automated animal cage feeding, watering, lighting, heating, and washing systems. |
| **Modern, Expensive, Large, Integrated, Complicated Systems** | Bioreactors, modern multi-channel chromatography workstations, newer HPLC systems, some newer ultraviolet/visible/infra-red spectrometers. Circular dichroism (CD), fluorescence, stop-flow, nuclear magnetic resonance (NMR), electron magnetic resonance (ESR), and mass spectrometers. Also evaluate densitometers, gamma and scintillation counting equipment, computerized axial tomography (CAT) scanners, NMR imagers, positron emission tomography (PET) scanners, and mass spectrometers |
| **Specialized Computer Code** | Homemade code/macros/programs might have date calculations built into them. Check out locally built applications, analysis tools, simulations, and data collection routines written in Matlab, Mathematica, SAS, Excel, Lotus, Fortran, C, C++, perl, and sh. |

**1. Computer-generated file dates can be wrong.** This problem particularly affects computers that interface with scientific equipment and collect raw data. Many, indeed most, NIH laboratories use this kind of equipment. Problems arise most commonly when the instrumental software misinterprets data with erroneous time stamps. For example, a spectrometer might use an incorrect base-line correction spectrum when more recent—but date-corrupted—data files are available. There are also potentially serious repercussions if the principal investigator is pursuing a patentable idea that for legal reasons needs a correct time stamp.

**2. Instrumentation software with built-in scheduled maintenance or other automatic operations can malfunction or shut down.** Software-enforced maintenance schedules more commonly occur in clinical-care equipment than in straight scientific laboratory equipment. Basic scientists often view instrumentation as a child views a set of Lego blocks: as something to be taken apart and reassembled in ways that fit the needs of the current experiment. They tend not to purchase instrumentation with software-automated maintenance controls. However, instrumentation software may perform other routine operations predicated on date calculations from an internal calendar and so may pose Y2K problems.

**3. Commercial and scientific databases that use date calculations are at risk. Homemade software, as it interfaces with scientific instrumentation, may perform improper date calculations leading to unintended errors.** This scenario seems less of a biomedical instrumentation problem than a straight computer software problem. But if software either directly communicates with instrumentation or analyzes the results of data collected from scientific instrumen-

## A WHALE OF A SERVICE FOR SEQUENCE-SEEKING SCIENTISTS

This week 77,000 new sequences were added to Genbank and about 3,000 to the major protein sequence databases. A researcher with a new nucleotide or protein sequence would compare it with these databases right away, but few can afford to spend hours each week repeating the comparison with the new database sequences.

Now, WHALES (Web Homology ALErt Service) will automatically keep NIH scientists informed of relevant new entries in these ever-growing DNA and protein sequence databases.

WHALES will be largely familiar to those who use Porpoise to keep up-to-date with the scientific literature. Like Porpoise, WHALES is a web-based resource that allows NIH scientists to define profiles—in this case, text terms or sequences. Once a week, these profiles will be searched automatically against new entries in the Genbank, Genpept, SwissProt, and PDB databases; the results will be returned via e-mail.

WHALES has two profile modes: text mode and homology search mode. In text mode, you can define a text string such as "tyrosine & kinase," or an author's name ("Wilson, I.A."), or even a protein size ("between 340 and 350" amino acids) or molecular weight ("greater than 60,000"). You can also specify an organism such as *Mus musculus*.

This search profile is compared against the text and reference data in the headers of new sequence entries. You can configure the weekly e-mail message to contain just sequence names, accession numbers, some selected fields, or even the entire sequence entry, at your choice.

In homology search mode, you enter your own nucleotide or protein sequence, which is then compared using Blast or Fasta with each week's new sequences. Your sequence should ideally be in Fasta format, but most other formats will be converted automatically. Short peptides, such as those produced from mass-spec analysis of protein digests, can also be entered, and the Fasta program will then look for (potentially noncontiguous) matches of all the peptides in the database sequences.

Access to WHALES is possible only from computers on the NIH network and from Parachute. All search results are returned to the e-mail address associated with each person's unique e-mail alias in the NIH Directory Service.

WHALES was developed by Helix Systems staff at CIT (Peter FitzGerald and myself). For more information, see the WHALES help pages, or call GO-CIT, or send e-mail to **<whales@molbio.info.nih.gov>**.

*—Susan Chacko*

### *Some URLs*

WHALES:
**<http://molbio.info.nih.gov/whales/>**
Citation and Literature Searching (Web of Science and Porpoise):
**<http://publishorperish.nih.gov/>**
NIH Directory and E-mail Forwarding Service:
**<http://directory.nih.gov/>**
NIH Molecular Biology Resources:
**<http://molbio.info.nih.gov/>**

tation, it should be evaluated for Y2K compliance. Particular attention should be given to any date calculations, date sorting, or data retrieval by date-logical test arguments.

**The Bottom Line**

Principal investigators should evaluate and upgrade the critical equipment now, or deal with the problems in January. It may be entirely appropriate to think about only a few of the most critical components in each laboratory now and put off the little stuff until later. A large fraction of the instrumentation with Y2K issues may have low impact or inconsequential effects. However, this is a case-by-case, PI-dependent decision. Walk around your lab and think about what equipment is critical to your own research. Call the manufacturers if necessary. Use the steps described in the box (p. 6, "A Process to Evaluate . . .") and the NIH Biomedical Clearinghouse Database:

**<http://oirm.cit.nih.gov/biomednih/>**

This web site provides a database of 12,000-plus scientific and biomedical instruments with detailed Y2K information for the scientific community. It has been carefully built, collated, and maintained by CIT personnel for over a year. It is an enormously valuable resource to the scientific and medical community here at NIH. It allows you to resolve immediately most instrumentation compliance questions.

But choices should be made now, with a deliberate and clear understanding of the consequences. If you put everything off until January, you will be in a boat with about $10^6$ other scientists worldwide, and all the rest of the technical, commercial, financial, manufacturing, transportation, and other sectors of the economy whose strategy is to wait until January. Each of these elements will be bombarding the industrial sector, including the scientific instrumentation industry, for equipment and software upgrade requests and for new equipment. **They will be swamped and there will be long backlogs. This may affect your research.** ■

*These comments are biased toward the perspective of physical and biochemical scientists; they do not reflect the interests of clinical research or practice. They derive from web searching and conversations with NIH scientists, particularly Charles Buckler, NIAID, Alec Eidsath, BEPS, and Dan Garrett, NIDDK.*

### *Y2K Service Offer And Awareness Day*

The Center for Information Technology is offering remediation services to the ICs to ensure that Unix systems achieve both Y2K compliance and an acceptable level of security. To take advantage of this offer, please contact Jim Sullivan at 6-7212; e-mail **<sullivan@alw.nih.gov>**.

All NIH staff are invited to attend **Y2K Awareness Day, October 29** from 9:00 am to 12:00 noon in Masur Auditorium, Building 10. Panel discussions feature representatives from NIH, PEPCO, financial institutions, local government, and private sector organizations. Information booths will be set up to help answer questions about how to prepare for Y2K both in the workplace and at home. For more details about Y2K plans at the NIH, visit

**<http://irm.cit.nih.gov/y2000/>**.

# Bounty of Summer Students On Display at Poster Day

*Text and photos by Celia Hooper, Ph.D.*
*Scientific Editor,* The NIH Catalyst

The rest of Maryland may have been enduring the worst drought in memory, but, judging from the bumper crop of energetic young scientists at this year's Poster Day, conditions at NIH this summer were perfect for raising future clinical researchers. Here is a random sampling of the research of this season's summer students.

A highly charged foursome from the NIMH laboratory of Ed Ginns and Brian Martin jointly produced and enthusiastically discussed their poster on scorpion toxin proteins. The collegiate member of the crew was **Madeleine Fersh**, a neuroscience major entering her senior year at the University of Pennsylvania in Philadelphia. Joining her on the molecular genetic study of scorpion venom were three Maryland high school students on the threshold of their senior year: **Elisa Hui**, of Gaithersburg High School, **Ronald Redmon**, of Gwynn Park High School in Brandywine, and **Natalie Regier**, of Bethesda–Chevy Chase High School in Bethesda. Working under Suzanne Winfield, the students helped identify novel bioactive components, including two active ingredients from the venom of *Tityus serrulatus*: hemolysin and a protein that appears to be a potassium channel blocker. All four aspire to medical school and to more research at NIH.


***The Gang of Four:*** *(left to right) Elisa Hui, Natalie Regier, Madeleine Fersh, and Ronald Redmon present "A Molecular Genetic Study of Scorpion Toxin Proteins."*

Someone down the hall from the lab of **Savita Dandapani**'s mentor at the Massachusetts Institute of Technology in Cambridge had worked with Harold Varmus and gave his lab a favorable review, so the MIT senior was urged to write the NIH director to see if a summer spot might be available. That was the start of a productive two months. Supervised by NCI-DBS' Yi Li in the Varmus lab, Dandapani worked on developing a mouse model of breast cancer, using a retrovirus from chickens to ferry the c-*myc* oncogene quite specifically into mouse mammary tissue. Following an approach developed by the Varmus lab, initially in collaboration with Steve Hughes and Mark Fetterspiel at NCI-FCRDC, the lab created a vector bearing the *gag*, *pol*, and *env* genes from the avian retrovirus, then added marker genes and the oncogene of interest—in Dandapani's work, this was a tagged *myc* or the gene for RCAS-luciferase. Normally, Dandapani notes, mammalian cells would not be a functional host for an avian virus, but the mice and mammary cells she worked with had a tissue-specific promoter linked to the avian virus receptor, allowing the virus to infect mammalian cells.

***Vectorious:*** *Savita Dandapani (center) attends with interest as two viewers discuss her research on "Modeling Cancer Using an Avian Retroviral Gene Delivery System."*

Ticking off the advantages of the avian viral vector over murine viruses, such as the Maloney virus, Dandapani points to high-titer efficiency in the transfer of genes and the tissue-specific targeting made possible by the tissue-specific transgene encoding the viral receptor. But perhaps the biggest advantage of the avian viral vector, as demonstrated by Eric Holland in his brain tumor model that used the chicken virus, is that it cannot crank out immunogenic proteins or replicate in a mammalian cell.

Over the course of the summer, Dandapani and her mentor were able to show that they could use the chicken virus system to insert the *myc* oncogene into mammary cells of the mouse, in vitro and also in vivo through intraperitoneal injection. As she wraps up her summer project, Dandapani will be testing other injection methods to maximize gene transfer. It will be up to the folks who remain in Bethesda after school resumes to follow the mice and see if they develop tumors. "This was exciting," she says, "and I learned a lot about retroviruses." Dandapani, who has previously done gene knockout research with her advisor at MIT, hopes to start an M.D. or M.D.-Ph.D. program that will allow her to combine research with clinical work after she graduates.

**Raymond Liu** was one of 12 college students in NIH's new Biomedical Engineering Summer Internship Program supported by The Whitaker Foundation, the Foundation for the NIH, and NIH itself (see interview with Constance Battle and Ted Colburn, page 1). Liu will be a senior next year at the University of California, San Diego, and hopes to continue on to medical school to become a physician or clinical researcher. "I did stuff here that people haven't done before, and that's really cool," Liu raves. "And I got to work with great people." Liu worked with Dan Sackett, Robert Bonner, and Al Yergy in NICHD. The group is developing a two-step approach to analyze protein isotopes and post-translational modifications, in this case, tubulin. After using high-resolution isoelectric focusing to separate the different forms in sharp gel bands, Liu cut and transferred the focused band, isolated the minute amount of protein in it, and subjected it to mass spectroscopy—which revealed two distinct peaks, suggesting at least two forms of tubulin within the band.

***Positively Tubulin:*** *Raymond Liu (right) shows Cherie Fisk, ORS, an "Analysis of the Post-Translational Modifications of Laser-Captured Tubulin Using Isoelectric Focusing and MALDI Time-of-Flight Mass Spectrometry."*


***Only the Beginning:*** *Duewa Williams' research on "EEG Relating to HIV+ Cocaine Patients" has instilled a desire in the high school senior to return to NIH.*

**Duewa Williams**' first summer at NIH has reinforced her fascination with science. "It's a real advantage," she says, "to get this kind of research experience" early in one's education. About to enter her senior year at Lakewood (N.J.) High School, Williams worked in the laboratory of NIDA's Jean Cadet and Ronald Herning under preceptor Kimberly Tate and alongside another student, Nishant Tella. Her research project involved deciphering the combined and individual effects of drug abuse and HIV on the brainwave patterns of drug abusers. Williams says she hopes to return to NIH, perhaps to continue studying HIV.


***Footprints:*** *Yvette Sandoval presents an "Analysis of the Density of Sympathetic Innervation of Target Tissues from Transgenic Mice with Altered p75 and NGF (Nerve Growth Factor) Levels."*

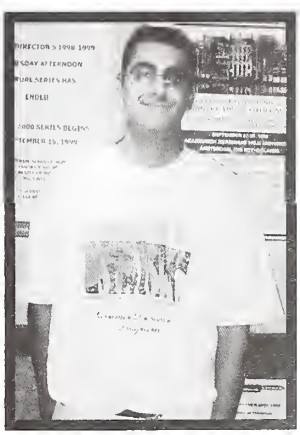
**Yvette Sandoval**, a senior at San Diego State, spent her first summer at NIH working with Christine Brennan in the laboratory of the NINDS scientific director, Story Landis. Sandoval was conducting research on the role of p75, one of two receptors for nerve growth factor, an essential neurotrophin for the growth and development of the sympathetic nervous system. She compared the numbers of sympathetic nerve endings in the thymus and spleen of p75-knockout and wild-type mice. Her data, combined with other data Brennan has published on foot-pad target tissues, have led the group deeper into mystery because they have concluded that p75 does not work either to cause apoptosis or to prevent it, as others have proposed. Sandoval, a cell and molecular biology major, hopes to return to NIH for a year as a postbac before going on to graduate school. "Christine Brennan," says Sandoval, "is a great mentor."

***On Neuropsychiatry's Threshold:*** *Elizabeth Thompson got a taste of neuropsychiatry and chocolate in the course of her research on "Regional Cerebral Blood Flow Changes in Response to Olfactory Stimuli in Alcoholic Patients and Normal Controls by [15-0] PET."*

**Elizabeth Thompson** has similar high praise for her more clinically oriented mentor, NIAAA's Wendol Williams. Thompson, who is going into her second year at Howard University Medical School in Washington, D.C., compared responses to olfactory stimuli in alcoholics vs. control patients using PET brain imaging and the subjective reactions of the patients to pleasant, unpleasant, and alcohol-like odors. Differences in the PET scans were subtle, but Thompson's hoping to see more marked differences between the groups in their subjective reactions, data that she was just beginning to sort out at the time of the Poster Day.

Although Thompson had not previously contemplated going into neuropsychiatry, she says she is now thinking about it. She says she especially enjoyed working with the patients. "I learned a lot about how to do research, about the diagnosis of patients," Thompson says. One patient sent her a box of Godiva chocolates to thank her for her help. "It's good to know you make a difference," Thompson says. "I definitely hope to end up in clinical research because that is what is ultimately going to help the most. I love being on the frontier, testing hypotheses, seeing what works. Not everything is going to work out, but at least you know you tried."

The poster next to Thompson's was presented by **Neil Sanghui**. Sticking around until the bitter end of the research fete, well after other students had taken down their posters, Sanghui was enthusiastic about his research with NIMH mentor David Jacobowitz and Francis Lau. "This was a wonderful experience," Sanghui said. Now going into his second year at the New Jersey Medical School in Newark, Sanghui says he jumped on the lab's major effort in applying differential display technology to identify altered expression of genes in the brains of people who have died with schizophrenia or bipolar disorder. The work this summer revealed that *OXA1*, a gene homologous to the mitochondrial oxidase assembly protein, is upregulated in postmortem brains from schizophrenics, compared with brains of age-matched people who did not have the disorder. Sanghui says the summer piqued his interest in clinical research and in some day incorporating it into a clinical practice. ■



***The Party's Not Over:*** *Neil Sanghui's research on "Alteration in Gene Expression in Neuropsychiatric Disease" may blossom into a career in clinical research and practice.*

**REMINDER: NIH RESEARCH FESTIVAL, OCT. 5–8**
HTTP://WWW.NHGRI.NIH.GOV/FESTIVAL99

*Hot Mouse Tips: a Three-Part Series*

# Part 2. The Ins and Outs Of Successful Surgery

*by Tory Hampshire, DVM, NINDS, and Judy Davis, DVM, NINDS*

*In the first article of this series (*NIHCatalyst, *May–June 1999), we provided an overview of normal physiological responses of mice and rats and expected deviations from normal after invasive procedures. We also made suggestions regarding preanesthetic preparation, anesthetic induction, and anesthetic maintenance. This article discusses in greater detail components of successful rodent surgery.*

Performing successful surgical procedures on rodents to obtain valid and relevant experimental data, depends on not only the necessary technical equipment but also surgeons with motivation, a certain persistence, and a genuine interest. Successful surgeries may also require training, qualified supervision, and a positive attitude to teamwork.

The investigator willing to learn to use state-of-the-art inhalation-anesthesia equipment will enjoy easily controlled anesthesia with remarkably fast induction and wake-up times.

For details beyond the "helpful hints" of this article, see the handout *Rodent Anesthesia for the Investigator,* available from the NINDS Animal Health Care Section.

### Seeing Is Believing

Tiny blood vessels and organs require good visualization and comfortable work surfaces to reduce frustration for the scientist. Key technical requirements include:

*Magnification glasses afford the user more freedom of movement and good field of view. These 4.5x glasses are by Designs for Visions.*

- *Microscopes.* Operating microscopes or magnifying glasses enhance vision and freedom of movement.
- *Microsurgical instruments and accessories.* A few high-quality instruments are usually sufficient; as a rule, the simpler the better. The choice of optimal clamps, retractors, catheters, and suture material and needles must also be considered.
- *A ventilator for long anesthetic procedures.*
- *A gas isoflurane anesthesia machine.*
- *Small-gauge catheters.*
- *Heparin solutions.*
- *Banked blood from like genotypes.*

### Basic Techniques and Considerations

The considerably higher success rate in human anesthesia compared to animal anesthesia seems to underline the inherent safety of properly administered anesthesia and highlights the value of intense monitoring during recovery that occurs with humans. In rodent surgery, many scientists are often guilty of poor selection of anesthetic agents and insufficient physiological monitoring and surgical techniques. **Helpful Hints** include:

- *Better Base-line Evaluations.* How does your mouse really look compared with a normal mouse? If you don't know what normal is, take the mouse out of the cage. Compare it with a wild-type mouse by assessing behavior, gait, and posture. Try to think of all the physiologic and anatomic outcomes your genetic alteration might cause. Talk to a clinician in that field and determine a test battery that might be used to explore that mutation in a human. Then discuss with your institute vet which tests could be done in mice. Details might surprise you. Physical conditions of genetically altered mice may significantly alter anesthesia regimens. For example, we are just beginning to get electromyographic and nerve studies on neurologically altered mice in our institute. The effect of peripheral neuropathies on respiratory function is a concern for anesthetic studies. Muscle weakness associated with these conditions reduces the functional residual capacity and depresses alveolar ventilation. Other contributing factors to consider would be health status, age, and animal position for surgery. For example, pregnant animals frequently do not have adequate tidal volume due to pressure on the diaphragm. Older animals with less compliant lungs may have inadequate gas exchange despite a relatively normal tidal volume and respiratory rate. Geriatric animals (a 1.5- to 2-year–old stock mouse or rat is old!) that are anesthetized and breathing room air are more prone to hypoxemia than young animals. Note that age ranges vary with strain; R111 mice, for example, typically live no longer than 8 to 10 months.

- *Chronic or Acute: Is the Mouse Here to Stay?* Most discussions of anesthesia in animals fail to distinguish the marked differences between the anesthetic technique for acute (nonrecovery) and chronic (recovery) experiments. The two experimental conditions require different techniques and anesthetic considerations. For example, interpretation of data derived from acute experiments using barbiturate or α-chloralose type anesthesia is difficult because of the animal's constantly changing homeostatic background. The popularity of α-chloralose for cardiovascular and neurophysiological studies is based on the drug's apparent lack of reflex and cardiovascular depression, an advantage over barbiturates. However, when attempts are made to keep a constant level of anesthesia by infusion over a three-hour period, there can be marked fluctuations in cardiovascular parameters.

- *Gentle Handling.* Atraumatic technique is an essential prerequisite for successful microsurgical procedures. For example, blunt dissection of arteries and veins and unnecessary manipulation must be avoided. The choice of clamps must be adapted to the type and size of the vessel; an optimal clamp exerts a minimal degree of compression, diminishing the risk of endothelial damage. Any instrumental manipulation with risk of intimal damage during preparation, as well as suturing, increases the possibility for thrombosis. When scheduling surgery, allow plenty of time to handle tissue gently.

### Machines and Agents

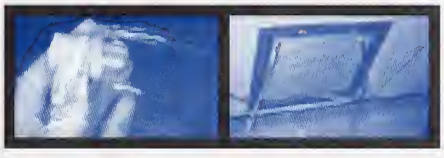
As we discussed in our first article, the most consistent and reliable anesthetic protocols for rodents involve inhalation anesthesia for both induction and maintenance, coupled with vigilant monitoring. Isoflurane with a calibrated vaporizer is the method of choice in terms of patient safety and lack of environmental contamination. We are *not* recommending the open-drop method (using ether, methoxyflurane, or halothane in bell jars), with its associated risks stemming from anesthetic flammability, waste-gas scavenging problems (including toxicity to humans handling methoxyflurane), and unacceptable mortality rates. As with injectables, open-drop methods are also associated with fluctuating anesthetic depth.

- *Purchasing the Machine.* You will need a standard isoflurane kettle with flow meter. Oxygen tanks and tank regulators of the "E-type" can be pur-

chased from Robert's oxygen. Other key procurement details are noted at the end of this article. **Helpful Hint**: Purchase a portable Rubbermaid cart to secure the oxygen tank, anesthesia machine, and tubing, as well as other monitoring equipment, so that you can move between laboratories if necessary. If scavenging isn't available at all your potential laboratory sites, purchase charcoal-scavenging cannisters from almost any veterinary source through blanket purchase agreement and follow the standard operating procedures for disposal when they reach weight capacity. The NIH Office of Research Services, Division of Public Safety (<**http://www.nih.gov/od/ds**>) will send a contract-monitoring representative to check the safety of this kind of system.

*A simple isoflurane induction chamber, oxygen flow meter, and kettle beside the flow meter*

■ *Induction Box.* Inhalation anesthesia can be delivered directly to the animal through use of a small Plexiglas box (induction chamber) with an inlet and outlet port. The chamber should be the appropriate size for the animal!



*This handmade face mask has two lines: one line from the inlet gas and the second to the facility exhaust.*

*A Plexiglas induction box inlet can be attached to the face mask when the mouse is asleep*

*You can even make a face mask with PVC or Plexiglas tubing. Take the mouse out of the induction chamber and connect the outflow directly to a scavenger system and the inflow port to the common gas outlet, using a "Y" connector and tubing. The tubing should have a stop-valve control for delivery to either the induction chamber or the face mask (or nonrebreathing tube). Induction chambers should allow continuous observation of the animal. Watch the animal!*

■ *Mechanical Ventilation: Endotracheal Tube Intubation.* Delivery of inhalation anesthesia can also be accomplished by using an endotracheal tube (ETT). Although this technique requires considerable practice, proper equipment and experience lead to successful, quick intubation in many animals. For projects requiring a ventilator or long-term anesthesia, an ETT may be your best choice. There is no ideal ETT. For rats, a 14-gauge or 16-gauge IV catheter can be used, as can a modified Cook Flexi-Tip® Urethral Catheter (V-021305). The advantages are that they require no stylet, are reusable, and are less traumatic. The disadvantage is that they are more expensive than IV catheters. You can also use silastic tubing (for a mouse, 0.025" i.d., 0.047" o.d., and for a rat, 0.040" i.d., 0.085" o.d.). IV catheters (20 or 22 gauge) will also work for mice. Measure the distance from the mouth to the cricoid cartilage (20 mm for a 25- to 35-g mouse; 26 mm for a 250- to 300-g rat).

Mechanical ventilation can be achieved by invasive or noninvasive means. Invasive mechanical ventilation requires deep induction with isoflurane or injectable components and rapid cannulation of the trachea using a 20-gauge IV catheter sleeve as an endotracheal tube. Again, this technique takes a lot of practice in mice and requires magnification. Risks include trauma to the tongue, cheek pouches, and pharynx and may become problematic. Catheters can be sealed by premeasuring from the bottom of the hub to the thoracic inlet and placing a thin bead of silicone around the catheter. Allow it to dry 24 hours before using.

Move the tongue to the side with a dry cotton-tipped applicator, with the animal resting on its breastbone. Insert an otoscope while tilting the animal's head to a 45° angle. Avoid traumatizing the soft palate but place the otoscope at the base of the tongue. Then elevate the head to an 80° angle, forcing

*Visualization of the trachea and insertion of the endotracheal tube: animal is supine, its jaws held open with rubber bands and cheek folds spread with a canary beak spreader—or by an assistant standing behind you.*

the epiglottis to protrude away from the mucous membranes and allowing complete visualization of the laryngeal folds. Carefully advance the ETT to the laryngeal folds and then remove the otoscope. Quickly advance the ETT past the laryngeal folds. If you feel some resistance, rotate the tube as you advance it. You will feel a "pop" as you enter the trachea. Check the tube for patency (wisp of cotton held at the end of the tube). Secure the ETT with tape or suture it to the lip.

*Multiple mice can be anesthetized simultaneously using a handmade manifold from the standard kettle and flowmeter over a downdraft surface.*

Proper positioning of the rodent and good magnification are key elements in successful intubation; 4X magnification glasses are perfect for this feat and give the scientist more freedom of movement (they can be purchased from Designs for Visions—516-585-3404). Alternatively, an operating scope with a horizontal arm 4X beam will also work. Both require practice. In the beginning, it is best to use injectable anesthetics to hold the mouse in a deep enough plane of anesthesia for training. Invasive me-

*Noninvasive mechanical ventilation is very attractive for chronic recovery models in the mouse. The procedure can be achieved atraumatically by setting the ventilatory rate around 120/min and the volume at 10 mL/kg and using a tightly sealed face mask. The mask must be custom-made and uses the mouse's loose skin as an "auto-seal." For the average 30-g adult mouse, a 6-mL syringe case works well.*

chanical ventilation by endotracheal intubation is best done in acute mouse models. Access may be gained by tracheotomy—a surgical incision into the trachea through which an ETT is inserted.

■ *Vaporizers.* Vaporizers facilitate evaporation of an anesthetic liquid and control the concentration of anesthetic in the carrier gas. Vaporization of volatile anesthetics depends on vapor pressure and ambient temperature. Vaporizers are calibrated for the agent for which they were designed because anesthetic agents differ in their vapor pressure and heat of vaporization. If you fill a vaporizer that was designed to deliver one agent, for instance, methoxyflurane, to deliver another agent, isoflurane, you cannot rely upon the concentration you set because the vapor pressure of methoxyflurane is 23 mmHg at 20°C, while that for isoflurane is 240 mmHg at 20°C. In contrast, the vapor pressure of isoflurane and halothane are similar; therefore, isoflurane can be accurately delivered from a vaporizer calibrated for halothane. However, companies (such as Anesthetic Vaporizer Services) will do changeovers for a reasonable cost.

As a rule, a vaporizer setting of 1 X minimum alveolar concentration (MAC) will produce light anesthesia. 1.5 X MAC will produce a surgical depth of anesthesia, and 2 X MAC will produce deep anesthesia. Inhalant potency is fairly constant across species, so vaporizer settings for inhalants will be fairly consistent with those of more familiar species.

■ *Rates and Volumes.* Commercial ventilators, like the CWE distributed through Harvard Biosciences, usually have either or both pressure and volume deliveries. Pressure-driven ventilators trigger a burst for inhalation that is based on negative draw of the chest wall; they require a tight seal of the ETT and no use of paralytic agents. These are advantages for short procedures because a balanced mouse patient will trigger the machine at will. Remember, if the rate slows drastically during anesthesia, the mouse will not compensate with increased tidal volume and is prone to develop hypoxia. It may also inspire less anesthetic gas and become less anesthetized. A pressure-driven ventilator also may cause less alveolar damage. Volume ventilation settings, on the other hand, deliver set volumes at established rates. They are ideal for long procedures and will work with imperfect seals. Many newer models have both options. Weigh the mouse to achieve the most reasonable tidal volume (10 mL X body weight in kg). Tidal volume is discussed in detail in our handout. Such methods as counting the respiratory rate and evaluating the color of mucous membranes are commonly used but are not practical in rodents. ■

**Normal Physiologic Variables For Mice and Rats**

| | Mouse | Rat |
|---|---|---|
| Body Temperature | 37.4° C | 38.0° C |
| Respiratory rate | 180/min | 90/min |
| Tidal Volume | 0.15 ml | 1.6 ml |
| Minute Volume | 24 ml | 220 ml |
| Resting Heart Rate | 570 bpm | 250 bpm |

From Flecknell, PA., Laboratory Animal Anesthesia , Academic Press, San Diego, CA, 1992

*Intravascular cannulation for measurement of blood gases can also be achieved with lots of practice. New catheters have been designed by Micron (available through A.J.Buck on BPA at NIH) that are 32 gauge and can be fed through a 26-gauge introducer needle in the internal carotid or jugular. With great practice, the femoral vein or artery can also be cannulated. Because of the small size of the vessels, it is imperative that a flush be at hand, and that the mouse is heparinized before manipulation (400 µg/kg subcutaneously five minutes before cannulation will work). If you plan on performing long procedures and sampling, you should have like-strain blood replacement available. Because of the small amount of blood volume in the mouse (1.0 mL) and the relatively large size of the sample for blood gas measurement (0.1–0.2 mL), combined with the dead space in the lines, the risk of hypovolemic shock warrants ready access to blood replacement.*

**Equipment Sources**

| Item | Source | Contact | Cost |
|---|---|---|---|
| Tabletop Rodent Anesthesia Machine | Vet Equipmment, Inc. | Doug DeMars 1-800-466-6463 | ~$,3700 with all attachments |
| Portable Surgical Lights (tabletop, flexible gooseneck) | Medical Diagnostic Services or Stoelting Research Instruments | 813-653-1180 630-860-9700 | ~$450 each |
| Omnicon f/air Scavenging System | A.M. Bickford, Inc. Wales Center, NY | 716-652-1590 (fax) 716-652-2046 | Call for quotation |
| Pulse Oximeter Model 200SL (small lingual sensor | Pennsylvania Veterinary Supply, Inc. | 1-800-233-0210 | $2,400 range |
| Rectal Temperature Probe | Yellow Springs Thermometer Co. | 1-800-343-4357 or 515-767-7241 | Call for quotation |
| Respiratory Gas Monitor (capnography, $O_2$ and gas concentrations) | Ohmeda Madison, WI | 1-800-652-2460 | Range: $2,500-$9,000 (call for quotation) |
| Mouse Respiratory Machine | CWE, Inc. Ardmore, PA | 610-642-7719 | $6,500 |
| Micron 32-gauge Intravenous Catheters for Mice | A.J. Buck; NIH BPA listings | Maryland local: 410-581-1800; veterinary: 1-800-638-8672 | $15 each |

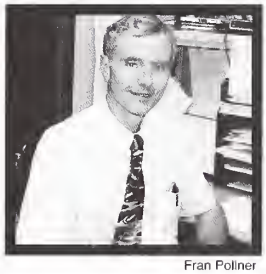
***Disclaimer:*** *Mention of specific products in this article does not constitute an endorsement of those products, nor does it signify that other similar products are less desirable.*

**Physiologic Influences on MAC Requirements During Anesthesia**

| Factors that Decrease MAC Requirements | Factors that Increase MAC Requirements | Factors that Do Not Alter MAC Requirements |
|---|---|---|
| Metabolic acidosis | Increased Neurotransmission (Stress) | Duration of Anesthesia |
| Hypothermia | Hyperthermia | Type of stimulation (noxious) |
| Hyponatremia | Hypernatremia | Sex |
| Hypoosmolality | Chronic Ethanol Use (NIAAA animals) | Hyperoxemia |
| Pregnancy | Geriatric Animals | Hyperkalemia |

# Recently Tenured

**Darrell Abernethy** *received his M.D. and Ph.D. in pharmacology from the University of Kansas School of Medicine in Kansas City in 1976. Following a residency program at Jackson Memorial Hospital in Miami, Fla., and a postdoctoral fellowship at Massachusetts General Hospital in Boston, he moved up the academic ranks at the medical schools at Tufts University in Boston, Baylor University in Houston, Brown University in Providence, R.I., and Georgetown University in Washington before assuming his current position earlier this year as clinical director and chief of the laboratory of clinical investigation at the NIA.*



Fran Pollner

*Darrell Abernethy*

My research has focused on the mechanisms of peripheral distribution of drugs, drug disposition in the context of obesity, and the pharmacokinetic and pharmacodynamic relationships of cardiovascular drugs in aging.

Recent studies center on the role of genetic polymorphisms that influence responses to cardiovascular drugs. We are exploring both phenotypic and genotypic changes that contribute to altered phenotype, as well as nongenotypic splice variant transcriptional changes that result in phenotypic changes.

These studies currently involve endothelial nitric oxide synthase (eNOS) and the L-type calcium channel.

We and other investigators have previously noted marked differences in forearm vascular relaxation due to alterations in endothelial-derived relaxing factor(s) in aged and in African-American subjects. The basis for this finding is a current line of investigation, with the hypothesis that polymorphic variants of eNOS make a major contribution to this variability over and above environmental and disease-based factors.

In laboratory studies, we have found expression of functionally different splice variants of the $\alpha_1$ subunit of the L-type calcium channel in young vs old human vascular smooth muscle cell lines. We are now exploring the basis for this observation in the laboratory and are pursuing its clinical correlates by characterizing the expression pattern of these splice variants in human surgical specimens.

**Robert Kotin** *received his Ph.D. in microbiology from Rutgers University in New Brunswick, N.J., and the University of Medicine and Dentistry of New Jersey in Newark in 1986. After a postdoctoral fellowship at Cornell University Medical College in New York, he worked at Lederle Laboratories in Pearl River, N.Y., and Genetic Therapy, Inc., in Gaithersburg, Md., where he developed recombinant adeno-associated virus vectors. In 1994, he joined the the Molecular Hematology Branch in NHLBI, where he is now a senior investigator in the Laboratory of Molecular Hematology.*

My research interests are focused on the molecular biology of adeno-associated virus (AAV), a human parvovirus. AAV is a deceptively simple virus with only two open reading frames—one encoding the structural proteins and the other the nonstructural, or Rep, proteins. The AAV genome consists of a linear single strand of DNA of approximately 4,700 nucleotides. One of the unique aspects of AAV is that it requires co-infection with a helper virus, such as adenovirus or herpesvirus, for productive infection. The only AAV gene product necessary for replication is the Rep protein(s). Helper virus infection renders the host cell permissive for AAV gene expression and replication, rather then complementing a genetic defect in AAV. In the absence of helper virus co-infection, the Rep protein represses AAV gene expression and the AAV DNA integrates into the cellular genome.

Fran Pollner

*Robert Kotin*

My early work examined the structure and organization of the AAV proviral DNA. Using the flanking cellular DNA as probes for genomic Southern blots, I discovered that the viral DNA integrated into a specific locus on human chromosome 19. Site-specific (or targeted) integration is still a very exciting discovery that, so far, is unique to AAV. This mechanism of targeted integration, however, remains obscure. A correlation between targeted integration and Rep protein expression is evident, but the known activities of Rep are not sufficient to explain targeted integration.

Our investigations of Rep have revealed several interesting activities that we think play an important role in AAV integration and virus replication and may explain some of the effects of Rep on the host cell.

Much of our research is driven by the desire to use recombinant AAV as a gene transfer vector. Understanding the functions of the AAV Rep proteins and how these proteins interact with the cell is central to advancing this goal. A major limitation for producing recombinant AAV results from the transient production methods used. Cells expressing Rep proteins display aberrant growth rates and phenotype. High concentrations of Rep appear to be cytotoxic to the cell, and it is not possible to produce a cell line that constitutively expresses Rep proteins. My laboratory has recently discovered that Rep binds and inhibits the cyclic AMP-dependent protein kinases, protein kinase A and protein kinase X (PrKX). The cellular role of PrKX is unknown, and we are currently investigating the molecular biology of this potentially important protein kinase.

The use of recombinant virus for gene transfer is a compelling idea for which the technology and applications have yet to be fully developed. The interaction between AAV and the host cell is complex, and a greater understanding of this system will be necessary before investigators can obtain transgene expression with therapeutic effect. Current AAV gene transfer vectors are based on AAV serotype 2; we have produced recombinant AAV type 4 and type 5. These three serotypes exhibit overlapping, but distinct, tissue tropism. Characterizing tissue specificity will enable investigators to select the appropriate vector for a given application.

In summary, my laboratory is making good progress on two fronts with AAV. First, we've been able to determine some of the ways that AAV interacts with cells—such as the interactions between Rep and PrKX and Rep and cellular DNA—which yield insights into viral and cell biology. Second, we've made strides in developing AAV as a gene transfer vector with great potential therapeutic value. AAV continues to surprise us in the ingenuity with which it manipulates the cell. I think this is very impressive for a simple, nonpathogenic virus.

# Recently Tenured

**Stuart Le Grice** *received his Ph.D. in biochemistry at the University of Manchester, United Kingdom, in 1976 After postdoctoral research in the UK, Germany, and the United States, he served as a senior scientist with Hoffmann-La Roche, Basel, Switzerland. In 1990, he joined Case Western Reserve University, Cleveland, where he directed the Center for AIDS Research (CFAR) from 1994 to 1999. He is now chief of the Resistance Mechanisms Laboratory in the HIV Drug Resistance Program at the Frederick Cancer Research and Development Center, NCI.*

My primary research interests are in protein structure and function in the regulation of gene expression. Studies of transcription in gram-positive and gram-negative prokaryotes laid the foundation for my current research. In these bacteria, multiple forms of sigma factors interact with the host RNA polymerase to control gene expression. We used chemical and enzymatic footprinting techniques to investigate RNA polymerase/promoter interactions in binary (polymerase/promoter) and ternary (polymerase/promoter/nascent RNA) complexes. We also studied cessation of transcription at sequence-dependent terminators to explore barriers to the use of heterologous signals in prokaryotes.

Wayne Main

*Stuart Le Grice*

These studies have allowed me to move into retrovirology and apply similar concepts to investigate mechanisms of reverse transcription in HIV and related lentiviruses, the focus of my research since 1987. My studies were aided significantly by the development of metal chelate technologies for purification of poly-histidine-extended proteins at Hoffmann-La Roche, where I reported the first single-step purification of p66/p51 HIV-1 reverse transcriptase (RT). The p66 and p51 subunits of this enzyme are derived from the same gene, but p51 undergoes COOH-terminal processing by the viral protease. Understanding the role each subunit plays within the parental heterodimer required preparing enzymes containing point mutations in a single subunit. A reconstitution protocol I developed made this possible and was first applied to prepare selectively deuterated p66/p51 heterodimers ([H]-p66/[D]-p51 and [D]-p66/[H]-p51) for analysis by neutron diffraction. Subsequently, I have been engaged in a program of subunit-selective mutagenesis and was the first to illustrate that the p51 subunit of p66/p51 RT was catalytically inactive; that is, all enzymatic functions reside within the p66 subunit. This mutagenesis strategy has been adopted by several laboratories studying structurally related lentiviral RTs.

The multifunctional nature of RT provides both its DNA polymerase and ribonuclease H (RNase H) activities as targets for development of antiretroviral agents. Thus, a second theme of my research is RNase H activity as it pertains to nonspecific and highly specific hydrolysis of the RNA-DNA replication intermediate. I was first to demonstrate that eliminating RNase H activity inhibited HIV replication in culture and that host enzymes could not complement an impaired retroviral function, thus suggesting this activity as a bona fide therapeutic target. In 1990, I showed that RNase H activity could be subdivided into polymerization-dependent and polymerization-independent modes, although their relevance was not immediately clear. Further research revealed mutants that retained exclusively polymerization-dependent RNase H activity. Eliminating the latter function significantly impaired DNA strand transfer, a process in retroviruses whereby nascent DNA is relocated between templates.

Despite such successful in vitro mutagenesis, the structural basis for phenotypes of many mutants prepared in the laboratory remains unclear. I am now developing bioconjugate strategies for probing retroviral replication complexes in solution and at high resolution. This approach entails site-specific tethering of photoactive, fluorescent, nucleolytic, and proteolytic probes to RT or its nucleic acid substrates. These probes will allow us to investigate contact between individual subdomains, "communication" between the p66 and p51 subunits, and potential interactions between RT and other viral proteins. Preliminary experiments have defined contacts between the extreme COOH-terminus of the RNase H domain and duplex DNA that cannot be detected through crystallographic analysis. The same approach indicates that RT accommodates duplex DNA and RNA-DNA hybrids differently. The fast-emerging field of bioconjugate chemistry provides the ideal complement to crystallographic approaches. The combined power of these disciplines should reveal features of the retroviral polymerase that lend themselves to therapeutic intervention.

**Robert Star** *received his M.D. from Harvard University-MIT Program in Health Sciences and Technology in Cambridge and Boston in 1980, did postdoctoral work at NIH, and then moved to the University of Texas Southwestern Medical Center in Dallas in 1987. He has returned to NIH as a senior investigator in NIDDK to form the Renal Diagnostic and Therapeutic Unit. He is also a Senior Scientific Advisor to the Division of Kidney, Urologic and Hematologic Diseases.*

I am interested in translational research for the diagnosis and treatment of renal disease. My initial bench studies at UT Southwestern wandered from renal transport mechanisms to mathematical modeling to nitric oxide; throughout, however, I attempted to translate my bench discoveries to solve clinically relevant problems. For example, I found that urea movement in the terminal part of the kidney is limited by the apical membrane. Reasoning that trapping of urea within cells might explain some symptoms in dialysis patients, we studied urea movement during dialysis. While incorrect, this led to the development of an easy method to measure the dialysis dose based on the simple concept of urea removal. We then showed that several widely used methods to quantify dialysis dose were inaccurate.

Most recently, we have been studying diagnostic and therapeutic approaches to acute renal failure, a condition that lacks a specific treatment and carries a 50 percent mortality. Again, serendipity played a role. While studying nitric oxide in the kidney, my colleagues and I discovered that nanomolar concentrations of the pituitary hormone α-melanocyte stimulating hormone (α-MSH) inhibited nitric oxide synthesis (NOS) in macrophages. Unlike standard NOS inhibitors that directly inhibit the enzyme, α-MSH inhibited the induction of the inducible NOS (iNOS). α-MSH darkens skin color in amphibians, but, more importantly, decreases fever and has profound anti-inflammatory effects in acute, chronic, and systemic inflammation. α-MSH is increased by systemic stress and is produced at sites of inflammation. We found that α-MSH is produced by inflammatory cells, indicating that α-MSH is an anti-inflammatory cytokine.

Fran Pollner

*Robert Star*

We worked on its mechanism of action in cultured and isolated cells, but switched to animal studies to search for clinically relevant uses. We found that α-MSH protected against endotoxin-induced liver

damage; unlike many other agents, α-MSH was effective even when given 30 minutes after endotoxin. We then tested α-MSH in a renal ischemia-reperfusion model of acute renal failure, a model in which injury is caused by inflammatory and cytotoxic (NO) injury cascades, both of which are inhibited by α-MSH. We discovered that α-MSH inhibits renal ischemia reperfusion injury. Again, unlike many other agents tested in animals, α-MSH was still effective even when started six hours after injury. We then investigated its mechanisms of action. As expected, α-MSH inhibited both inflammatory (neutrophil, chemokines, adhesion molecules) and cytotoxic (NO) pathways; however, we found it also simulated an endogenous protective mechanism involving IL-10.

Based on its multiple effects and mechanisms of action, we conducted a Phase I dose-escalation trial to establish α-MSH safety in humans. α-MSH was safe; however, we unexpectedly found that α-MSH increased blood pressure. This "side effect" could be useful in treating patients with ischemic acute renal failure and adds another mechanism of action of α-MSH.

Human acute renal failure is difficult to diagnose, because currently available tests are insensitive and do not detect renal damage for several days. We are, therefore, pursuing new diagnostic tests for acute renal failure and have developed and validated a bedside method for measuring glomerular filtration rate that takes only 45 minutes—five times as fast as the current method. We are using a variety of biochemical and molecular techniques—akin to a renal CPK test—to search for molecules that specifically appear in the injured kidney or the urine it produces. For example, we are using laser-capture microdissection to study gene expression in specific portions of the injured nephron. We are using this technique to identify genes that are differentially expressed in different portions of the kidney in response to ischemia. We hope that some of these genes may point to novel protective agents and that some may serve as markers for renal injury. We plan to use advanced imaging techniques to measure renal function, inflammation, and fibrosis.

**Gisela Storz** *received her Ph.D. in biochemistry from the University of California, Berkeley, in 1988 and did postdoctoral work at NCI and Massachusetts General Hospital in Boston before joining the Cell Biology and Metabolism Branch of NICHD in 1991.*

My main interest is in how cells sense and protect themselves against oxidative stress. Reactive oxygen species—such as superoxide, hydrogen peroxide, and hydroxyl radical—have the potential to damage almost all cell components, including DNA, lipid membranes, and proteins. Oxygen radicals have been implicated as causative agents in several degenerative diseases.

Fran Pollner

*Gisela Storz*

Most organisms have an adaptive response to defend against oxidants. For example, treatment of bacterial or yeast cells with low doses of hydrogen peroxide induces expression of a distinct group of proteins, the decreased expression of other proteins, and resistance to killing by subsequent higher doses of hydrogen peroxide. My group at NIH has focused on this adaptation.

The *Escherichia coli* and *Salmonella typhimurium* responses to hydrogen peroxide involve at least two regulators, OxyR and OxyS. The expression of nine hydrogen peroxide–inducible proteins is controlled by the OxyR protein, the first transcriptional regulator found to be directly sensitive to the redox environment of a cell. Studies of the DNA-binding properties of oxidized and reduced OxyR showed that the two forms have different binding specificities, allowing OxyR to act as an activator after oxidative stress and as a repressor during normal growth.

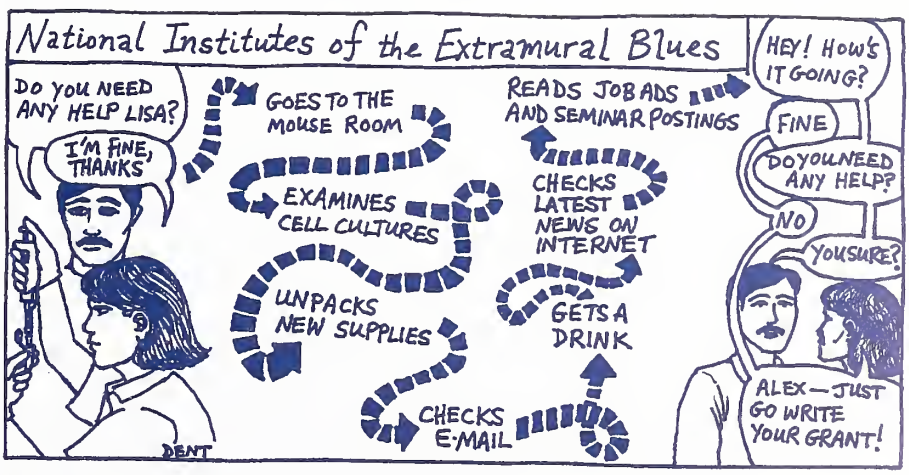
We recently discovered that OxyR is activated by the formation of a disulfide bond and is deactivated by enzymatic reduction by glutaredoxin. Because the formation of a disulfide bond in the reducing environment of the *E. coli* cytosol was unexpected, we are now examining the nature of the exquisite redox sensitivity of OxyR. Our studies should give new insight into cysteine chemistry and the regulation of other redox-sensitive proteins.

Hydrogen peroxide treatment also leads to the induction of a novel 109-nucleotide, untranslated RNA regulator denoted OxyS. OxyS is conserved between *E. coli, S. typhimurium,* and *Shigella flexneri.* We found that the OxyS RNA activates and represses the expression of as many as 40 genes in *E. coli,* and we identified eight targets. This unusual RNA also acts as an antimutator, reducing chemically induced mutagenesis. Thus, we propose that OxyS integrates adaptation to hydrogen peroxide with other cellular stress responses and helps to protect cells against oxidative damage.

We are now using mutational studies of the OxyS RNA and its target sequences to investigate the mechanism of the regulation by this RNA. In addition, we are conducting genetic and biochemical assays to identify and characterize proteins that interact with OxyS. We have also begun to examine the functions of other small RNAs of unknown function in *E. coli.*

Compared with the bacterial response to hydrogen peroxide, relatively little is known about the cellular mechanisms used by higher cells to sense and protect against oxidative damage. To initiate studies of the oxidative stress response in eukaryotes, we constructed isogenic *Saccharomyces cerevisiae* strains carrying mutations affecting known signal transduction pathways.

We are now comparing the oxidant sensitivities and whole genome expression patterns of these mutants and are genetically screening for yeast mutants with altered sensitivities to hydrogen peroxide in order to identify new components of the eukaryotic response to oxidative stress ■



# Call for Catalytic Reactions

In this issue, we are asking for your reactions in four areas: The Foundation for the NIH,millennial research challenges, hot methods, and campus security.

**Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 1, Room 209.**

***In Future Issues...***

- Grad Students: Once and Future
- The Unretiring Bernhard Witkop
- Campus Security

1) The Foundation for the NIH is eager to hear from NIH scientists as to how best it can further the work of the NIH. In which critical areas do you believe the Foundation should be concentrating its efforts in seeking otherwise difficult-to-obtain funding?

2) What would be on *your* top-ten list of research challenges (see page 4) for the new millennium?

3) Hot methods, anyone?

4) *The NIH Catalyst* plans to examine relations between the NIH community and the NIH police. Do you have any questions or security issues you'd like explored?

*The NIH Catalyst* is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 209, 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, Warren Grant Magnuson Clinical Center, and Associate Director for Clinical Research

Lance Liotta
Chief, Laboratory of Pathology, NCI

**Scientific Editor**
Celia Hooper

**Managing Editor**
Fran Pollner

**Copy Editor**
Shauna Roberts

**Contributing Writers**
Cynthia Delgado



U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health
Building 1, Room 209
Bethesda, Maryland 20892