Electronic Communications and Collaboration

The NIH Catalyst

A Publication for NIH Intramural Scientists

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Collins Begins Post as New Genome Project Head

by Seema Kumar

Geneticist Francis Collins plans to cut a new facet in NIH, the "crown jewel of biomedical research." Recently appointed head of the National Center for Human Genome Research (NCHGR), Collins will set up and run NIH's newest intramural program, focusing on the medical benefits of the Human Genome Project—identifying disease-related genes, developing diagnostics based on those genes, and following through with gene therapy to treat hereditary disorders.

"I am extremely enthusiastic about this new program," said Collins at a news conference at the Stone House on April 8. "The purpose of the program is not to create an insular program but to recruit a critical mass of scientists collaborating and working together on genetic diseases in ways that will benefit the entire NIH

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This Just In

The U.S. Court of Appeals has struck down the federal government's honoraria ban, which has restricted federal employees from accepting remuneration for writing or speeches not related to work activities. On March 30, the D.C. Circuit Court of Appeals declared the ban unconstitutional because it violates the First Amendment. The Appeals Court's decision will not go into effect until May 14 and could be stayed, pending an appeal by the federal government. Scientists should continue putting their honoraria in escrow and not accept payments until further notice. Further details, and implications of the Court's decision for intramural scientists, will be covered in the next issue.

SDs Request Scientists' Input on NIH Tenure-Track Policy

Here it is for your review: a copy of the tenure-track policy for intramural scientists approved by the scientific directors (SDs) at NIH. Remember—this is a draft, and we want your input before it is finalized. Please write your comments on the FAX-BACK response sheet provided on p. 20 and fax or mail it back to us by June 1. Our fax number: 402-4303. Our address: Bldg. 1, Room 114. ■

NIH Intramural Tenure-Track Draft Policy

Preface

by Lance A. Liotta M.D., Ph.D.

The concept of tenure in intramural research has evolved gradually over the years as a policy and process to ensure the highest attainable quality in the scientific staff engaged in intramural research and related medical care. The conferring of tenure in intramural research is a vote of confidence in the achievements and potential of the candidate, and the research support which is implied by that decision requires that it be made with maximum care.

—John Carol Eberhart, Ph.D.
Tenure in the NIH Intramural Program
Dec. 30, 1987

Tenure at NIH, defined as the long-term commitment of salary, personnel, and research resources, lies at the heart of the creative freedom we value so much here. The flexibility to seize new scientific opportunities, to act immediately on a new idea, and to undertake long-term and highly innovative projects accompany the privileges of tenure. In addition to protecting scientific freedom, as it does in the academic setting, tenure at NIH also conveys resources to take advantage of that freedom. The security of support provided by our tenure system, the freedom from the time demands and uncertainty of grant applications, are our most important inducements in recruiting and retaining the best scientists in the world.

Tenure at NIH differs from tenure at a university. University tenure does not typically include long-term commitment of salary, resources, and personnel, as it does at NIH. Indeed, according to Bickel (Academic Med. 66, 219, 1991) 42% of academic tenure comes without any salary support whatsoever. Bickel also reports that academic institutions are moving to institute post-tenure evaluations. This need is strongly met by the Intramural Research Program boards of scientific counselors who review the

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NEW "FAX-BACK" FEATURE TO PROVIDE SCIENTISTS A VOICE


Lance A. Liotta

Thank you for all the letters and phone calls supporting the concept of the *Catalyst* and offering suggestions for articles. The *Catalyst* is a forum for voicing opinion and a showcase for intramural science. In this issue, in addition to our usual scientific reports, we are launching a new feature, "FAX-BACK" : a page on which you can write your opinion and then fax it back to the Deputy Director for Intramural Research. The purpose of the FAX-BACK is to gather feedback in the spirit of a year-round electronic town meeting. The fax page will provide the opportunity for everyone at NIH to voice their opinions on any subject. Your opinions and suggestions will be important in developing policies, establishing a consensus on certain issues, and decision making at all levels. The FAX-BACK page will list several questions or issues relating to current policies or projects under evaluation. Your opinions will provide a boost for the good ideas and give us a reason to curtail or eliminate the bad ones.

WE ARE LAUNCHING A NEW FEATURE, "FAX-BACK" : A PAGE ON WHIGH YOU CAN WRITE YOUR OPINION AND THEN FAX IT BACK TO THE DEPUTY DIRECTOR OF INTRAMURAL RESEARCH. YOUR OPINIONS WILL PROVIDE A BOOST FOR THE GOOD IDEAS AND GIVE US A REASON TO CURTAIL OR ELIMINATE THE BAD ONES.

In the current FAX-BACK page, we are asking your opinions on four issues. The first issue is our new tenure-track draft policy, published for your comment in this issue. Our most important investment in the future comes from supporting the career development and independence of junior scientists. The new tenure-track policy is designed to protect that vital investment and is part of a larger career development initiative that also addresses issues of concern to women and minority scientists, including stop-the-clock, family-leave, and extend-the-clock provisions. An NIH request to implement the stop- and extend-the-clock provisions has been sent to PHS for approval. These and other additional recommendations made by the Task Force on the Status of Women Scientists at NIH (see task force report on page 4, February issue) constitute the second part of the career development initiative that will be published in the next issue of the *Catalyst*.

The second issue for the FAX-BACK relates to the "Task Force Report On The Intramural Program" chaired by Rick Klausner. Copies of the report have been distributed through the scientific directors to the laboratory or branch chiefs. We have extra copies of the report in our office. A major recommendation of the task force was to establish a series of interinstitute advisory faculties focused on specific cross-cutting scientific disciplines. The task force suggested that each faculty elect a chairperson and that a faculty council oversee all the faculties and provide a central organization link. The recommended purpose of the advisory faculties would be to further stimulate NIH's intellectual environment, to identify a cadre of transinstitute expertise for recruitment of scientists, to provide a voice for the scientific community; and to host seminars and workshops. The scientific and institute directors are continuing to discuss the advisory faculty concept and welcome your input.

The third issue is a parking-policy idea under evaluation. NIH employees across the campus are faced with a hardship in the availability of parking. However, the most frequent complaint I receive comes from employees with young children who must be dropped off at school or day care. By the time the school or day-care facility opens, all of the non-car-pool parking places are filled on the NIH campus. Why not have a special car-pool parking exemption for employees faced with this special family need? Obviously, the logistics of setting up and monitoring such a system would have to be carefully considered. Steve Ficca, director of the Office of Research Services, has stated that such a system is feasible. Do you support this idea?

The last issue raised on the FAX-BACK page relates to a tuition-loan project that we are now studying. Many NIH employees are burdened with the financial responsibility of tuition payments for their children attending college. Other employees will be faced with tuition payments in the near future. Consequently, the tuition support offered by many universities becomes a tempting part of a package used by recruiting officials trying to attract NIH employees. To counter this, the Foundation for Advanced Education in the Sciences (FAES) is exploring the possibility of setting up a low-interest or no-interest tuition loan program for NIH employees. Would you take advantage of this program if it existed? Do you think it would make a difference in retention?

Join the fax town meeting and be heard! We look forward to seeing your comments. Depending on the number of responses, the suggestions will be tallied or excerpts will be published in the next issue. ■

Lance A. Liotta, M.D., Ph.D.
Deputy Director for Intramural Research

NIEHS REORGANIZES: ECTOPIC INSTITUTE EMBRACES BASIC SCIENCE

by Celia Hooper

In this age of commuter marriages, E-mail, faxes, and teleconferencing, researchers at NIH's southernmost annex take a pragmatic view of their remote status. The 280 doctoral-level scientists at the National Institute of Environmental Health Science in Research Triangle Park are doing exciting research, but they miss some of the intellectual and professional perks of working in Bethesda, and suspect their northern colleagues may—sometimes—forget them. "As an 'ectopic site,' we still feel close, even though we are 300 miles from what is going on on [the Bethesda] campus. And I think people have become even more attuned [to NIEHS] in the last year and a half," says NIEHS Scientific Director John McLachlan.

Twist the arm of an NIEHS researcher and another truth emerges: many scientists enjoy many of the benefits of NIH, but without the cost-of-living, traffic, space, and parking problems of their Bethesda colleagues. "Please don't tell anyone," said one NIEHS scientist who requested anonymity, "but we have more space down here than you do up there. It's a well-kept secret, but we don't have to do experiments on top of our centrifuges."

NIEHS is now completing a reorganization, combining its former three intramural divisions into one. The goal, says McLachlan, "is to improve interdisciplinary work and communications" within the North Carolina facility. "With the reorganization, we can have applied toxicologists working with molecular biologists, cell biologists working with epidemiologists, and molecular modelers working with lab scientists—that is the driving force behind the reorganization," McLachlan says.

Although just an hour's plane ride separates NIEHS from the main campus, limited travel funds mean that most contacts with Bethesda colleagues must be by E-mail, fax, and phone. "That is not optimal," says McLachlan. "It's more collegial to meet at a lecture or walking down the hall to the lab. . . .What we miss most is access to seminars—the ideas from all over the campus and the wealth of techniques and core facilities."

In addition to fostering interdisciplinary interaction, the reorganization should boost basic science at NIEHS, McLachlan says. "For the first decade and a half, we have been thought of as a toxicology institute, but in the last two to four years, we have been increasing our basic-science base." In collaboration with Duke University in Durham, N.C., and the University of North Carolina in Chapel Hill, NIEHS is also adding a clinical component to its research portfolio. And on Earth Day—April 20—NIEHS will be unveiling its revamped *Environmental Health Perspectives* journal which will contain original research articles and news. Previously, the journal's primary emphasis was on publishing NIEHS symposia.

McLachlan says despite these changes, the NIEHS mission is the same. "Our mantra is that human disease and dysfunction is related to a complex interaction between genetics and the environment, factored over time. It's hard to do anything to change genes or time, but we can do something about the environment."

An NIEHS Research Sampler

Imaging the movements of cellular calcium:

James Putney, chief of the NIEHS Laboratory of Cellular and Molecular Pharmacology, says scientists at Research Triangle Park recruited him seven years ago for his background in cellular calcium metabolism because experts believe that environmental toxins like lead, cadmium, and tributyl tin do their damage to cells by interfering with the regulation of calcium metabolism. One major problem stood in the way of studying the effect of the toxins: there was no foundation for the research—scientists knew very little about the normal regulation of calcium metabolism in cells, starting with the entry of calcium into the cell, which was Putney's area of special interest.

Even after seven years, Putney remains focused on basic research, and he has made a significant and practical discovery that could help in understanding the fundamentals of calcium metabolism. In December 1992, Putney and his colleagues published a paper in the *Journal of Biological Chemistry* on their discovery that fluorescent calcium-indicator dyes could be loaded into organelles in living cells. "This is really the first successful use of fluorescent indicators to get information on calcium distribution in live cells," says Putney.

Five to 10 years ago, researchers thought that mitochondria were the key organelles in moving calcium quickly into and out of cytoplasm. Putney and colleagues found no evidence for mitochondrial involvement and were surprised to find that the nuclear envelope appears to store and release calcium. Putney's technique could not resolve details of the endoplasmic reticulum (ER), which fills the cytoplasm, nor other putative non-mitochondrial organelles called calciosomes. Scientists now think that portions of the ER or the calciosomes play the lead role in rapid calcium fluxes in the cell. Putney also has yet to determine whether the nuclear membrane (which is contiguous with ER membranes) is releasing calcium into the nucleus or out to the cytoplasm.

Calcium regulation is important in the relay of signals in response to peptide hormones (such as angiotensin), growth factors (such as platelet-derived growth factor), and neurotransmitters (such as acetylcholine or norepinephrine). When these ligands bind to receptors on the cell membrane, inositol trisphosphate (IP-3)—the so-called second messenger—is fired into the cytoplasm. Scientists believe that IP-3 binds to the cell's calcium-storing organelles, opening membrane channels and flooding the cytoplasm with calcium. A change in cytoplasmic calcium-ion concentration sets in motion the cell's response to the hormonal signal.

Putney and others at NIEHS are now trying to improve their calcium imaging technique. Robert London and Elizabeth Murphy in the Laboratory of Molecular Biophysics are developing versions of the calcium indicator that would be visible in living patients via nuclear magnetic resonance. Individual organelles would not be visible with this technique, but NMR might be used to study calcium fluxes in tissues of the beating heart, for example.

Putney says his next goal is to develop an indicator with better binding kinetics that would flag calcium-ion release instantaneously. The current indicators "allow us to identify regions of the cell that respond, but not to measure the release in real time—not until most of the calcium is gone" from an organelle, Putney says. "There is lots of controversy surrounding how calcium is released and taken back up in oscillations in the cell. If we could measure calcium in the lumen of the ER—or wherever—in real time, we could get some information about what is driving this process."

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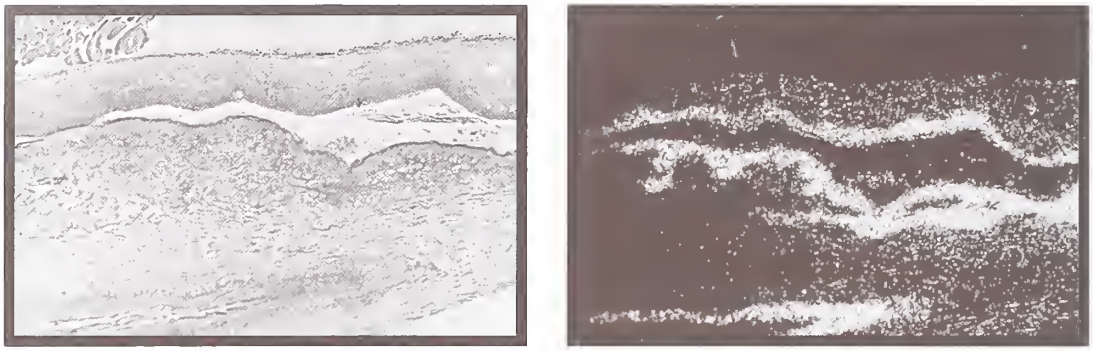

Light and dark-field microscopy shows the persistant expression of epidermal growth factor (EGF) in the vagina of an ovarectomized, 52-day-old mouse that was exposed neonatally to diethylstilbestrol (DES). Control animals, which have been ovarectomized but not exposed to DES, do not express EGF unless they are treated with estrogen.

NIEHS REORGANIZES
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Tracking estrogen's alternates

Working with McLachlan, who heads the Laboratory of Reproductive and Developmental Toxicology, Karen Nelson and Diane Ignar-Trowbridge are studying the cross talk between estrogen, a steroid hormone, and two peptide hormones—epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-∝). Estrogen and other steroid hormones interact with specific receptors in the cell, triggering transcription of specialized suites of genes. The peptide growth factors operate only at the cell membrane, but several types of experiments by Nelson and Ignar-Trowbridge suggest that the extracellular peptides may act as stand-ins or partners for estrogen.

As with Putney's intracellular calcium studies, McLachlan's lab's work had its inception in a toxicological catastrophe—reproductive-tract cancers in the daughters of women who had taken the synthetic steroid, diethylstilbestrol (DES), during pregnancy. Searching for basic mechanisms to explain DES' action, the researchers began looking at the role of hormonal interactions in the reproductive tract of fetal and neonatal mice. Nelson was recruited to NIEHS five years ago to help fill in the background—to study how normal estrogen affects growth of uterine and vaginal tissue in mice.

What has emerged from Nelson's studies and collaborations are good animal models for studying the growth- and tumor-promoting interactions of hormones, and an increasingly complicated picture of estrogen's interactions with TGF-∝, EGF, and other peptide hormones. Nelson finds that when female mice are 17 to 19 days old—before their ovaries have begun producing estrogen—uterine and vaginal epithelial and stromal cells "are exquisitely sensitive to the growth-inducing effects of estrogen." Within 16 hours of getting their first exposure to estrogen, an astonishing 80% of the epithelial cells move into a grand, synchronous cell division. Estrogen induction of peptide growth factors, such as TGF-∝, plays an important role in mediating this profuse growth.

Younger mice—one to five days old—lack estrogen receptors, but Nelson finds that she can still elicit an estrogen-like growth response in the reproductive-tract cells by treating the mice with EGF or TGF-∝. "TGF-∝ is mimicking estrogen," says Nelson. In a collaboration that defies the Maryland-North Carolina divide, Nelson is working with NCI's Bethesda-based Glenn Merlino, who has produced mice with a TGF-∝ transgene. In these mice, TGF-∝ is constantly produced. Experiments with the transgenics suggest that overexpression of TGF-∝ makes the mice more susceptible to estrogen-induced neoplastic and pre-neoplastic lesions of the reproductive tract. The TGF-∝ transgenic mice develop DES-induced tumors much more quickly than do other mice. Nelson hopes that further study of the interaction of estrogen and peptide hormones in vivo and in vitro and the identification of other important genes involved in the growth of reproductive tissue will lead to the development of markers that will aid in the diagnosis and therapy of endometrial, vaginal, and cervical cancers, fibroids, and endometriosis.

Reexamining the cell cycle

About a year ago, Robert Maronpot made a discovery that has enmeshed him simultaneously in basic research on the cell cycle and in applied, clinical research on chemicals that may induce cancer simply by increasing the number of times a cell divides.

Maronpot, who is chief of the Laboratory of Experimental Pathology, discovered that in animal cells, an antibody called anti-PCNA binds to the DNA polymerase-Δ complex—and possibly an assortment of other cyclins, molecules thought to play a central role in governing cell division. Previously, scientists had seen the binding in yeast, but Maronpot found that it also occurred in animal cells—from fish to rodents to humans—including cells in fixed tissue.

The binding has a great practical result: it makes anti-PCNA a good indicator of stages of the cell cycle that are otherwise not distinguishable to the eye. Cells in S phase concentrate anti-PCNA densely in the nucleus, whereas cells in G2 bind the antibody in both the nucleus and cytoplasm. Cells in G1 show light binding of the antibody in the nucleus only. "This gives us a win-

dow into the cell cycle," says Maronpot. "We can learn so much more than with the standard labeling that only shows the S phase."

Practically, the result means that tissue that has been collected and preserved years ago can be checked for signs of over-active, precancerous cell division. The technique is rapid and robust. "Everybody can get this to work," says Maronpot. The difficult part is the time it takes to count the variously stained cells after the procedure.

Maronpot is using the anti-PCNA technique to develop a method for assessing the carcinogenic potential of non-genotoxic agents. "More and more, the people who make new chemicals have stopped development of genotoxic agents in favor of non-genotoxic chemicals, such as chlorinated ethylene, carbon tetrachloride," and chemicals in the current generation of pesticides. Studies show that despite their lack of direct genetic effects, these agents nevertheless increase tumors in animals by stimulating cell division. "Does that mechanism occur in humans, or is it unique to the mouse and rat?" asks Maronpot. By studying so-called archival tissue taken over many years from workers exposed to the chemicals, Maronpot hopes to get an answer.

On a more basic level, Maronpot is using anti-PCNA to understand the operation of DNA polymerase-Δ and associated cyclins during the course of cell division. "This is still in the experimental stages. We are trying to understand better and better how [anti-PCNA] works," Maronpot says. Some evidence suggests that proteins in tumor cells may interact differently with PCNA than do proteins in noncancerous cells. "We're on a rapid learning curve as to what these proteins do. The technique is telling us all kinds of things. We just have to be smart enough to figure out what they are."

Delving into Drosophila's dual-function repair and recombination enzyme

Miriam Sander of the NIEHS Laboratory of Genetics has found a very curious, multitalented enzyme in *Drosophila*. Sander discovered the gene *Rrp1* in 1989. Since then, as she studies how the enzyme works, it just keeps looking more and more unusual. Rrp1 has a dual function: it both repairs DNA and catalyzes recombination.

Comparison of *Rrp1*'s gene sequence put it in a distinct family of five repair enzymes. The family includes proteins in human, mouse, and cow cells, and exonucleases in *Escherichia coli* and *Streptococcus pneumoniae*. These enzymes snip out sections of DNA that have been damaged by free radicals or alkylation. Insertion of *Rrp1* into *E. coli* has demonstrated that, like the other members of its family, Rrp1 also catalyzes DNA repair.

But a distinctive part of Rrp1—the 420 N-terminal amino acids—are what really make the enzyme interesting. Sander finds that this section of the enzyme catalyzes recombination in vitro —the binding of single strands of DNA, strand transfer, and renaturation of the helix. "Those enzymatic functions are not known to be associated with any other members of the family," says Sander. To date, no genes with a sequence homology to the N-terminal region of Rrp1 have been identified.

Working with Shu-Mei Huang and Liya Gu, Sander is now looking at Rrp1 in vivo—confirming its functions in the fruit fly and observing its levels of expression over the course of *Drosophila's* development. "We may be able to identify separate functions associated with expression at different times in development. This may have a parallel in other, more complex, multicellular organisms, but it is much easier to address in a system like *Drosophila*," Sander says. Sander is also attempting to boost concentrations of Rrp1 above those normally found in *Drosophila* to see if this gives the flies increased resistance to oxidative and alkylation damage.

Sander is guessing that *Rrp1* was formed by a fortuitous fusion of repair and recombination genes some time during the course of evolution. "When there is DNA damage, it can certainly be of value to the organism to use an undamaged strand of DNA as a source of information for the repair," says Sander. Although she has not yet confirmed that Rrp1's repair and recombination functions operate contemporaneously in vivo, it would be reasonable to suspect that they work together to excise damaged DNA and replace it through recombination with DNA from an undamaged homologous strand.

Sander, who came to NIEHS from MIT in 1990, summarizes the feelings of many researchers at Research Triangle Park: "It's a very easy place to get science done." ■

The medical-pathological waste (MPW) boxes have taken on a new look and are safer and easier to use. Replacing the old brown MPW boxes are new, self-assembling boxes developed by the NIH Division of Safety (DS). Unlike the old ones, the new MPW boxes will not require tape. A ridged, self-assembling base and three interlocking tabs make assembling and closing the new boxes easier. Simple instructions are printed on each box.

The new MPW box is conveniently packaged in kits that contain five boxes, 10 bags and 12 plastic draw-tie closures. This eliminates the need to purchase seperate boxes, bags, and tape. The kits will also make it simpler to meet a new requirement to double-bag MPW to prevent leakage during transport and disposal.

Look for the new MPW box in the NIH Stock Catalog and in the Building 35 Self-Service Store. ■

Hibernation— A Novel Approach to Stroke Therapy?

by Seema Kumar

Think of stroke as a vast riot raging in the brain, suggest John Hallenbeck and Kai Frerichs of NINDS. By that analogy, current stroke therapies are doing less than the Los Angeles authorities did in the 1992 L.A. riots — barely containing the fires, while looting, robbing, and assault continue unabated. Hallenbeck, Frerichs, Michael Brenner, and colleagues at the NINDS Stroke Branch point out that many different mediators, including excitotoxins, calcium, free radicals, and leukocytes, have been implicated in stroke, and therapies targeted at one or a few of these mediators have shown only modest clinical success. Because these agitators may all combine to cause brain damage through a complex network of minor destruction with no apparent order or direction, a better approach, according to the researchers, might be to calm the riot globally rather than to control individual elements. Taking this approach, the NINDS scientists are studying mammalian hibernation — a natural state in which adaptive changes allow the brain to completely quell havoc from stroke-like conditions.

"During hibernation, animals' metabolic rates drop to extremely low levels, their heart rates drop, followed by a gradual decline in body temperature, and blood flow to the brain decreases to levels below the ischemic thresholds" that occur when blood flow is cut off during stroke, says Frerichs. "Levels of oxygen use during this stage are low, and glucose metabolism is greatly depressed." Yet after several weeks in this state — which would be lethal after a few hours in non-hibernating animals — hibernating animals return to normal activity without any evidence of brain damage. The researchers say low body temperature, or hypothermia, alone does not account for the protection because artificially inducing the low body temperatures found during hibernation is actually harmful and can only be survived for a short time. Hibernation, the researchers conclude, entails more than a passive loss of temperature. They see hibernation as a regulated condition during which the animal actively shuts off metabolism and production of heat and makes adaptive changes before allowing the ambient temperature of the environment to gradually and safely lower body temperature.

Hibernation of the thirteen-lined ground squirrel may help NINDS scientists find a way to protect brain tissue from severe damage after a stroke.

"There is something extremely protective and controlled about hibernation that is different from hypothermia," that has been tried as a treatment for stroke, explains Hallenbeck. "The mechanism involved in hibernation has pleiotropic actions and makes the necessary modifications in all systems and cells to preadapt the brain to reduced blood flow and oxygen availability." Identifying the factors that regulate hibernation and the mechanism that protects hibernating animals from ischemic damage might enable researchers to prevent the damage that follows cerebral ischemia in other mammals — including human stroke patients.. Frerichs says that preliminary evidence suggests that humoral factors may be involved in the regulation of hibernation and that the changes may ultimately be regulated in the area of the brain that controls temperature and general autonomic homeostasis — the hypothalamus.

Hallenbeck, Frerichs, and colleagues began to study hibernation in 1991 when they realized that a nonconventional, global approach might be needed in the search for new stroke therapies. Currently, therapy for stroke in many hospitals is still supportive — keeping the patients comfortable, hydrated and infection-free. However, after scientists learned that brain cells do not immediately die after stroke and that there might be a short window of time to revive dying cells in the ischemic penumbra — a shell of tissue that surrounds the blood-starved core of brain tissue at the epicenter of a stroke — approaches to therapy began to focus on ways to prevent cell death in this zone. However, these approaches, including excitotoxin blockers, calcium-channel blockers, antioxidants and hypothermia, generally assume that one or a few dominant factors, out of many that have been implicated, mediate most of the early and delayed damage.

"If there would turn out to be in stroke something equivalent to insulin in diabetes, then this approach could do well in therapy for stroke," says Hallenbeck. "But studying the basis of stroke is, to paraphrase NIMH researcher Louis Sokoloff's concept, like trying to study the chemistry of a compost heap — what goes wrong in stroke seems to be a mishmash of numerous factors, including platelet adhesion and aggregation, endothelium activation, leukocyte accumulation, excitotoxic neurotransmitters, calcium, and cytokines."

If it is true that several factors cause damage in the penumbra, "and chances are that it is," says Frerichs, "it would seriously degrade the likelihood of success of conventional stroke-therapy approaches." Hallenbeck and Frerichs therefore decided to take an alternate approach. "Instead of trying to interfere with mediators of ischemic damage, we thought it may be useful to study mechanisms of natural tolerance to cerebral ischemia" by analyzing hibernation in thirteen-lined ground squirrels *(Citellus tridecemlineatus)*. Although the physiology of hibernation has been studied before, cerebral blood flow (CBF) during hibernation had not been measured. Scientists knew that heart rates drop during hibernation, but they had not made the connection to stroke.

In the past year and a half, the NINDS researchers studied brain changes in hibernating squirrels by implanting radiotransmitters under the squirrels' skins and placing the animals

in a hibernaculum — an environmental chamber in constant darkness at 5 °C. The telemetry devices allow continuous recording of body temperature and heart rate and a continuous electroencephalogram (EEG). The researchers also measured local CBF, glucose metabolism, and protein synthesis by using autoradiographic methods developed by collaborator Sokoloff's lab at NIMH with help from NIMH researchers Charles Kennedy, Gerald Dienel, and Carolyn Beebe Smith.

The NINDS team found that when the animals entered into hibernation, their heart rate dropped from about 350-400 bpm to 20 bpm within minutes. This was followed by a gradual decline in body temperature from about 35 °C to about 6 °C within two to four hours. The animals' blood pressure, which normally averages about 90-100 mm Hg, dropped to shock levels of 35-30 mm Hg instead of increasing or staying normal to compensate for the low pulse rate. With almost no pressure or pumping, the CBF dropped drastically, to levels normally associated with irreversible ischemic damage. Further, in a two-minute autoradiographic study of CBF, investigators found regions that were not being perfused at all. "So technically, at that time, the animal should have died," says Frerichs.

Instead, the changes reversed fully when the animals aroused from hibernation, and the animals showed no sign of brain damage. In ongoing collaborative studies, the researchers are attempting to find differences in proteins and peptides from the brain and plasma of hibernating and active ground squirrels. Janet Joy and Bill Wallace in Carl Merril's laboratory at NIMH are performing two-dimensional gel electrophoresis and in vitro protein-translation studies. Howard Jaffe in Harold Gainer's laboratory is further purifying and sequencing candidate proteins. Further, cDNA libraries and a subtraction library have been developed under Brenner's guidance. The ongoing studies, researchers say, might turn up a protein or peptide that governs entrance into the hibernation state.

In addition, the researchers observed other changes during hibernation that indicated that the animals were avoiding coagulation and inflammation. Platelets and leukocytes seemed to be sequestered in the spleen and the liver during hibernation and were returned to the blood after hibernation. "The decrease in circulating levels of white blood cells may help blunt the initiation of any proinflammatory self-destructive processes," says Hallenbeck. Other experiments showed that conductivity of membranes in hibernating animals is reduced — a step that must be necessary to maintain ion gradients during reduced metabolism. The result of these combined changes enables the animal to avoid edema and maintain excitability of neurons and cardiac cells, Frerichs says. Hallenbeck notes that it would be valuable to know what controls the orchestrated changes during hibernation, and how the controls operate.

FACTORS FROM THE BRAIN OR FROM THE BLOOD THAT PLAY A ROLE IN REGULATING THE TRANSITION TO HIBERNATION COULD BE USEFUL IN A STATE THAT HAS LOST HOMEOSTATIC CONTROL, LIKE STROKE

"We hope to mimic the mechanisms of tolerance these animals exhibit during hibernation. It may be possible to isolate factors from the brain or from the blood that play a role in regulating the transition to hibernation. Those factors could be useful in a state that has lost homeostatic control, like stroke," says Frerichs.

Temperature is not the regulatory factor, says Hallenbeck. Measurements of oxygen consumption and other indexes of metabolic rate show that during entrance into or emergence from hibernation, changes in body temperature lag behind the decline or rise in metabolic rates. "The temperature is not slowing down the metabolism, but the metabolism is being actively slowed down, and this allows the reduction in temperature," says Hallenbeck. The controlled drop in temperature distinguishes hibernation from induced hypothermia. Although moderate induced hypothermia may be beneficial to stroke victims, the extreme hypothermia of hibernation is detrimental and ultimately lethal when artificially induced because of the dissipation of ion gradients and the unregulated and uncoordinated depression of metabolic and cellular functions. Hibernating animals have found a way to coordinate and regulate these functions to accommodate the low temperature, say the researchers.

In the hibernaculum, animals decide on their own when to go into hibernation; the researchers have no way of forcing the animals into this state. The researchers believe that hibernation may be partly voluntary and partly induced by some annual cue. "Hibernation occurs mostly in wintertime; however, under simulated laboratory conditions, it also occurs during the summertime," explains Frerichs. "This demonstrates that the environmental factor is important, but that the animal can make successful use of hibernation at any time of the year—which means that it does not require lengthy seasonal preparations to hibernate." Although an animal can hibernate at any time throughout the year, it has a predilection for winter months and will attempt to hibernate then even at room temperature, with abundant food and water. "This would support the presence of an annual rhythmmaker that tells the animals it is time to hibernate; this, however, does not seem to be a crucial factor for the animal's ability to hibernate," says Frerichs.

The next step for the NINDS team is to demonstrate that the control mechanism of hibernation could be protective by comparing levels of damage from induced stroke in hibernating animals and non-hibernating controls. Here, the researchers seem to have hit a potential roadblock. "The problem," says Frerichs, "is that to compare ischemia in hibernating and non-hibernating animals, we would have to cool down the control artificially,"—a process that the researchers already know induces damage. "We have to find other experiments that circumvent this problem to test the idea that the hibernating brain is tolerant, even though just observing the state itself offers a simple test," says Frerichs. ■

NIH Researchers Find Genetic Treasures in Egypt

by Elia Ben-Ari

Last fall, three NIH researchers — a geneticist, a molecular biologist, and a clinician — tapped into one of the largest genetic databases in the world. The database, assembled by their Egyptian collaborator, Nemat Hashem, at Ain Shams University in Cairo, contains epidemiologic and demographic information on thousands of individuals with genetic disorders. Traveling to Cairo, Sherri Bale and John Compton of NIAMS and John DiGiovanna of NCI launched a unique collaborative study to determine the genetic basis of one or more rare, autosomal-recessive forms of a group of acquired and inherited scaling skin disorders known as the ichthyoses.

"The great utility of Hashem's population is due to the high rate of consanguineous marriages [intermarriage] in Egypt, which results in an increased prevalence of autosomal-recessive disease if the genes for that disease are in the population," says Bale. Linkage analysis using inbred pedigrees is advantageous, she says, because "it has been shown that a single affected child from an inbred marriage provides as much information as three affected children from an outbred marriage."

"Because of the consanguinity, we have found a clinical resource that doesn't exist anywhere else," says DiGiovanna. He says it would not have been possible to do linkage analysis on the recessive skin diseases "without having the appropriately structured families, and they just don't seem to exist here [in the United States] because of custom." To do linkage analysis with outbred pedigrees, the researchers would have had to find a large number of families with at least two affected siblings. However finding such families in the United States was difficult because autosomal-recessive ichthyoses are rare.

"Discovering the wealth of information in the Egyptian database was like finding a hidden treasure," says DiGiovanna. The researchers were able to get DNA samples from 48 people from 16 families. This material will be used for genetic-linkage analysis and molecular-genetic studies to identify the disease-causing gene or genes.

Among the ichthyoses, which are characterized by hypertrophy of the cornified layer of the skin, are two severe autosomal-recessive forms, lamellar ichthyosis and congenital ichthyosiform erythroderma (CIE). Because these disorders are genetically and clinically heterogeneous, it is sometimes difficult to distinguish one from another. In this regard, the many affected individuals in Egypt provide another benefit to researchers. "Because we now have the opportunity to examine a large number of patients with these diseases, we're hoping to be able to better classify them," explains DiGiovanna.

Creating a computerized database has been part of Hashem's mission to help her patients, who suffer from a wide variety of inherited disorders. Over the past 25 years, Hashem, director of the Medical Genetics Center at Ain Shams, has created a database of over 5,000 pedigrees. She can easily search this database to obtain a list of patients or families with a specific disorder. In this manner, she located several families with congenital ichthyosis and asked them to come to her clinic during the week of the NIH team's visit.

Sherri Bale (left), Nemat Hasbem (right) and Egyptian colleagues

"We spent 15 hours a day for the first three days examining patients and collecting blood samples," Compton says. "We barely saw the daylight." Compton set up a laboratory to isolate DNA and white blood cells (for establishing permanent cell lines) from each sample. Performing these relatively simple procedures was a challenge at the genetics clinic, which has limited laboratory facilities and supplies (although it does have a still-functioning 50-year-old clinical centrifuge!). Nevertheless, says Bale, "there was nothing we needed that our Egyptian collaborators didn't get for us."

Despite the challenges, the U.S. researchers returned to NIH with many viable samples. They plan to return to Egypt in June to obtain samples from people they missed the first time around, as well as from additional families, and to revisit some patients to answer clinical questions that remain. DiGiovanna notes that because of the clinical and genetic heterogeneity of autosomal-recessive ichthyoses and because this study involves multiple families, rather than one large family, patients must be carefully examined and their disease type properly classified. Otherwise, this type of genetic study won't work.

To identify the genetic basis for the autosomal-recessive ichthyoses, the researchers will attempt an approach similar to the one that they and their colleagues at NIAMS and NCI used to show that a specific mutation in the keratin 1 gene can cause an autosomal-dominant form of ichthyosis, epidermolytic hyperkeratosis (EHK). In the studies of EHK, the researchers used genetic-linkage analysis to identify the candidate disease gene, followed by polymerase chain reaction (PCR) amplification and sequencing of genomic DNAs from affected and unaffected family members to identify the putative disease-causing mutation. In the study of the autosomal-recessive ichthyoses, the researchers will use a method of linkage analysis specific for inbred pedigrees.

The researchers hope that identifying the genetic basis of these diseases will enable prenatal diagnosis and, ultimately, improved treatments, perhaps via gene therapy. In addition, says DiGiovanna, "understanding the underlying abnormalities in these diseases will help us [to] understand how normal skin functions and to understand other skin diseases of abnormal proliferation and differentiation that are more widespread — for example, psoriasis."

Hashem has information on families and patients with a wide variety of genetic diseases in her database, including genetic skin disorders; dwarfism syndromes; hematopoietic errors, especially the thalassemias; neurologic disorders, including microcephaly syndromes, familial mental retardation, and seizures; inborn errors of metabolism, including phenylketonuria and glycogen-storage diseases; neurofibro-

continued on page 17.

McKAY ET AL.: PROBING THE PROMISE OF STEM CELLS

by Celia Hooper

Ron McKay and an international crew of biologists, now moving into their quarters on the third floor of Building 36, are launching more than a new lab at NIH—they are launching a new approach to studying the development and differentiation of cells in the mammalian brain.

Ron McKay

"The main reason I got this job is because the work we have done here [at his previous position at the Massachusetts Institute of Technology in Cambridge] is interesting in its own right, but also because it provides a very general approach to the molecular biology of the mammalian brain," says McKay. The overarching theme of the work McKay and colleagues will be bringing to the NINDS Laboratory of Molecular Biology is transplantation of neuronal stem cells—multipotential cells from embryonic brain tissue. McKay and his co-workers discovered a protein marker, called nestin, that distinguishes undifferentiated neuronal precursors from cells whose fate has been set.

The researchers used genetic-engineering techniques to insert into rat neuronal epithelial stem cells an enhancer that drives expression of reporter genes, making the stem cells easy to identify and manipulate. They also added a gene that gives the cells temperature-conditional multipotentiality. This allows researchers to grow and manipulate the undifferentiated cells at cool temperatures in laboratory culture. The cells then differentiate at body temperature, when transplanted into the brain, for example. Transplant experiments have shown that the particular cellular environment into which the cells are placed determines their fate, and that transplanted stem cells are functionally integrated. Stem cells that McKay and colleagues transplanted and allowed to grow in the hippocampus of the brains of newborn rats expressed proteins that are uniquely hippocampal, and the transplanted cells responded to glutamate agonists in exactly the way hippocampal cells would react to this signal. Stem cells transplanted to the cerebellum showed no hippocampal traits.

At birth, most neuronal cells in the mammalian brain have lost their multipotentiality and ability to divide, so the still-unfated embryonic "stem cells present a very interesting problem at the molecular level," says McKay. "And the ability to manipulate them promises a new molecular and genetic technology." McKay has great hopes for this technology. He wants to use it to understand fundamental biological problems—such as the molecular complexity of the mammalian brain—and to develop transplantation and other procedures that might be useful in treating degenerative brain diseases, such as Parkinson's, or brain tumors. The fact that stem cells "know what to do"—to differentiate into the correct cells for the environment they find themselves in—suggests to McKay that it may some day be possible to "rebuild animals from cultured stem cells. That is quite a lot of fun as an idea," he says, likening cultured stem cells to an endless box of Lego parts that could be used to rebuild deteriorating tissue throughout the body.

McKay says he is excited about the possibilities of tapping into the expertise of other NIH scientists, core facilities, and sustained, broad support for research. "NIH offers the possibility of doing really big projects—taking a broad approach to problems—that is not possible in a university," says McKay, who will be chief of the NINDS lab. Coming from MIT with McKay will be postdoctoral scientists Tim Hayes, Diana Collazo, Carlos Vicario, Richard Josephson, Martha Marvin, and Alice Brown. Uwe Maskos will be coming from the University of Oxford; C. Oliver Brüstle, from the University of Zurich; Luis Manuel Delgado-Rivera, from the University of Oviedo in Spain; Tom Hazel from the University of Illinois at Champaign-Urbana; and Shigeo Okabe and Yasushi Maeda will be coming from the University of Tokyo. ■

IS AN ACCIDENT BREWING IN YOUR LAB?

One day last December, a chain reaction of potentially life-threatening lab accidents was touched off in an NIH laboratory.

It began when a researcher poured trifluoroacetic acid (TFA) into a waste container that held ethanolamine in methanol and, possibly, methylene chloride and dimethylformamide. The violent exothermic reaction that followed splashed the researcher with the mixture of chemicals. Startled, he dropped the container holding the remaining TFA. A nearby bottle of formic acid also fell and broke on the floor. As he moved to get away, the researcher slipped and fell into the caustic puddle of formic acid and TFA. The strong acids burned through his clothing. The researcher should have been able to use a safety shower in the room — but it was not working. A co-worker called the 116 emergency number and helped the scientist to a functional safety shower in another nearby room. The scientist, who was seriously burned in the accident, was transported to a treatment facility and has since recovered. Ironically, had the shower closest to the accident scene worked, the scientist might have been electrocuted: Beneath that shower was a tangle of power strips and electrical cords.

Does you lab look like this? If it does, an accident may be brewing in your lab.

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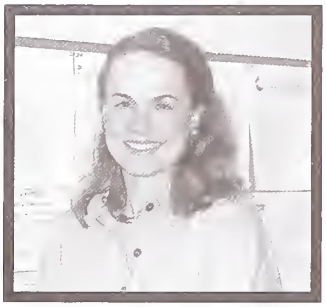
RECENTLY TENURED

by Seema Kumar

Scientists tenured December 1992 to Date

Jacqueline N. Crawley, NIMH
Alan P. Wolffe, NICHD
Patrick C. Elwood, NCI
Kirk R. Gustafson, NCI
Andrew C. Larner, CBER/FDA
Peter L. Nara, NCI
Robert E. Walker, NIAID
Michael J. Hamilton, NCI
Paul E. Klotmen, NIDR
Hau Su, NIAID
John Oshea, NCI
Marsha Merrill, NINDS
Frederic Kaye, NCI
Philip M. Murphy, NIAID
Arun K. Seth, NCI
Michael Steller, NCI
Michael A. Norcross, CBER/FDA

Susan Bates, M.D.



At NCI's Medicine Branch, Susan Bates and her colleagues are exploring novel ways to reverse multiple-drug resistance in human cancer cells by modifying the function of P-glycoprotein (Pgp), a protein thought to mediate drug resistance by pumping chemotherapeutic drugs out of cancer cells, thus decreasing the amount of drug retained inside the cell.

"Our hope is to define the role of P-glycoprotein in patients, to find a way to overcome it, and to explore other pathways of drug resistance," says Bates.

In one set of studies, Bates and her colleagues are examining Pgp's expression in biopsies of patients' tumors to determine its relevance to cancer therapy. Using RNA in situ hybridization, immunohistochemistry, Northern analysis, or the polymerase chain reaction, the researchers determined the levels of Pgp expression in tumor samples from breast cancer and lymphoma patients. They found that half of the breast cancer biopsies express the protein at moderate levels and that the lymphoma samples express the protein at low levels, but this often increases after treatment failure, indicating that Pgp expression is indeed related to drug resistance.

In a second set of studies, Bates and her colleagues are trying new ways to reverse drug resistance caused by Pgp. Previous attempts to reverse drug resistance in vitro have used Pgp antagonists that block drug efflux by competing for binding to Pgp. However, the concentrations of antagonists that can be attained in patients are often too low to boost drug accumulation in the cell. Bates and her colleagues are now studying the mechanisms of reversal of drug resistance by using new, less-toxic Pgp antagonists. Clinical studies with R-verapamil and PSC833 (a cyclosporine analog) are already under way.

In a second approach, Bates and her colleagues are using differentiating agents and protein kinase inhibitors to impair Pgp function or to decrease its expression. Current studies suggest that increased Pgp phosphorylation, which increases the range and effectiveness of Pgp as a drug-efflux pump, most likely occurs when the cancer cells become malignant. Because differentiating agents and kinase inhibitors can decrease phosphorylation of Pgp, and thereby impair Pgp's pumping capability, this work may be the basis of a novel therapeutic strategy for reversing drug resistance. In a third approach, Bates and her coworkers are using growth-factor antagonists to reverse multidrug resistance. Studies have demonstrated that breast cancer cells that have been selected for drug resistance show altered growth-factor-receptor expression, again suggesting a target for treatment. Researchers plan to determine whether the observed alterations in growth-factor biochemical pathways contribute to drug resistance. The laboratory plans to focus its future research on unraveling the underlying mechanisms.

Bates received her M.D. in 1978 from the University of Arkansas for Medical Sciences in Little Rock. She joined NCI in 1981 and since 1982 has been working in the Medicine Branch. She was recently selected to be one of NCI's women scientist advisors — part of a recently appointed group of scientists who will work to improve the status of women scientists at NIH by increasing their visibility and providing mentorship.

Kathleen Kelly, Ph.D.

Kathleen Kelly works in the Laboratory of Pathology, Division of Cancer Biology, Diagnosis, and Centers (DCBDC) at NCI, where, during the past few years, she has been studying how lymphocytes are activated. When a resting T cell in the peripheral blood is stimulated by an antigen or a mitogen, a genetic program inside the T cell is activated, causing the cell to proliferate and specialize—expressing characteristic proteins appropriate to differentiated function. Kelly and her colleagues have cloned several genes encoding proteins that appear to function as pleiotropic regulators of the signal governing this differentiation.

"A vast array of functions are turned on when a lymphocyte is stimulated to proliferate and function," says Kelly. "The presence or absense of certain key proteins provides us with a glimpse of some of the processes that are regulated in the cell."

In particular, Kelly and her colleagues are analyzing the structure and function of a novel family of phosphotyrosine phosphatases (PTPases) that are located in the nucleus of the cell and that may play a role in regulating transcription. Last year, they cloned the mitogen-induced gene (PAC-1) that encodes one of these novel PTPases in human T cells. PAC-1 shares sequence homology with a phosphatase induced by mitogens or heat shock in fibroblasts, a yeast phosphatase (YVH1), and a tyrosine-threonine phosphatase (VH1) in the vaccinia virus. This PTPase family is structurally distinct from several previously cloned PTPases exemplified by CDC45.

Kelly and her colleagues also are characterizing a cell-

This regular feature will list the names and affiliations of recently tenured scientists. A brief description of scientists' works will be featured, with their approval.

surface receptor that contains seven membrane-spanning regions characteristic of signal-transducing receptors that couple to G proteins. The receptor also contains repetitive sections at the amino terminus that resemble repeats in epidermal growth factor. These repeats are thought to be involved in protein-protein interactions and are often found on receptors and their ligands. The surface protein that Kelly found may play a role in T-lymphocyte adhesion and, possibly, in the linked recognition of a secreted ligand. This cell-surface receptor is induced by activation, and it couples with a G protein.

"This receptor has several unique structural aspects that make it a curiosity from an evolutionary point of view," says Kelly. "Our immediate goal, of course, is to identify the ligand or ligands of this activation-induced receptor that will directly determine the immune and/or proliferation-related function. Fortunately, with molecular-biological techniques, there are some rather straightforward approaches to begin testing potential ligands."

Finally, Kelly and her colleagues are studying a novel GTP-binding protein that is distantly related to the ras family of proteins and is located on the inner face of the plasma membrane, positioned for a signal-transducing function following an initial stimulus. A gene encoding this protein — GEM — was cloned from mitogen-induced human peripheral-blood T cells. Although GEM displays extensive homology to ras-family proteins, it clearly defines a new structural class of GTP-binding proteins. GEM is bigger (35kDa) with additional amino acids on the C and N terminals. GEM protein is transiently expressed in activated fibroblasts and human peripheral blood T cells, and the message is detectable in activated human B cells and monocytes. The protein is phosphorylated by tyrosine, indicating that it is a signal-transducing protein. Kelly and her colleagues hypothesize that GEM may be responsible for mediating signal transduction at the plasma membrane during activation in cells of multiple lineages. This is the first report of a mitogenically inducible GTP-binding protein occurring in hematopoietic cells.

"The structural characterisitics of GEM and its expression pattern really suggest a signal-transduction role in mid-G1 [phase of the cell cycle], and this is a relatively uninvestigated area," says Kelly. "Although it is clear that cells are receiving extracellular cues and feedback throughout the cell cycle, most of the attention has been focused on the signal-transducing events that initiate the G0-to-G1 transition." Kelly adds that the signalling pathways that operate during G1 are important checkpoints for determining whether a cell commits to DNA synthesis.

Kelly received her Ph.D. in microbiology from the University of California at Irvine, working in James Watson's laboratory. She first joined NIH in 1980 as a junior staff fellow in Philip Leder's laboratory at NICHD. She left to work at the Department of Genetics, Harvard Medical School, Boston, for two years and came back to NCI in 1984.

Glenn Merlino, Ph.D.

Over the last six years, Glenn Merlino of the Laboratory of Molecular Biology, DCBDC, NCI, has independently established and directed a highly successful transgenic-mouse facility on the NIH campus. Transgenic technology, in which foreign DNA is stably introduced into the mammalian germ line, has provided a powerful means to study fundamental biological questions. Merlino's group uses this technology to investigate the role of growth factors, receptors, and oncogenes in the initiation and development of cancer, and to establish animal models for the study of pathogenesis of human disease.

Overexpression of the epidermal-growth-factor (EGF) receptor and its ligands (transforming growth factor-∝, or TGF-∝, and EGF) can transform cells in culture. To determine the in vivo consequences of perturbing the EGF-receptor signal-transduction pathway, Merlino and his group made transgenic mice by using foreign TGF-∝ and EGF receptor genes. In one series of experiments, a construct bearing the human TGF-∝ cDNA was injected into one-cell embryos. Mice bearing this transgene expressed the human TGF-∝ RNA and protein in a majority of tissues, including the liver, pancreas, stomach, and breast. Elevated levels of TGF-∝ were detected in the blood and urine of transgenic mice, paralleling the TGF-∝ elevations that have been found in some cancer patients. TGF-∝ transgenic mice progressively developed several dramatic lesions. Merlino believes the animals will serve as useful models for some common human cancers. Merlino and co-worker Hitoshi Takagi have discovered that TGF-∝ combines with diverse oncogenic environmental and genetic agents in producing these lesions.

In another series of experiments, Merlino and his colleagues made transgenic mice bearing the human EGF-receptor cDNA. In one line of mice, the scientists detected pronounced expression of the human EGF receptor only in the testis. Homozygous male mice carried genetic aberrations that resulted in sterility due to sperm malformation and paralysis. Similar malformations have been observed in the sperm of sterile men. Merlino says that this unique line of transgenic mice could provide a model for studying male infertility.

More recently, Merlino's efforts have focused on the suspected role of TGF-ß1 in the maturation and function of the mammary gland. Important regulators of development and cell growth, the versatile TGF-ß superfamily of cytokines both inhibits and stimulates cell growth, and its repertoire of activities includes promotion of wound healing and bone reformation. Scientists have long been interested in TGF-ß because its malfunction has been implicated in some cancers. Drugs that mimic its growth-inhibitory effects may be useful in cancer therapy. To investigate the in vivo effects of TGF-ß overexression on mammary-gland function, Chamelli Jhappan in Merlino's lab made transgenic mice that expressed TGF-ß1 in the mammary gland of pregnant mice. These animals were unable to lactate because their breasts did not develop and their milk production was suppressed, suggesting that TGF-ß1 has a role, in vivo, in regulating the development and function of the mammary gland. Merlino's group is now mating TGF-ß1-expressing mice with TGF-∝ expressing mice to create female mice that express both TGF-ß1 and TGF-∝. The mice will be used to determine whether TGF-ß1 can suppress the development of breast cancer induced by excess TGF-∝.

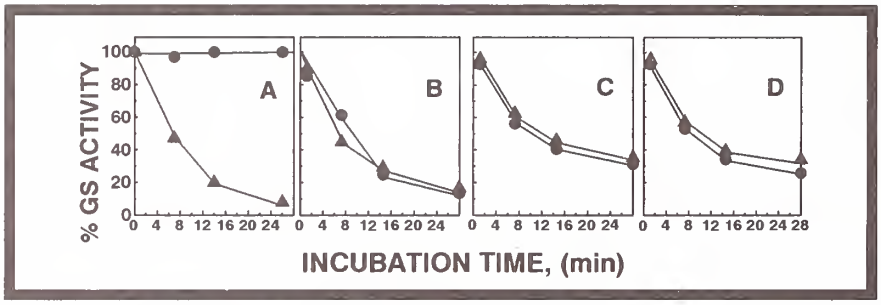
continued on page 17.

Thiol-Specific Antioxidant Enzyme

by Sue Goo Rhee and Earl R. Stadtman, Laboratory of Biochemistry, NHLBI

Our laboratory is investigating a novel, thiol-specific antioxidant enzyme (TSA) that protects proteins and lipids from oxidative damage by metal-catalyzed oxidation (MCO) systems. Collaborating with Sue Goo Rhee and Earl Stadtman on this research are Kanghwa Kim, Il-Han Kim, Ho Zoon Chae, Moon Bin Yim, and Luis Netto, also in NHLBI's Laboratory of Biochemistry.

It has become increasingly evident that a number of highly reactive cytotoxic oxygen species (e.g., H_2O_2, $O_2^{\bullet -}$, $HO^{\bullet}$, and singlet oxygen) are produced as inevitable side products of molecular oxygen during normal metabolic electron-transport processes. The interaction of these active oxygen species with lipids, nucleic acids, and proteins is likely to be implicated in the etiology or manifestation of several pathological processes, including atherosclerosis, diabetes, aging, muscular dystrophy, ischemia-reperfusion injury, the formation of cataracts, cancer, various inflammatory disorders, and, most recently, amyotrophic lateral sclerosis. In particular, Fe^{2+} and Cu^{2+} have catalytic roles in these processes (for review, see Stadtman and Oliver 1981). To guard against this oxygen toxicity, organisms have developed a battery of anti-oxidant defenses. These include the synthesis of enzymes such as catalase, glutathione peroxidase, superoxide dismutase, and glutathione-S-transferase that catalyze destruction of some active oxygen species. Additional defense against oxygen radicals is provided by small molecules—such as uric acid, ∝-tocopherol, ascorbate, carotenoids, and glutathione—that act as scavengers of free radicals. As summarized here, studies in the Laboratory of Biochemistry at NHLBI have led to the discovery of another antioxidant enzyme that, in the presence of sulfhydryl compounds, can protect proteins and lipids from oxidative damage by a variety of active oxygen-generating systems. This protective effect of TSA could play an important role in oxygen-radical-mediated disease and deterioration of tissues.



Effect of TSA on inactivation of glutamine synthetase by various metal-catalyzed oxidation systems. Mixtures contained 10 µg yeast glutamine synthetase (GS) and either TSA (▲) or variable amounts (0.18-0.4 mg) of TSA (•), and MCO systems comprising (A) dithiothreitol (DTT), Fe(III), and O2; (B) ascorbate, Fe(III), and O2; (C) xanthine oxidase, hypoxanthine, Fe(III), and O2; (D) NADH oxidase, NADH, Fe(III), and O2. See Kim et al. (1988) for details.

TSA Is a Physiologically Important Antioxidant

In the course of our studies on the regulation of glutamine synthetase (GS) activity in *Saccharomyces cerevisiae*, we noted that partially purified preparations of GS and several other enzymes are rapidly inactivated by an MCO system comprising a thiol compound, (RSH), Fe(III), and O_2 (K. Kim et al. 1985). This thiol-mediated inactivation was associated with fragmentation of the polypeptide chain, and in the case of GS, with gradual loss of histidine, tyrosine, and arginine residues and with the oxidation of some amino acid side chains to carbonyl derivatives. The further observation that GS was resistant to oxidation in crude yeast extracts led us to the discovery that crude extracts contained an enzyme that could protect GS and other enzymes from oxidative damage by the RSH-Fe(III)-O_2 MCO system, but not by an MCO system in which the RSH component was replaced by either ascorbate or any one of several enzymatic electron-donor systems (see Fig. 1; K. Kim et al. 1988). Subsequently, a similar enzyme was found to be widely distributed in mammalian tissues, with particularly high concentrations in brain, spleen, pancreas, and lung.

An important physiological role for TSA is indicated by the fact that its intracellular concentration in yeast decreases when cultures are shifted from an anaerobic to an aerobic environment, and increases when cultures are shifted from an aerobic to a hyperaerobic (95% O_2, 5% CO_2) environment (K. Kim et al. 1989). Moreover, supplementation of the culture medium with high concentrations of either one of the three substances — O_2, 2-mercaptoethanol, or Fe(III) — leads to accelerated synthesis of TSA. The induction of TSA synthesis by these three compounds is particularly significant because together they constitute a highly effective MCO system whose ability to damage proteins is inhibited by TSA.

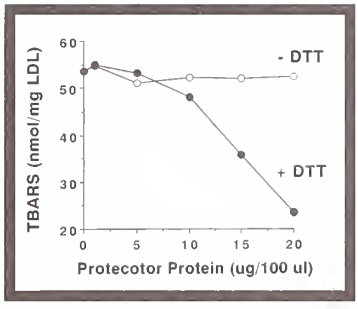
The physiological significance of TSA was highlighted further by the recent studies of H.Z. Chae, I.-H. Kim, K. Kim, T.B. Uhm, and S.G. Rhee (unpublished observations) showing that wild-type and tsa– strains of yeast grow at equal rates under anaerobic conditions, but that the tsa– strain grows more slowly than the wild-type strain under aerobic conditions, and even more poorly under conditions of oxidative stress, as provoked by the presence of hydroperoxide, alkyl peroxides, or methyl viologen. That TSA may have an even wider antioxidant role is suggested by recent studies of K. Uchida, H.Z. Chae, and E. R. Stadtman (unpublished observations) showing that TSA inhibits the Cu(II)-catalyzed oxidation of lipids in low-density lipoproteins (LDLs), as measured by the production of thiobarbituric acid-reactive substances (TBARSs) (Fig. 2). The fact that protection against LDL oxidation is dependent on the presence of a sulfhydryl compound, dithiothreitol (DTT), focused attention on the importance of sulfhydryl compounds as an essential component of the TSA-protection system, and cast doubt on the proposition that TSA protection is restricted to oxidative damage provoked by the RSH-Fe(III)-O_2 system. Indeed, L. Netto, H.Z. Chae, S.G. Rhee, and E.R. Stadtman (unpublished observations) recently demonstrated that contrary to the result shown in Fig. 1B, TSA can protect GS from inactivation by the ascorbate-Fe(III)-O2 MCO system if DTT is also present.

Proteins Homologous to TSA

Chae et al. (1993b) isolated TSA as homogeneous proteins from yeast and from rat brain. These proteins have molecular weights of 25 and 26 kDa, respectively. The same investigators cloned the genes from both sources, and the gene from yeast was overexpressed in *Escherichia coli* (Chae et al., unpublished observations). In contrast to other antioxidant enzymes, TSA does not contain a tightly bound metal ion, nor does it possess a heme or a flavin prosthetic group. Moreover, it does not chelate added Fe(III) or Cu(II) (K. Kim et al., 1988).

Although there is no homology between TSA and any of the other conventional antioxidant enzymes, an examination of gene bank data led to the discovery that several proteins with unknown functions have been described that exhibit considerable homology with TSA (Chae et al., unpublished observtions). These proteins fall into two classes. One class consists of three gene products from either transformed mammalian cells or pathogenic bacteria, including Mer5, which is preferentially expressed in immature murine erythroblastoma cells; a 29-kDa cysteine-rich surface antigen of pathogenic *Entamoeba histolytica;* and a 26-kDa antigen from the gastric pathogen *Helicobacter pylori.* The second class includes products of genes that are located upstream from genes encoding proteins involved in oxidation-reduction and anti-oxidant functions. These include genes upstream from an alkylhydroperoxide reductase in *Salmonella typhimurium,* the manganese superoxide dismutase in *Methanobacterium thermoautotrophicium,* an NADH dehydrogenase in Bacillus sp. strain YN-1, and a gene in *Clostridium pasteurianum* that is associated with the thioredoxin reductase gene.

In particular, the homologous proteins all share two conserved cysteine residues, which correspond to Cys47 and Cys170 of the yeast TSA. By means of site-directed mutagenesis, H.Z. Chae and S.G. Rhee (unpublished observations) showed that replacement of Cys47 with serine led to complete loss of TSA protective activity, whereas replacement of Cys170 with serine had little effect.



Role of dithiothreitol (DTT) and TSA in the inhibition of the Cu(II)-catalyzed oxidation of low-density lipoprotein (LDL). Reaction mixtures (0.1 mL) containing 10 µmol CuSO4, 50 µg LDL, 50 mmol sodium phosphate buffer (pH 7.2), and various concentrations of the protector protein (TSA), as indicated, were incubated in the presence (•) and absence (O) of 0.1 mmol DTT. TBARS is the amount of thiobarbituric acid-reactive substance that was formed.

TSA Reduces the Level of Sulfur-Containing Radicals in vitro

Plausible mechanisms for the action of TSA are suggested by the recent studies of M.B. Yim, H.Z. Chae, S.G. Rhee, and E.R. Stadtman (unpublished observations), who used electron- paramagnetic-resonance-spectroscopy (EPR) to show that sulfur-containing radicals formed in the presence of the RSH-Fe(III)-O_2 system and in the reaction between H_2O_2 and RSH, as catalyzed by horseradish peroxidase, are eliminated by the presence of TSA. Thus, TSA either prevents the formation of sulfur-containing radicals ($RS^{\bullet}$, $RSSR^{\bullet-}$, or $RSOO^{\bullet}$) or is able to scavenge them once they are formed. The latter possibility invites speculation that TSA may have a more important role in the protection of cells against free-radical damage than had been originally surmised. The intracellular production of thiyl radicals is well-established by EPR measurements (Ross 1988). Thiyl radicals are produced as intermediates in the oxidation of glutathione (GSH) by H_2O_2 or organic hydroperoxide, as catalyzed by various peroxidases (e.g., thyroid peroxidase, myeloperoxidase, lactoperoxidase, and horseradish peroxidase) and by prostaglandin-H synthetase. In addition, glutathionyl radicals ($GS^{\bullet}$) can be generated by having GSH interact with hydroxyl radical, superoxide anion radical, Fe(III), Cu(II), and alkyl radicals ($R^{\bullet}$) and by irradiation. As a working hypothesis, we propose that various kinds of free radicals ($X^{\bullet}$) generated by MCO systems, by other biological processes, or by environmental factors react with GSH or other intracellular sulfhydryl compounds to yield thiyl radicals (reaction 1).

$$X^{\bullet} + GSH \longrightarrow HX + GS^{\bullet} \quad (1)$$

This may then be followed by the kinetically favored reaction of the thiyl radical with thiolate anion (RS–) to form the disulfide anion radical (reaction 2)

$$GS^{-} + GS^{\bullet} \longrightarrow (GSSG)^{\bullet-} \quad (2)$$

Finally, in a free-radical chain-breaking reaction, TSA catalyzes dismutation of the disulfide-anion radical (reaction 3) to form nontoxic products

$$2(GSSG)^{\bullet-} + 2H+ \longrightarrow 2GSH + GSSG \quad (3)$$

This is, of course, only one of many mechanisms by which TSA might exert its effect. However, the likelihood that a dismutation type of mechanism is involved is supported by the demonstration that the rate of Fe(III)-catalyzed autooxidation of DTT in the presence of TSA is approximately one-half the rate observed in the absence of TSA (Chae et al., unpublished observtions).

The protective effects of TSA could play an important role in oxygen-radical-mediated disease and deterioration of tissues. However, both the mechanism of action and the physiological function of TSA remain to be established. Current studies in the laboratory are being carried out to determine whether the action of TSA can be augmented by other factors present in crude extracts of yeast, to elucidate the mechanism of action of TSA, and to explore further the kinds of radical damage TSA is able to prevent. The knowledge that high concentrations of purified TSA are required to obtain protection against free-radical damage to proteins and that TSA is highly homologous to several other gene products with unknown functions should serve to caution researchers about assigning physiological functions before more is known about these enzyme systems. ■

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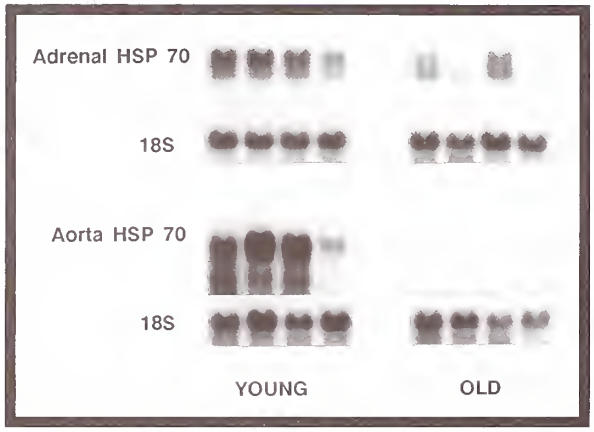
Aging, Stress, and the Heat-Shock Response

by Nikki Holbrook, Laboratory of Molecular Genetics, NIA

Human aging is accompanied by a progressive decline in physiological processes and particularly by a decreased ability to respond to the stresses of life. Current concepts suggest that aging results at least in part from damage to molecules, cells, and tissues by a variety of toxic factors that are either endogenously produced or that come from the environment. Genetic systems have evolved to detect specific forms of molecular and cellular damage and to activate the expression of genes whose products increase the resistance of cells to such damage and aid in their repair. The particular genes that are induced depend on the nature of the stress and the resulting damage. For example, heat stress induces a particular set of genes, oxidative stress, another, and DNA damage induces still other genes, although there is clearly some overlap in the responses. The continued effectiveness of these genetic responses to environmental damage may be a major factor in resistance to disease and aging. Support for this notion has come from recent findings in my own as well as other laboratories indicating that the ability of cells to express the heat-shock genetic response declines with age. Our research, conducted in NIA's Laboratory of Molecular Genetics over the past several years, has focused on understanding the cause for this age-related decline.



Age-related decline in restraint-induced HSP70 m RNA expression. Results shown are for four separate young (six-month-old) and old (24-month-old) Fischer 344 rats are restrained for one hour.

Although originally named after their increased expression following heat stress, the heat-shock proteins (HSPs) are also induced by a wide variety of toxic and metabolic stresses. HSPs are also expressed during normal cell growth and are thought to function as chaperone proteins that assist in the intracellular transport, assembly, and folding of other proteins. There is considerable evidence that these proteins protect against various damaging factors and are part of a protective homeostatic response.

We demonstrated in rats that expression of the major HSP, HSP70, is induced in vivo in response to a variety of stresses, including mild elevations in body temperature, anesthesia, and surgery, and by the stresses engendered by restraint, (Blake et al. 1991a, 1991b; Udelsman et al. 1991, 1993). We have concentrated our recent efforts on the response to restraint where HSP70 is induced only in the adrenal gland and vasculature. In situ hybridization demonstrated that the expression within the adrenal gland was localized to the cortex, whereas expression within the vasculature was restricted to smooth muscle cells in the media. Of particular interest was the finding that the responses of both tissues are greatly attenuated in old rats. These findings raise several important questions. What controls the response? What role does HSP70 play in these selective tissues? And what is the cause of their age-related decline?

Endocrine and molecular control of the response

Because immobilization alone activates the response, an endogenous factor modulated by restraint may lead to the induction of HSP70 expression in these tissues. In mammals, virtually any stress results in the activation of both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Furthermore, both of these endocrine axes appear to undergo alterations with age. Therefore, we examined whether these systems were linked to restraint-induced HSP70 expression . HPA activation results in secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which stimulates the adrenal cortex, causing the synthesis and release of glucocorticoids into the peripheral circulation. Activation of the sympathetic nervous system results in the elevation of plasma catecholamines. We demonstrated that restraint-induced HSP70 expression is linked to these acute hormonal responses, but that the adrenal cortical and vascular responses are regulated differentially. Adrenal HSP70 expression after restraint is dependent on an intact HPA axis and requires ACTH, whereas vascular HSP70 expression appears to be regulated through alpha1 adrenergic receptors. In fact, administration of either ACTH or phenylephrine, an alpha1-receptor agonist, is sufficient to induce HSP70 expression selectively in the adrenal cortex and vasculature, respectively. These findings demonstrate that restraint-induced HSP70 expression in both adrenal cortical and vascular tissues is intimately linked to the acute neuroendocrine stress response and suggest that HSPs are likely to play a fundamental homeostatic role in the organism's ability to cope with stress.

Induction of HSPs in response to heat and other classical stressors occurs as a result of activation of one or more heat-shock transcription factors (HSFs). These proteins bind to a specific DNA sequence, the heat-shock element, in the promoter regions of HSP genes, which increases their rates of transcription. Using an electrophoretic gel-shift assay, we have provided evidence that restraint increases HSF binding activity in both adrenal and vascular tissues, and this, presumably, is the mechanism whereby HSP70 gene expression is elevated following restraint. Importantly, the magnitude of HSF binding activity (a measure of the degree of activation) was found to be significantly lower in aged rodents than in young animals.

Functional significance?

An important question regarding the selective induction of HSP70 in adrenal glands and blood vessels during stress is, what role does it serve? For the adrenal gland, it may serve to protect cortical cells from locally produced high glucocorti-

coid concentrations because such concentrations are toxic to most tissues. The adrenal gland appears to be the most immunocompromised organ in the body because of the locally produced high concentrations of glucocorticoids. Thus, the adrenal gland is prone to metastatic carcinomas and opportunistic infections. Although glucocorticoid synthesis is necessary for mammals' ability to cope with stress, chronically high concentrations of glucorticoid in the adrenal gland could be detrimental if there were not some mechanism to protect the gland itself. We hypothesize that HSPs could play a key role in such a mechanism.

Causes for the age-related attenuation of the response

There are several possible explanations for why aged animals show a reduced response to restraint. The finding that HSF binding activity following restraint is reduced in aged animals suggests either that lower amounts of HSF are present in the tissues of aged rats (i.e., HSF protein concentrations decline with age) or that there is a deficit in the signal-transduction pathway(s) leading to HSF activation. Current work leads us to favor the second possibility, because a preliminary examination of HSF protein concentrations indicates that they are similar in young and old rats. Two alternative explanations for the decline in the response with age are either that aged rats do not perceive restraint to be as stressful as do young rats, or that the lower induction in aged rats occurs secondary to alterations in endocrine function. We have addressed these possibilities with respect to adrenal HSP70 expression and the HPA axis. Measurements of plasma ACTH and corticosterone (the major glucocorticoid in rodents) concentrations in young and old stressed animals do not support either of these views, because both age groups were found to respond to restraint with a similar rise in ACTH and corticosterone concentrations.

Transplantation model for studying the vascular response

In collaboration with Robert Udelsman of the Department of Surgery at the Johns Hopkins University and Hospital in Baltimore, we have developed a cross-transplantation model for use in studying the mechanism(s) responsible for the age-related decline in HSP70 expression. Specifically, thoracic aortas from young rats are transplanted into old rats, and vice versa. One month after transplantation, animals are subjected to restraint. This aortic-transplantation model should allow us to answer a fundamental question: is the aging process inherent to the aged aorta, or is the environment in which the aorta resides responsible for the age-associated decline in stress-induced HSP70 expression? Our preliminary results demonstrate that aged aortas show a restored HSP response when transplanted into young animals, whereas young aortas behave like old tissues in old hosts. These data indicate that the environment in which the aorta resides is a major factor in determining the amount of HSP70 expression in the stressed tissue. It is possible that some negative, presumably circulating, factor is present in the old host that depresses responsiveness or, alternatively, that aging leads to loss of a positive factor necessary for maintaining the response. Future studies will address these possibilities.

In summary, our in vivo findings support observations made with cultured cells that there is a decline in the heat-shock response with aging. We believe that this in vivo stress response represents an important homeostatic function and that a general decline in the ability to mount this response renders the aged individual more vulnerable to stress, further contributing to the aging process. These studies also raise the question of whether or not other host defense mechanisms show a similar decline with age.

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IS AN ACCIDENT BREWING IN YOUR LAB?
continued from page 9.

"This accident shows us how hazardous laboratory conditions can develop when laboratory personnel don't appreciate the potential dangers of accumulated chemicals, jerry-rigged apparatus, or malfunctioning safety equipment," says Robert McKinney, director of the Division of Safety (DS). "Any resulting accident could affect other labs, even entire buildings. Fortunately, the impact of this accident on other research groups in the building was minimal."

The DS evaluated the scene of the accident and found a variety of unsafe conditions:

- inappropriate storage of flammable liquids,
- storage of potentially dangerous substances on open shelves and carts,
- inappropriate storage of excessive amounts of combustible paper products,
- use of incense,
- surplus of unnecessary and outdated chemicals,
- inoperative safety shower, and
- various electrical hazards.

The laboratory remained closed for almost a week while DS officials worked with scientists to correct the unsafe conditions (a quick reference list of DS services is on page YP-52 of the NIH phone book). "The result is a cleaner, better-organized, less-cluttered, and above all, a safer laboratory," says McKinney "To avoid accidents, investigators should regularly assess their labs for potential dangers. The Division of Safety is ready to help other researchers identify and eliminate hazardous conditions." ■

COLLINS AS NEW PROJECT HEAD
continued from page 1.

campus." With considerable experience in forging collaborations, Collins says his goal at NIH will "not be to create another crown jewel," vying with existing programs, but rather, "to put another facet on the diamond." Collins said he chose medical genetics as a focus for the intramural program because NIH "with its Clinical Center and its vast array of strengths in many disciplines, is ideal for this goal." Creating a purely mapping-oriented genomics program, a la MIT's, in the midst of the Bethesda campus would amount to putting "a square peg in a round hole," says Collins. "It just wouldn't be a very good fit."

Until about a month ago, the NCHGR was exclusively extramural. Now, the extramural Human Genome Project will be combined with the new intramural program, and the Center is expected to be expanded into NIH's newest institute, to be called the National Institute on Genomics and Medical Genetics (NIGMG). Collins will head the new institute but will also continue an active research program, moving his laboratory from the University of Michigan at Ann Arbor to Building 49, the Silvio O. Conte Building.

Collins' own research priority will continue to be chasing disease genes — a major theme of his research at Michigan — and determining their function in order to treat diseases. Collins and his crew recently contributed to efforts to identify the Huntington's disease gene and currently are hot on the trail of the familial breast-cancer gene on chromosome 17. An estimated one in 200 women inherits an alteration in this gene that predisposes her to increased risk of breast cancer and to a higher risk of ovarian cancer. If found, the gene will enable development of a screening test for predisposition to breast cancer in pre-symptomatic stages, allowing cancer-prone women to intensify detection efforts, says Collins. He predicts that this test will be the first in a wave of diagnostics that will shift medical emphasis from costly treatment to prevention.

Another focus for Collins' lab will be gene therapy. The cystic fibrosis (CF) gene that Collins co-discovered in 1989 has already led to gene-therapy protocols. Clinical trials on CF gene therapy are scheduled to be launched next month at five different institutions, including Michigan and the intramural research program. Collins continues to explore the neurofibromatosis gene, which he also co-discovered, in 1990. Collins would also like to see his lab start hunting genes for common diseases, such as adult-onset diabetes. Sifting through the genetic causes of such polygenic diseases (diseases influenced by several different genes), says Collins, is the next frontier in positional cloning, a gene-finding technique he pioneered that uses inheritance patterns within families to locate the gene, even when no information about its function or biochemistry is known. Finding common disease genes "is going to be a very hard problem to tackle and may take many years, but it is time to start," says Collins.

Francis S. Collins, M.D., Ph.D. Director, National Center for Human Genome Research

At Building 49, Collins has already begun the process of setting up the intramural facility whose research agenda will include identifying common disease genes, developing DNA- and cytogenetics-based diagnostics, conducting linkage analyses and gene-therapy, and establishing core facilities to conduct genotyping, statistics, cytogenetics, physical mapping, and sequencing. Collins says his goal is to "set up a very open, interactive environment for human genetics, to supply a hub of resources [that] other institutes can also benefit from, to stimulate seminars and training programs, and to cause human genetics to flourish in all of the buildings and all of the institutes, not just in Building 49."

For now, one of Collins' top priorities is to find a scientific director for NIGMG. In the past several months, he has also been negotiating with a several senior investigators about the scientific director and the laboratory chiefs slots. Although no offers have been formally accepted, at least seven world-class, well-respected geneticists are seriously considering joining the intramural program, says Collins. Introducing Collins at the news conference, NIH Director Bernadine Healy quipped, "No one says 'no' to Francis Collins." According to Collins, "These are people that everyone at the NIH campus will welcome into their midst as scientists of the highest caliber." The bulk of Collins' own lab will move from Michigan in September. By 1995, Collins plans to bring in about 20 independent investigators, each with his or her own research activity.

The extramural Human Genome Project, says Collins, will not change in a major way. Collins plans to revisit and rethink some of the goals of the genome project in light of the fact that "only two and a half years into its 15-year track, the genome project has gone farther than anticipated—particularly in the area of genetic and physical maps." The project "is ahead of schedule and under budget," Collins says. He predicts that the genetic map will be finished next year and that the first-pass physical map of the entire human genome, based on yeast artificial chromosomes (YACs), will probably be finished in another two years. The map, although not perfect, will serve as a first approximation, says Collins.

The much-touted mega-YACs (containing a catalog of the entire human genome), completed by the French Généthon effort at Centre d'Étude du Polymorphism Humain in Paris, have been a subject of recent controversy. Researchers have found that pieces of DNA are missing or misplaced in the YAC libraries compared with where they occur normal on the human chromosome. Although researchers had expected some glitches, the percentage of mismatches is turning out to be higher than expected.

"Virtually everybody working on the human genome would love to see a better system," says Collins. "But we can live with" the imperfections, "as long as we are aware of [them] and recognize that this is a first approximation." Collins adds that "the genome project has been

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RECENTLY TENURED
continued from page 11.

Merlino's future plans include studying the rate and type of mutations in transgenic mice exposed to environmental carcinogens, and their role in tumor development and metastasis, which is poorly understood. Merlino is also collaborating with NIEHS researchers (see story on page 4) to study the effect of TGF-∝ overexpression in female reproductive organs and the role TGF-∝ plays in the presence of chemical carcinogens.

Merlino says he loves to collaborate with other NIH scientists. "I get a lot of requests" to collaborate or to create transgenic mice, says Merlino. "Although it is important to forge collaboration so that better science can result, we have to temper that enthusiasm with trying to remain focused scientifically." Merlino says the major goal of his lab is "to study the role of potent growth and differentiating factors in oncogenesis" and to continue the quest for optimal models for studying cancer. Merlino adds that "NIH provides scientists with a unique opportunity to do experiments that are risky and not necessarily goal-oriented." Occasionally, a shot-in-the-dark approach, he says, can result in unforeseen but useful byproducts, especially in transgenics, in which scientists cannot always control the in vivo effects of the transgene.

Merlino received his postdoctoral training under Ira Pastan at NCI. He has received several honors and awards, and he recently helped organize an international meeting and a course on transgenic-mouse technology, "Mouse Developmental Genetics," offered annually at the Einstein College of Medicine in New York. He is also an adjunct associate professor in the Department of Pathology, Georgetown University, and in the Department of Biochemistry, George Washington University. Merlino received his Ph.D. in 1980 from the University of Michigan at Ann Arbor. ■

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very careful not to put all of their eggs in this one basket," and is increasingly emphasizing STS mapping, based on sequence-tagged sites or reference points as a way of building physical maps independent of vector systems such as YACs.

To help identify new opportunities and technologies for Human Genome Project, NCHGR scheduled a meeting in Baltimore on April 23 and 24. Collins hopes to use the meeting to solicit input from a broad range of scientists, including geneticists, cell biologists, microbiologists, evolutionary biologists, population geneticists, and clinicians. He hopes to update the goals for the genome project by mid-summer. ■

GENETIC TREASURES IN EGYPT
continued from page 8.

matosis; and eye defects. The advisory council for the database project, which is funded by the U.S. Agency for International Development, is willing to consider proposals to develop new collaborations with Hashem if there can be some demonstrated advantage to the Egyptian patients — for example, molecular diagnoses that can be used in genetic counseling or for determining treatment options. Interested investigators may contact either of the U.S. principal investigators, Sherri Bale at NIH (301/402-2679) or Kenneth Rosenbaum at Children's National Medical Center in Washington, D.C. (202/745-5480). ■

NIH INTRAMURAL TENURE-TRACK
continued from page 1.

accomplishments of tenured scientists every four years. This retrospective review was recommended by Kornberg (1992) as the best way to maintain high-quality science.

But until now, academic tenure has had certain features that were absent from the NIH system. These include defined tenure tracks, with written policies and regular review, stop-the-clock provisions, special programs for women and minority scientists, and appeal and grievance procedures. The goal of NIH's new career-development initiative is to enhance the NIH tenure process by adding these features. In doing so, we will improve our support for the independence and creative freedom of young scientists and thereby invest in the future scientific excellence of the intramural program.

Scientists, as is true of athletes and artists, should be awarded contracts on the basis of what they have achieved rather than for what they promise to do. Scientists working at a frontier of science or creating a new one must rely on intuition, serendipity, and a capacity to move quickly in new directions to exploit findings that emerge from their research and that of others.

—Arthur Kornberg
Science Editorial
August 14, 1992

NIH intramural tenure-track policy

The Board of Scientific Directors of NIH has established a tenure-track system to allow unambiguous identification of candidates for scientific tenure at the NIH. The goal of this system is to provide all necessary resources and encouragement to tenure-track scientists, so that these investigators will have a fair opportunity to demonstrate their creativity and productivity as independent scientists.

Definition of tenure track

Tenure track is a position designation for independent investigators whose research abilities and focus make them candidates for the NIH permanent staff of independent scientists. Individuals in a tenure-track position are on a career path that, if successful, will lead to formal consideration for tenure. Award of tenure-track status implies that an institute anticipates a long-term commitment of space, dollars, support staff, and other resources required for the investigator to demonstrate a high level of independent scientific productivity and innovation. Investigators enter tenure-track positions after completing advanced research experience, that is, some form of postdoctoral training or its equivalent. Such training may have occurred outside NIH or in one or more of the NIH institutes. There are no formal criteria for the duration of pre-tenure-track experience, but the candidate's record should allow for a thorough evaluation of his or her potential as a tenure-track scientist. However, due to limited resources, only about 5% of the scientists who arrive as postdoctoral fellows achieve tenure.

Initiation of tenure-track positions

In many cases, tenure-track positions will be designated prospectively, that is, when the need for an individual of high caliber to perform a particular level of research is recognized, even though an individual to fill the position has not yet been identified. However, tenure-track decisions may also be filled retrospectively when an individual of outstanding ability has been identified at NIH and a position is created to accommodate that individual. The decision to initiate the position is made through the confirmed, mutual agreement of both a laboratory or branch chief and the scientific director of the institute. This agreement is necessary to ensure that sufficient resources are available to allow the tenure-track scientist to develop an independent research program and to satisfy the general long-term programmatic goals of the institute's intramural program as a whole. Thus, although the scientific excellence of a prospective tenure-track scientist is a paramount consideration, the long-term goals and priorities of the institute must be taken into consideration before a tenure-track position can be established.

Recruitment and appointment to tenure-track positions

The initiative for such recruitment may come from a laboratory or branch chief or from the

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scientific director, but the decision to proceed requires the mutual agreement of both. Additional sources of input (e.g., from appropriate scientific senates) may be obtained, and/or a formal search committee may be formed. Although it is strongly recommended that a search committee be used to assist identification of tenure-track candidates, the scientific director retains broad discretion in the determination of when a search committee is necessary and when it is not. The laboratory or branch chief will inform tenure-track candidates of the magnitude of allocated resources such as space, positions, and budget. These resources will be drawn from within the existing laboratory allotment or by additional provision from the scientific director. Other senior staff in the candidate's laboratory or branch should participate in the decisions about resource commitment. When a search committee is requested by the laboratory or branch chief or by the scientific director , the committee will include members from outside the laboratory or branch doing the recruitment. The scientific director should be consulted when the field of candidates has been narrowed, and should have an opportunity to meet final candidates and participate in final decisions (ranking of candidates). Final negotiations, including salary issues, are the responsibility of the scientific director .

Changes of Titles for NIH Scientists

Current		New	
Position	**Title**	**Title***	**Comment**
PERMANENT STAFF		**ICD****	
Tenured independent Scientist (GS-13 - GS-15) commissioned officers	Senior investigator	Senior investigator (GS-13 - GS-15)	Tenured position, independent investigator
Collaborative investigator	Senior investigator	Staff scientist	Permanent position
Clinician, dentist, veterinarian	Senior investigator	Staff physician	Permanent position
NONPERMANENT STAFF			
Tenure-track scientist	None; commonly use senior staff fellow, and visiting associate	Investigator	Tenure-track, independent investigator
Research associate staff fellow Senior staff fellow		Research associate	
Clinical associate		Clinical associate	
IRTA***, visiting fellow, NRSA, PRAT		Postdoctoral fellow	

*PHS commissed officer billets will be changed to use these titles. **ICD, Institute, center and division. ***IRTA, Intramural research training award fellows; NRSA, National research service award fellows; PRAT, Pharmacology research associate program fellows.

Resource commitment for tenure-track positions

Upon entry into a tenure-track position, the investigator is given (and will cosign) a written agreement signed by the laboratory or branch chief and the scientific director outlining the resources to be given him or her and the timetable leading to tenure consideration. Although such resources may vary considerably depending on the resources available and the promise of the work, they should be sufficient to allow the tenure-track investigator to develop an independent research program.

Timetable for tenure-track positions

Tenure-track positions will be designed to allow the tenure-track scientist sufficient time (six years, unless special circumstances such as family care necessitate an extension) to become established as an independent scientist. The initiation of a tenure-track position starts a new career time clock at NIH. Tenure-track scientists who are granted extended leave because of a personal or family emergency, or to care for an infant, shall have the normal, six-year period leading to tenure evaluation extended by a period equal to the time taken for leave. The period leading to tenure evaluation for tenure-track scientists who are granted part-time status because of personal or family responsibilities will be adjusted according to the fraction of their appointment; thus, half-time employees will be evaluated for tenure after 12 years, and three-quarter-time employees will be evaluated after nine years.

As outlined below, after the equivalent of three years in a tenure-track position, individuals will be reviewed for progress, which will include outside review by a board of scientific counselors and a decision will be made about their continuation in the tenure-track position. There is no minimum time a person must be in a tenure-track position before conversion to tenure. If the appropriate authorities decide not to continue a tenure-track individual, he or she will be allowed one terminal year.

Evaluation of tenure-track scientists

Although the goal of the tenure track is a tenured position, tenure-track scientists do not automatically advance to consideration for tenure. The latter requires continued research progress and professional growth consonant with institutes', centers', and divisions' (ICD) programmatic goals, so that when the tenure-track scientist is considered for tenure, he or she has achieved very considerable standing in the research community.

Individuals will be evaluated for tenure on the merit of their research, the relevance of their work to the laboratory or section, their scientific independence, their productivity, their leadership, and their potential for sustained intellectual growth, productivity, and excellence in their contributions to the intramural program. In their evaluations, committees that are making tenure recommendations may consider the candidate's publications and the impact of those publications on other scientists, participation in and direction of scientific conferences and symposia, development of innovative techniques or patentable products, overall contribution to intramural research at NIH, mentoring and leadership within the laboratory, branch, or section, and adherence to the highest ethical standards in the conduct of science and letters of recommendation from NIH and non-NIH scientists.

Tenure-track scientists are evaluated at several levels. First, they are evaluated continually by either the section chief or the laboratory or branch chief. One of these individuals will meet annually with the tenure-track scientist to provide feedback on performance. Second, each tenure-track scientist will be formally evaluated at least every three years. These formal reviews, initiated by the scientific director, can be carried out by any of several mechanisms agreed to by the ICD director. For example, the progress review might be performed by a board of scientific counselors and/or by a specially convened review team. A review team could be composed of representative tenured scientists appointed by the scientific director .

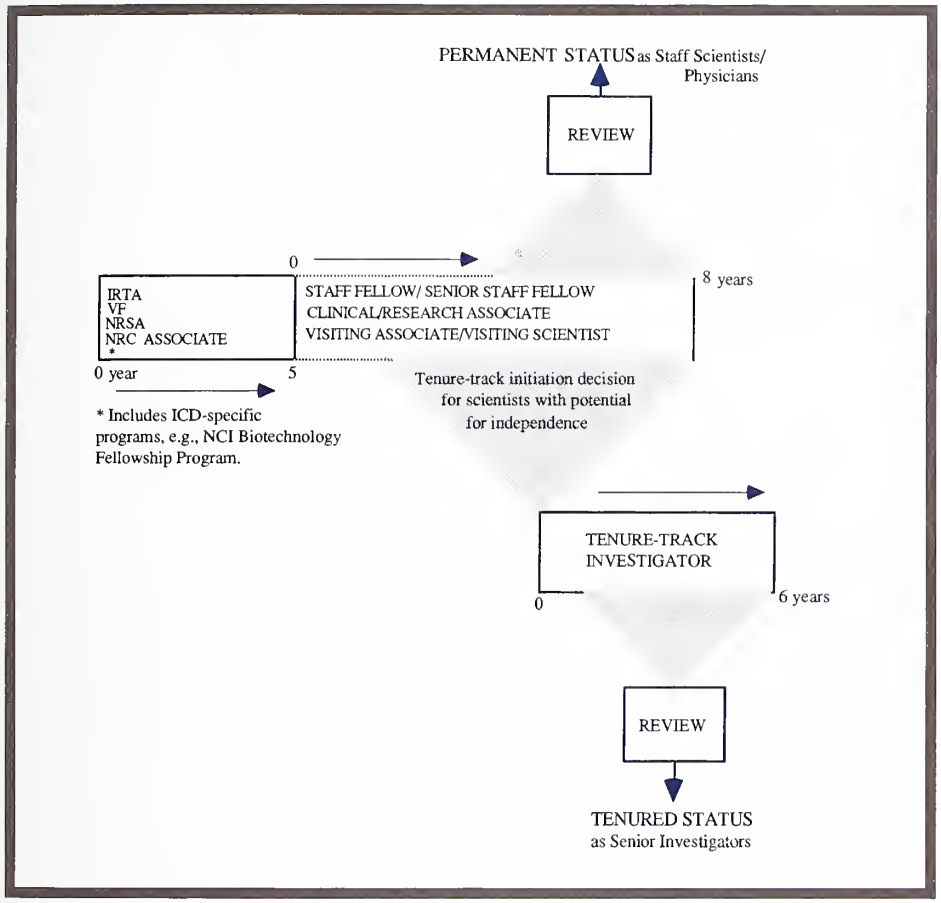
General comments regarding recruitment of tenure-track and tenured scientists

In developing lists of candidates, every effort will be made to include women and minorities. Although it is highly desirable to obtain wide-ranging input during the recruitment process, the practical aspects of negotiating with final candidates dictate that the laboratory or branch chief and the scientific director bear responsibility at that stage.

Staff scientists: criteria for permanent positions

Within the past few years, the scientific directors have discussed formalizing the concept of a permanent staff scientist. These discussions arose out of the recognition that a traditional benchmark for the tenured scientist has been "independence," and letters are requested from peers outside NIH asking for their opinion on the independence of the tenure candidate. Indeed, most tenured university-based scientists are truly independent because they receive grant support in their own name, and the expectation that this will be the case weighs heavily in the university tenure process.

Career Pathways for Postdoctoral Investigators at NIH



At NIH, there has been a need, on occasion, to assemble multidisciplinary research teams led by one truly independent principal scientist. In the absence of a steady flow of graduate students and long-term postdoctoral fellows, awarding permanent positions to "collaborative" investigators offers stability to such teams. Moreover, certain types of modern research require highly specialized, often unique, skills that are not yet developed in trainees; this, too, is a rationale for collaborative investigators. Thus, there is now recognition of the need to have an evaluation and promotion process for such members of research teams—staff scientists—distinct from the tenure and promotion process for clearly independent principal investigators.

Collaborative investigators, to be termed "NIH staff scientists," have highly specialized and uncommon technical expertise. These may include certain X-ray crystallographers, protein chemists, electron microscopists, "high-end" computer scientists, statisticians, and epidemiologists. Staff scientists may also be investigators with superb but more general scientific skills and expertise, performing a critical team function. These scientists may operate major core resource facilities or work with more limited resources. They function as key members of a team of researchers whose project goals are defined by a principal, independent scientist. Resources assigned to such a staff scientist are those of the resource facility or principal investigator. Although collaborative investigators of this type may follow an independent research theme, as well as providing a resource-facility service or serving a "team" function, the scientific director is free to assign the staff scientist to any research team to which his or her special expertise would make a valuable contribution. Some scientists within highly specialized disciplines are exceptionally creative, and their technology per se has the power to shift scientific paradigms; they are independent investigators, not staff scientists.

When a permanent position for a staff scientist is proposed, it should be based as much on consideration of the strength of the research program as on the candidate. Thus, the program itself must be very strong scientifically, and the candidate must have demonstrated an exceptional ability to be highly productive within this established research program. Moreover, a permanent position should be awarded to such an individual only when it is likely that he or she will continue to be highly productive even if the current program is terminated. Staff scientists given permanent standing in this circumstance must be aware that they can be reassigned to another lab if there are major changes in the program, and in this event, the collaborative investigator will be expected to address the research goals of the new principal investigator.

In general, NIH will discourage the practice of appointing permanent staff scientists at the expense of recruiting and retaining independent investigators. As an alternative to a quota on staff scientists, diligent case-by-case review of such scientists will be conducted. A subcommittee of the scientific directors will be constituted to examine these permanent-position candidates rigorously before recommendations about them are made to the scientific directorate as a whole.

In evaluating candidates for permanent staff scientist positions, the following questions will be asked: 1) What are the research contributions of the candidate's laboratory with respect to originality and imporance to the field? 2) What is the candidate's contribution to the research program, and are his or her skills or expertise sufficiently uncommon that he or she could not be replaced with a reasonable effort? 3) What is the laboratory's potential for future significant contributions? Would this potential be seriously and durably compromised if the candidate were to leave?

The scientific directors have adopted these definitions and descriptions of staff scientists to reduce the ambiguity that may currently exist. Importantly, these distinctions in no way diminish the value of any scientist's contribution, but they do encourage an honest and explicit evaluation of each person's particular assets and responsibilities. In considering the award of a permanent position to a staff scientist, the same guidelines will apply as in the case of an independent scientist: the institute must have, or anticipate having, sufficient resources so that the candidate will be able to fulfill his or her creative potential. Given the availability of these resources, a candidate's research skill must be of high programmatic priority to the institute. Finally, given available resources and research priorities, the question must still be asked: Is this the best possible candidate—at NIH or elsewhere—available for the position?

The awarding of a permanent position either as director of a highly specialized resource facility or in general support of a principal investigator's research program does not preclude the individual from later being nominated for a tenured, independent-investigator position. However, a second review process will then be required, as described for independent investigators. There is general agreement that the word tenure should be applied only to independent investigators as a useful and familiar indicator of institutional commitment to the concept of total academic freedom. As a result, only independent scientists ("senior investigators") will be tenured, and staff scientists will be permanent. Therefore, only independent junior scientists ("investigators") will formally be placed in a tenure track. ■

In the Next Issue...

- Reversing the brain drain: status of women scientists at NIH
- Part II of the career development initiative
- Details on the status of the honoraria ban
- IRTA Fellows: Coping with FTE restrictions

FAX-BACK

Starting this issue, we are launching a FAX-BACK page on which you can write your opinions on issues, policies, or programs under evaluation and then fax it back to the Deputy Director for Intramural Research.
This feature will serve as a year-round electronic town meeting, gathering feedback to boost good ideas and weed out the bad ones.
This time, we are asking for your opinion on four issues detailed in Lance Liotta's column on p. 2 and for your suggestions on the various sections of the newsletter. Fax your comments to 402-4303 or mail it to us at Building 1, Room 114.

What do you think about

1) The NIH intramural tenure-track policy?

2) The interinstitute advisory faculties recommended by the taskforce report on the intramural program?

3) The special car-pool parking exemption for employees with young children who must be dropped off at school or day care?

4) A low-interest or no-interest tuition-loan program for NIH employees?

5) The newsletter: should any sections be expanded, changed, or deleted?

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