FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

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CELEBRITY SCIENTISTS: PERSPECTIVES FROM NIEHS NEWSMAKERS

by Seema Kumar

Although most biomedical researchers labor in quiet obscurity, fame is no stranger to some members of the NIH intramural community. Two of the latest intramural scientists to catapult into the headlines are from NIEHS: Martin Rodbell, who shared the 1994 Nobel Prize in physiology or medicine with Alfred Gilman of the University of Texas Southwestern Medical Center at Dallas for work on G proteins, and postdoc P. Andrew Futreal, who, along with Senior Staff Fellow Roger Wiseman, was part of the group that isolated the long-sought breast cancer susceptibility gene, *BRCA1*. *The NIH Catalyst* recently conducted interviews with these two scientists to see how they're handling their sudden notoriety.

Martin Rodbell

Q: *How does it feel to become an overnight celebrity?*

Rodbell: There's no sensation more bizarre than receiving a phone call at 6 a.m. from someone who says that you've been selected for the Nobel Prize. If my wife, daughter, two granddaughters, and son-in-law hadn't been there with me, I might have considered the whole thing

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MEDICAL PATHOLOGICAL WASTE DISPOSAL
THINK TWICE BEFORE YOU THROW IT OUT

by Occupational Safety and Health Branch Staff

Since NIH shut down its incinerators last spring, medical pathological waste (MPW) disposal has become a growing — and expensive — problem. Currently, scientists and others generating MPW package the waste in the MPW "burn box," which is then handled as infectious waste — even if the items inside are not contaminated. Every day, NIH transports an average of 719 boxes of MPW, weighing about 5 metric tons, to a private waste incinerator for disposal at a cost of more than $500 per ton. In an era of budget crunches, the high cost of off-site MPW disposal has intensified the need to minimize the volume of MPW.

Here are a couple things that intramural scientists should keep in mind. First, all lab waste does not have to be disposed of in an MPW box. Surveys of NIH use of MPW boxes have shown that the boxes often contain materials that could be appropriately disposed of in other, less costly ways. Secondly, research labs themselves can often decontaminate MPW and dispose of the treated waste by non-MPW routes. With a few exceptions, MPW can be decontaminated by using methods like chemical treatment or steam sterilization, and then safely discarded in the general waste, disposable-labware box, or sink. For example, it is often possible to decontaminate used disposable labware with bleach or Wescodyne solution before placing it with uncontaminated gloves and labware in the disposable-labware box (NSN # 8115-01-154-2305) for removal as general waste. For routine tissue culture and bacteriology, autoclaved waste can be discarded as general waste. MPW boxes should be used only for disposal of non-radioactive, biologically contaminated materials; sealed "sharps" containers (3/4 full); and small animal carcasses.

Newspapers, food and beverage containers, and office paper should be disposed of in regular trash cans or recycling containers, where available. You might consider stocking up on reusable labware (especially unbreakable plasticware), which reduces waste and supply costs over time.

The *Guide to NIH Waste Disposal*, often referred to as the Waste Calen-

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CONTENTS

Gazing Into the Crystal Ball: NIH's Clinical Research Future, Part I

John Gallin

There has never been a scientific resource like the Clinical Center. Therapies incubated here have had far-reaching impacts on the quality of world health for more than 40 years. As the scope and sophistication of clinical research have increased over the past decades, so have the requirements of a physical plant to house that research. And as scientific inquiry and medical care have evolved, so has the vision of what the Clinical Center needs to provide. This evolution is central to revitalizing clinical research within the NIH intramural programs.

At the core of our current plans to revitalize clinical research is the construction of a new 250-bed research hospital. The Department of Health and Human Services, acting on recommendations from the External Advisory Committee, is considering funding a competition to develop a concept for this facility. The complex will include a prominent day hospital; contiguous laboratory space — a hallmark of the Clinical Center since its inception; and access to the current Ambulatory Care Research Facility, as well as diagnostic and surgery suites. As currently conceived, the $380 million needed for this facility would come from existing and future intramural funds.

In preparing for the new facility, we must streamline current Clinical Center programs. Consolidations of patient-care units now under way are designed to reduce the number of beds from 416 to 325 over the next few years.

PATIENT-CARE UNIT CONSOLIDATIONS WILL RESULT IN ANOTHER MAJOR SHIFT — A CULTURAL ONE.

This reduction will help alleviate another pressing concern for intramural researchers: shortage of laboratory space. Cutting the number of patient beds will free an additional 15,000 square feet of space throughout the building for reassignment. We plan to move offices that now encroach on laboratory space into the newly vacated areas. Laboratories can then be added and expanded, contributing to a much-needed NIH Director's reserve of space to support new recruitment and scientific initiatives. We have also initiated a massive program to provide essential maintenance and repair to the existing facility.

The patient-care unit consolidations will result in another major shift — a cultural one. Institutes will, by necessity, share space for patient care, an arrangement that should foster a new era of intellectual cross-fertilization, a concept that has traditionally served as the foundation for creativity here at NIH.

We are also exploring ways to control the cost of providing Clinical Center services. One new policy allows us to structure an accurate, detailed measure of how much it costs for the Clinical Center to support an institute's protocol. Those accounting steps, coupled with efforts to better coordinate with the institutes to anticipate support needs, should enable us to work more efficiently.

The Clinical Center does more than provide services for the institutes. Staff members also conduct their own high-quality research, which has yielded important results. I encourage this research, and have instituted new policies to support it, including clearly defined budgets for Clinical Center research and rigorous quality review by the intramural Boards of Scientific Counselors. These changes have been a tremendous morale booster for the scientists and health-care providers at the Clinical Center. An additional incentive for improving the efficiency of Clinical Center operations is a new policy that earmarks a percentage of money saved in service functions to be applied to research activities. Other efforts to breathe new life into clinical research here include development of a program to identify and recruit minority patients, a step crucial to achieving diversity in our patient populations.

A revitalization of medical information systems is opening doors for innovative patient evaluation and consultation. Digitized images such as X-rays will soon be available on desk-top computers for patient-care providers throughout the Clinical Center. This technology can also extend the digital images to remote locations, allowing referring physicians to follow the care of patients here. The system will also be able to transmit other clinical data, such as retinal photographs and electrocardiograms, to in-house computers and to monitors in the offices of referring physicians.

Developing strategies for electronic transmission of clinical images will pave the way for another innovative aspect of medical care, remote assessment and monitoring of patients. Telemedicine technology could enable patients to go to nearby regional centers to be interviewed and examined by NIH physicians via video links, thereby reducing travel-related expenses, always a substantial component of clinical-care costs.

We are in a unique position to help define the roles of computer technology in clinical research, roles that will strengthen protocol monitoring and the interactions between extramural and intramural research. A regional linkage will establish a framework for clinical trials not previously possible. It will enhance patient and data monitoring, as well as increase the involvement by referring, primary-care physicians. This direct interaction between the principal investigators and the primary-care physicians will help provide consistency of clinical decisions during trials and improve the quality of clinical research.

It makes sense to place these regional centers in the existing network of General Clinical Research Centers. I am exploring this possibility with the Directors of the National Center for Research Resources and the Division of Computer Research and Technology.

We will continue to develop these and other ideas to strengthen research at the Clinical Center. In the next issue of *The NIH Catalyst,* we will discuss the second part of NIH's clinical research future: a major new training initiative that the Clinical Center plans to launch later this winter.

John I. Gallin, M.D.
Director
Warren Grant Magnuson Clinical Center
Associate Director for Clinical Research

JUST THE TICKET FOR NIH FELLOWS

If you read the DDIR's Bulletin Board, you may have noticed that the NIH Fellows Committee recently received support from the Scientific Directors for a merit-based travel-award program and for a one-day symposium.

The activities of the Fellows Committee include promoting education and career development, fostering communication among fellows, keeping fellows informed about policies affecting them, acting as a liaison to the administration on issues concerning fellows, and recognizing excellence in research and education. Since assuming its current form about a year ago, the Fellows Committee has sponsored career development seminars and Fellows Forums on the NIH tenure-track policy. This year, it is also sponsoring three speakers in the NIH Wednesday Afternoon Lectures; conducting a survey to assess fellows' access to, and knowledge of, computing resources at NIH; and working with the Office of Education to develop a fellows directory, fellows handbook, and the annual NIH Clinical Teacher Award.

The new NIH Fellows Award for Research Excellence was established to offer 30 travel awards to outstanding clinical and postdoctoral fellows. These awards will provide up to $1,000 toward travel expenses to a domestic scientific meeting attended in fiscal year (FY) 1995. The Scientific Directors of the Institutes, Centers, and Divisions have offered to support the fellows from their institutes who receive the award. All NIH postdoctoral and clinical fellows, including

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NIH Fellows Committee

Name	Degree	Institute
Jay Pearson	Ph.D.	NIA (Co-Chair)
LaRoy Penix	M.D.	NINDS (Co-Chair)
Rose Aurigemma	Ph.D.	NCI-FCRDF
Carolyn Bouma	Ph.D.	NIDR
Allen Braun	M.D.	NIDCD
Don Button	Ph.D.	NIMH
Edward Cupler	M.D.	NINDS
Charles Chu	Ph.D.	NIAID
Michelle Evans	M.D.	NIA
Jean Fraser	M.D.	NIMH
Susan Fueshko	Ph.D.	NINDS
Scott Hall	M.D.	NIAAA
David Hawver	M.D.	NIMH
Chris Hussussian	M.D.	NCHGR
Sari Izenwasser	Ph.D.	NIDA
Samir Khlief	M.D.	NCI-DCE
Tom Kristie	Ph.D.	NIAID
Nancy Leidy	Ph.D.	NINR
Stu Levine	M.D.	CC
Patti Lodi	Ph.D.	NIDDK
Kathryn Munoz	Ph.D.	NCI-DCPC
Linda Nebeling	Ph.D.	NCI-DCPC
Jose Pando	M.D.	NIAMS
Kathy Partin	Ph.D.	NICHD
Ronald Petralia	Ph.D.	NIDCD
Jill Ray	Ph.D.	NCI-DCBDC
Tom Selvaggi	M.D.	NIAID
David Sulciner	M.D.	NHLBI
Richard Nelson	M.D.	NIDA
Chris Walton	M.D.	NEI
Stephen Wiener	M.D.	NHLBI
Awa Wu	M.D.	NIDR
Tong Wu	M.D.	CC ■

FAX-BACK FEEDBACK

Below is a FAX-BACK comment we received for a topic raised in the September issue.

How postdocs regard mentorship at NIH

"*Floundering* is not the proper term. A more accurate description is that postdocs at NIH are PISSED OFF. An essential problem with this notion of mentorship is that it assumes that the postdocs don't know what is good for them and that the "mentors" act in the best interest of the postdoc. This is rarely the case. Quite often, mentors act in their own best interest. They need the work done in the lab. In many cases, the people who should advise new postdocs are the old, worn-out postdocs who have been through the wringer, often many times. A way to improve the mentorship at NIH could be to have a forum for post-docs not sanitized by the Office of Education. It would deal with the realities of the postdoc experience, academic appointments, job prospects, etc. I don't want to hear the experiences of the one in 100 who got that tenured position — I want to hear from the other 99." — *Kevin G. Becker, NINDS* ■

LETTER TO THE EDITOR

Seema Kumar's advice on writing grant applications (July 1994 issue) teaches scientists things they should not have to learn. Good research scientists know how to discover things and invent things. Such people can sometimes be identified in advance, but not by any tidy procedure that a bureaucracy would be comfortable with. Rather than fitting itself to the job of spotting talent in often strange and difficult personalities, NIH forces scientists to conform to its bureaucratic mechanism. The mechanism selects those who are good at following orders, at making long-range plans for exploring the unknown (and not noticing the contradiction), at self-promotion, and at currying the favor of their peers on the study sections. It is all very sad.

Sincerely yours,
Charles W. McCutchen, NIDDK

Editor's Note

In the September 1994 issue, two words were omitted on page 11 from the conclusion of Celia Hooper's article, "Chutes and Ladders: NIH Scientists Discuss the Art and Strategy of Biomedical Publishing." Harvey Pollard's concluding quote should have read, "The reason why people are more concerned with where an article is published, rather than its intrinsic merits, is that many readers cannot evaluate the latter anymore outside their own narrow fields."

Establishing a Science Ethics Framework

by Joan P. Schwartz, Ph.D., NINDS, and Philip Chen, Ph.D., OD

The increasing interest and concern about cases of misconduct in science not only on the part of scientists, but also members of Congress and the press, make ethics in science a timely subject. Ethics in science is the code of behavior that governs the manner in which scientists relate to each other and the process by which they acquire their data and ideas and communicate them to other scientists and to the public at large. Most of us strongly believe that good science demands high ethical standards. The Office of Intramural Research wants to develop better mechanisms both for educating intramural researchers about these ethical standards and for dealing with issues of misconduct in science. We hope that this forum will serve an educational function, and we would like to include contributions from intramural scientists about their viewpoints on — and their real-world experiences with — ethical issues in science.

Joan P. Schwartz

Philip Chen

Misconduct in science, as currently defined by the Office of Research Integrity (ORI), includes "fabrication, falsification, plagiarism, and any other practice that seriously deviates from what is commonly accepted in the scientific community ..." It **does not** include honest error or differences in interpretation or judgment of data, authorship disputes, human- or animal-subject protection issues, discrimination, or criminal activities, each of which is handled by another specific process. However, some of these other issues fall into the category of "questionable research practices ... in areas such as allocation of credit, the treatment of research data, respect for intellectual property, and mentorship responsibilities" [Alberts & Shine, *Science* **266**, 1660 (1994)]. The ORI definition of misconduct in science is currently undergoing review by the Public Health Service Commission on Research Integrity.

ORI has issued a set of guidelines on how to handle allegations of scientific misconduct (entitled *Scientific Misconduct in Intramural Research*, available from ORI, (301) 443-3400). Basically, three sequential steps are involved when an allegation is raised in the NIH intramural program.

1. The process begins when a Lab or Branch Chief or Scientific Director receives an allegation of scientific misconduct. After consultation with Philip Chen, the NIH Agency Intramural Research Integrity Officer (AIRIO), an "allegation assessment" is carried out by the Scientific Director, with the cooperation of the Lab or Branch Chief.

2. If this assessment suggests that there is sufficient evidence or information to support the need for further evaluation, the Deputy Director for Intramural Research (DDIR) notifies ORI and establishes a committee to carry out a formal inquiry. At this point, all notebooks, records, and data related to the case are secured for review by the Inquiry Committee. The purpose of the inquiry is information gathering and fact finding to determine if the alleged conduct is within the definition of misconduct in science and substantial enough to allow a specific finding of scientific misconduct. However, the Inquiry Committee **does not** make a finding of misconduct in science.

3. If the Inquiry Committee recommends that an investigation be carried out, the case is turned over to ORI, which carries out a formal investigation.

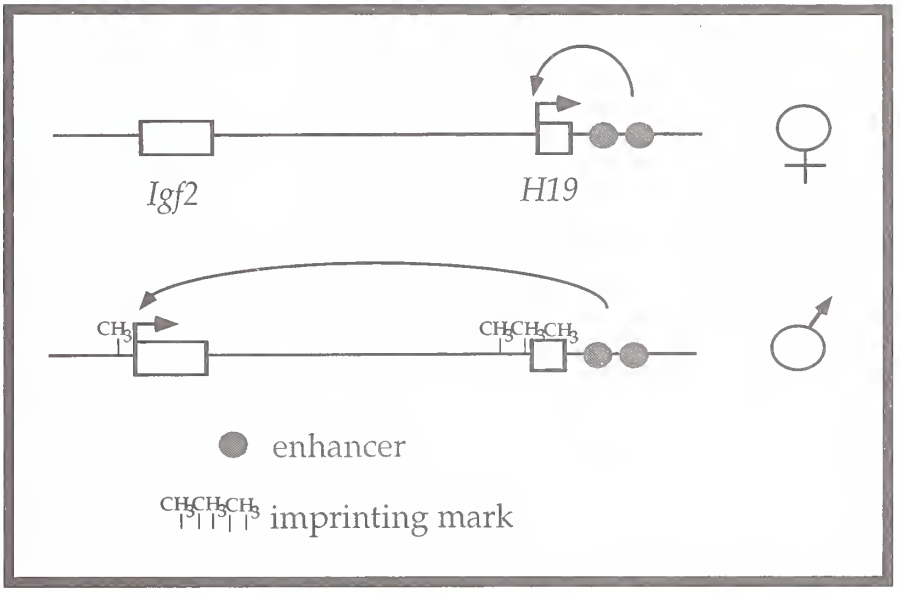
Given this overall process, what principles should underlie how the NIH Intramural Program deals with cases involving misconduct in science, as well as other ethical issues in the conduct of science? Three principles seem paramount: **timeliness, confidentiality**, and **fairness** to all parties involved. To ensure that cases are dealt with expeditiously and in a completely impartial way, the Office of Intramural Research plans to establish an NIH Committee on Scientific Conduct and Ethics. Members will be named from each Institute, Center and Division to ensure that a broad range of scientific disciplines is represented. This committee will help refine the ORI guidelines, as well as the intramural guidelines on research conduct, if necessary. Procedures for the protection of the rights of both "whistleblowers" and scientists accused of misconduct in science will be developed by this group. A subcommittee would be formed immediately, from members of the committee, to address each case that arises involving allegations of misconduct in science or disputes concerning authorship or publication practices, record keeping, sharing of materials and data, and mentoring and supervision. Arbitration may be offered as a settlement mechanism in some cases. Finally, the Office of Intramural Research is drafting a confidentiality statement to be given to any person with a "need to know" about a case, reminding that person of the importance of keeping allegations and other information confidential. We believe that these mechanisms will allow a timely, fair, and confidential evaluation of every case that comes to the attention of the Office of Intramural Research. ■

Coordination Between the Imprinting of Insulin-like Growth Factor 2 and H19

by Philip Leighton, Karl Pfeifer, Ph.D., Jennifer Saam, Andrea Webber, Tamara Caspary, Marisa Bartolomei, Ph.D., and Shirley M. Tilghman, Ph.D., Tilghman of Howard Hughes Medical Institute at Princeton presented this talk at NICHD's Distinguished Alumni Symposium on Sept. 19, 1994

ABSTRACT

Embryological and genetic studies conducted over the past 15 years have suggested that the male and female genomes of mice are not inherited in an equivalent state. The reason for this nonequivalence is now clear: there are several genes that are inherited in silent states from either mothers or fathers. Two such genes are insulin-like growth factor 2 (*Igf2*), a fetal-specific growth factor expressed almost exclusively from the paternal chromosome, and *H19*, an unusual gene that codes for a 2.5-kb RNA and whose expression is exclusively dictated by the maternal chromosome. The genes lie within 90 kb of DNA of one another and are co-expressed throughout development. This led us to propose that the genes are reciprocally imprinted through competition for a common set of regulatory elements, presumably enhancers (Fig. 1). The bias toward *Igf2* on the paternal chromosome is set up by sperm-specific DNA methylation of the promoter and structural *H19* gene, which acts to suppress its transcription. We propose that on the maternal chromosome, which is apparently unmethylated at either gene, the *H19* gene is favored because it is closer to key regulatory elements and/or it has an inherently stronger promoter. To test this model, mutations were generated in the *H19* gene itself, as well as in the presumptive regulatory elements that govern expression of *H19* and *Igf2*.



The Enhancer Competition Model to Explain Reciprocal Imprinting

The Igf2, *and* H19 *genes (open boxes), their transcriptional orientation, and chromosomal transcription site (horizontal arrows). Two enhancers which lie downstream 3' of the* H19 *gene are indicated by the closed circles, and the arrows leading from them indicate which of the genes is transcribed on the maternal and paternal chromosomes. Allele-specific methylation sites are indicated by the CH_3 symbols.*

QUESTIONS

Q: *What was the starting point for this work?*

A: The starting point for this work was not a fascination with imprinting, but a fascination with a mysterious gene. We had cloned the *H19* gene in the course of trying to understand the regulation of another gene, a-fetoprotein. *H19* bore no resemblance to any other gene that had been described before. Although it was transcribed and processed through the classical mRNA pathway, it lacked an open-reading frame of any length. Normally, that would have been enough to discourage us from pursuing it further because the most likely conclusion was that it coded for a pseudogene. However, two things kept us in the game: the fact that the RNA was transcribed at a very high rate during embryogenesis and the fact that the RNA structure (but no open-reading frame) was conserved in evolution. Finally, the observations that led us to *H19's* imprinting were that extra copies of the gene were lethal in mice, suggesting that its dosage was being carefully controlled, and that it mapped to an imprinted region of the mouse genome.

Q: *Which findings have been most surprising to you or to other scientists?*

A: The first surprise to us was that the *H19* gene was imprinted in the opposite direction from its nearest neighbor, *Igf2*. The paternal-specific expression of *Igf2* had just been uncovered by Argiris Efstratiadis, Tom DeChiara, and Elizabeth Robertson at Columbia University in New York City, and we had guessed that if *H19* was imprinted, it would be imprinted in the same direction. The fact that it was not, immediately eliminated the X-chromosome-inactivation model, whereby a chromosomal domain is simply shut down. The reciprocal nature of the imprinting of *Igf2* and *H19* led directly to the enhancer-competition model.

Q: *What were the greatest stumbling blocks, and what new observations, techniques, reagents, or insights helped you to get past them?*

A: The general problem in studying imprinted genes is devising ways to examine the expression of each allele separately. Until our work with *H19*, the only two imprinted genes that had been identified were uncovered by mutations. In the case of *Igf2* the mutation was generated on purpose using homologous recombination. For the

continued on page 16.

New Membrane-Based Cell-Motility Mechanism Yields Insight In Hearing Research

Scientists studying cell motility have traditionally focused on conspicuous systems, such as muscle cells or cilia. These investigators have succeeded in identifying motors — the mechanisms whereby chemical changes are converted into movement. But in the past decade, otologists studying the cells responsible for human hearing have discovered a much less conspicuous type of cell motion: a membrane-based contraction of the outer auditory hair cells.

Our laboratory has discovered and is now investigating the unique molecular mechanism underlying the operation of this most recently described cellular motor. This mechanism could be important in a variety of movements controlled by the cellular membrane, and is likely to prove vital to our understanding of the cellular basis of hearing disorders.

Historically, several lines of evidence have suggested that the hair cells of the mammalian hearing organ — "the organ of Corti" — are not simply passive transducers but also serve as amplifiers of the mechanical input they receive. As early as 1948, Gold (1) predicted that some active mechanical process must take place to overcome the damping effects that occur inside the fluid-filled cochlea, thus permitting a high degree of subjective frequency discrimination. He postulated that sound might emanate from the ear as a byproduct of the amplification process. In 1978, Kemp (2) recorded acoustic emissions from the human ear that were evoked by an acoustic-click stimulus. This discovery gave experimental support to the theory of an active cochlea. Recently, several reports have provided data about the way auditory hair cells can amplify sound within the cochlea. Propelling this renewed excitement in auditory research is our discovery of a novel force-generation mechanism located in the lateral plasma membrane of the outer hair cells of the organ of Corti in the cochlea. During sound stimulation, this membrane-based, voltage-dependent motor is capable of providing mechanical feedback that appears to modulate the sensory transduction response of inner hair cells, which are responsible for the bulk of sound detection and discrimination.

Outer hair cells were a complete enigma until the mid-80s

Sensory Transduction in Hair Cells

Hair cells are the receptors that convert mechanical movements into electrical signals in the auditory and vestibular sensory organs of the inner ear. These sensory cells were named "hair cells" because of the bundle of sensory stereo cilia — resembling hairs — that projects vertically from the apical cell surface. Each "hair" bundle is composed of about 100 stereo cilia (1 to 5 m long) and the hairs of each bundle are grouped in a staircase-like arrangement. The tips of the shorter hairs are connected to the sides of their longer neighbors by thin cables called tip-links (3). During an incoming mechanical stimulus, the hair bundle tilts toward the taller cilia, stretching the tip-links. Each stretched tip-link pulls open a mechanically gated ion channel, allowing an influx of cations that depolarize the cell by tens of millivolts (3). This depolarization is transmitted to the central nervous system by excited afferent synaptic connections.

Outer Hair Cells of the Mammalian Cochlea

In the mammalian cochlea, pure tone stimulation evokes mechanical waves that travel through the organ of Corti, from the base of the basilar membrane toward its apex, peaking in amplitude at a specific point along the organ's approximately 3-cm-long path. Inner and outer hair cells of the mammalian cochlea distributed in parallel rows along the length of the basilar membrane cooperate to analyze this wave. The row of inner hair cells is wired with afferent innervation and delivers the bulk of auditory sensory information to the brain. The three or more rows of outer hair cells in mammals receive only about 5% of the afferent innervation (4) but possess extensive efferent innervation. Outer hair cells were a complete enigma until the mid-1980s, when Brownell and others discovered that the cells boast an unusual talent: the ability to change length when subjected to changes in membrane potential. Both inner and outer hair cells respond to vibrations by producing such a change in membrane potential (5,8). However, only outer hair cells are also equipped to do the opposite, that is, to vibrate in response to a change in membrane potential, lengthening and shortening with small changes in membrane potential.

Another important difference between inner and outer hair cells is their location in the organ of Corti. Inner hair cells are located near the attachment site of the basilar membrane, an area where the membrane vibrates very little. Outer hair cells have a long, cylindrical shape and are positioned above the section of the basilar membrane that vibrates the most in response to sound. Researchers now believe that the outer hair cells can increase basilar-membrane vibrations by making contact with this sensitive section of the membrane as it vibrates, an action analogous to pushing a child on a swing. The outer hair cells could thus act like miniature amplifiers, sustaining the vibrations of the basilar membrane. This mechanism can indeed generate forces large enough to influence the basilar membrane (9) and can operate at frequencies that span the range of those produced by the human voice.

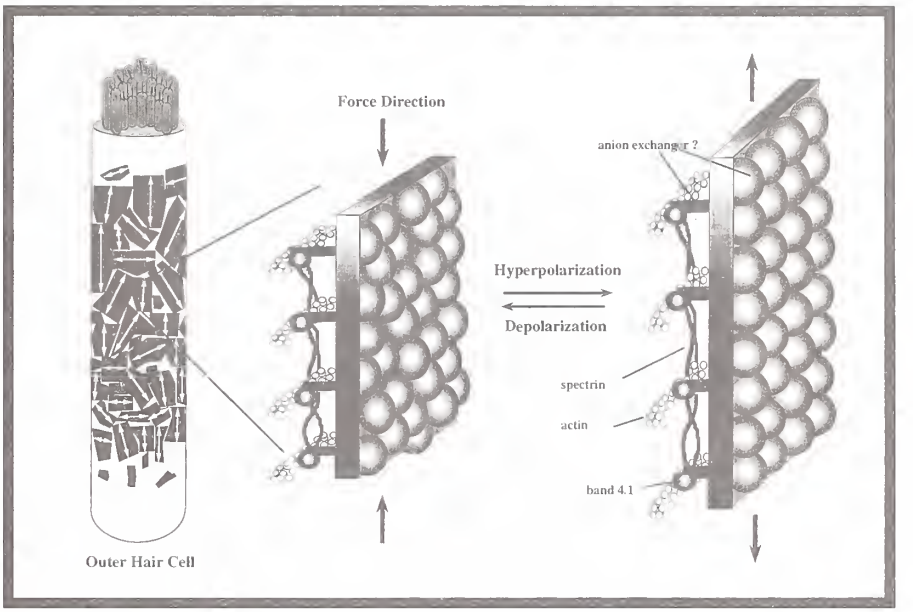
Fast Cellular Motor of Outer Hair Cells

The lengthening and shortening of outer hair cells depends on a unique motor that senses changes in membrane potential. The molecular mechanism behind this motor differs radically from those found in muscle-cell contraction and the motion of cilia, which require energy in the form of ATP and are based on filament structures such as actin and mictrotubules in the cytoskeleton. In contrast, the mechanism driving contractions of outer hair cells uses no ATP and is membrane based (6). The immediate questions that

by Bechara Kachar, Chief, Section on Structural Cell Biology, Laboratory of Cellular Biology, NIDCD

this novel cell-motility mechanism raise for us are: How do the cells detect voltage changes across their membranes, how do they convert voltage changes into mechanical forces, and where are the molecular motors that generate movement?

By combining patch-clamping and microscopy techniques (10), we found that the movement associated with outer hair cells can be observed directly in isolated membrane patches from the lateral surface of cells. In this way, we observed that the curvature of membrane patches inside a patch pipette increases (i.e., the membrane bows out) when the membrane is hyperpolarized — regardless of the hydrostatic pressure on the patch. This observation demonstrates that the electric field across the membrane is providing the energy for the motion, leading us to conclude that the motor and voltage sensor may be one and the same molecule within the membrane.



The lateral plasma membrane of the outer hair cells of the organ of Corti contain "force-generation units" composed of small domains of a semicrystalline array of motor proteins. Pillars, connecting these motor proteins to an actin-spectrin meshwork inside the membrane, convey the forces generated in the plane of the membrane to the cell's interior. We propose that the pillars are composed of the anion-exchanger protein and 4.1-band proteins, but it's unclear whether these proteins are just force conveyors or are themselves the motor proteins.

The distribution of motor activity along the lateral plasma membrane of the outer hair cell coincides with the distribution of arrays of closely packed transmembrane proteins (10). The high density of such molecules should allow lateral interaction among them, so changes in the subunits or reordering of the particle array could generate lateral expansion of the membrane. For example, simple calculation shows that an alteration in the subunit packing of a tetrameric aggregate observed in the hair cells could produce a change in area of at least the required amount predicted by the data. Researchers in several labs have observed that the lateral plasma membrane is visibly connected by pillar-like protein structures to an anisotropic actin-spectrin meshwork on the interior of the cell (11). The actin meshwork extends the length of the cell and could convert membrane-area changes into axial cellular forces.

Ashmore (7) and others recorded charge movements in the lateral plasma membrane during outer-hair-cell electromotility. This intramembrane change in charge distribution is analogous to the gating current of voltage-dependent ion channels and confirms, in neurons, for example, the presence of an extremely high density of voltage-sensing proteins that can undergo conformational change.

Using immunocytochemistry, we have shown that the anion-exchanger protein (AE) and band 4.1 proteins are associated with the plasma membrane at the site of force generation (12) in contractions of outer hair cells. We have also proposed that these proteins form pillars that connect the plasma membrane to the cortical actin-spectrin lattice. Members of the AE-protein family are known to be involved in regulation of cellular volume and pH, as well as in the mechanical coupling of the plasma membrane to the cortical cytoskeleton. This coupling is essential for funneling forces generated in the plane of the plasma membrane into the longitudinal axis of the cell. Indeed, sulfhydryl agents, which selectively bind to the hydrophobic pockets of AE proteins, inhibit electromotility of outer hair cells.

Using a different approach, we screened an organ of Corti cDNA library for isoforms from the AE-protein family, and we identified a transcript that encodes an isoform with an unusual membrane-spanning domain. We have determined with specific anti-peptide antibodies that this transcript is indeed expressed in outer hair cells along the lateral plasma membrane. The characterization of this novel AE is currently the focus of our effort to understand the role that these proteins might play as membrane-cytoskeletal linkers in hair cells and their potential involvement in the force generation in outer-hair-cell electromotility.

This novel molecular mechanism for force generation, so conceptually different from other mechanisms of cell motility, may well prove important beyond the realm of auditory cell biology. Some form of this mechanism may be expressed in other cells that undergo membrane-potential changes. Understanding the mechanism by which voltage is converted into membrane protein-conformation change in this system may add to our knowledge of other analogous systems, such as the gating of voltage-sensitive channels.

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Arf Proteins: From Cholera Toxin Cofactors to Masters of Membrane Regulation

Most recent reviews of the properties of biological membranes convey one of two overly narrow views. Some portray biological membranes as molecular putty readily fashioned into different shapes (vesicles, tubes, and sheets) by proteins or protein complexes. Others portray the biophysical properties of the amphipathic lipids themselves as sufficient to explain all the diverse and dynamic changes occurring at each biological membrane surface. A remarkable convergence of recent information from cell biology, neurobiology, and studies of the yeast secretory pathway have yielded several proteins (e.g., NSF, SNAPs, and synaptobrevins) that appear to serve a common role in membrane traffic, synaptic transmission, and organelle remodeling (1). Although this exciting development should not be minimized, what is lacking from the emerging models of regulated and constitutive endo- and exocytosis is an integrated view encompassing the roles of these proteins and of the lipid components in membranes.

One reason this synthesis has not emerged is that we simply don't know how the cell "reads" and processes information inherent in the lipid composition of a membrane and integrates it into interactions with integral and peripheral membrane proteins. Now, a family of regulatory GTP-binding proteins has been observed to possess many of the features predicted for integrators of such interactions. These proteins, called ADP–ribosylation factors, or Arfs, have recently been found to 1) interact with specific lipid components of membranes, 2) modify the lipid composition and release potential second messengers through activation of phospholipase D (PLD), and 3) regulate the assembly of at least a subset of protein complexes or membrane coats (for reviews, see refs. 2 and 3). In short, Arf proteins appear to be both sensors of the lipid environment and transducers of information (including information inherent in lipids), which results in changes in Arf activities and the consequent assembly of protein structures on the membranes.

Arfs are ubiquitous in eukaryotes

Genes for GTP-binding Proteins

The name ADP-ribosylation factor derives from the discovery, in 1984, of the factor that confers sensitivity of the stimulator of adenylate cyclase (Gs) to cholera toxin, an ADP–ribosyltransferase (4). In the ensuing years, the number of distinct genes discovered that encode low-molecular-weight GTP–binding proteins has increased to more than 100. The products of these genes can be conveniently (but not always accurately) lumped into four sub-families of the Ras superfamily: the Ras, Rab, Rho, and Arf proteins. Six mammalian Arf proteins have been identified, five of which appear to be expressed in all cells and tissues; Arf2 has, so far, been found only in cows. Arfs are ubiquitous in eukaryotes, are very highly conserved both structurally and functionally, and exchange guanine nucleotides in a complex, highly regulated fashion that is sensitive to lipids, salts, and divalent metals. A larger sub-group in the Arf family is the Arf-like proteins, which are structurally related (typically 40–60% identical to any Arf) but lack defined Arf activities, such as acting as the cholera toxin cofactor for Gs (5).

A role for Arf proteins in membrane traffic has now been confirmed by several independent techniques. Arf proteins' role in protein secretion was first observed in the yeast *Saccharomyces cerevisiae* (6). Localization of Arf proteins to the cytosolic side of (predominantly) *cis*-Golgi structures, and later other Golgi-related structures, further supports the conclusion that a major site of Arf action in mammalian cells is the Golgi complex. However, Arf plays other roles in membrane traffic, and its other sites of action now include the endoplasmic reticulum, the nuclear envelope, and elements of the endocytic pathway, likely the plasma membrane and early endosomes. We learned this from in vitro assays of membrane traffic, including those for intra-Golgi transport and nuclear vesicle and endosome fusion, which are inhibited by GTPgS and require the addition of cytosol or a cytosolic factor (7,8). In two of these cases (intra–Golgi transport and nuclear vesicle fusion), the cytosolic factor was purified to homogeneity and shown unambiguously to be an Arf protein. Independent studies have confirmed roles for Arf proteins in the movement of proteins out of the ER and Golgi and in endocytosis. Expression of dominant activating alleles of Arf1 blocks proteins from exiting the ER (9) and causes dramatic expansion of the ER lumen (10), expansion and vesiculation of the Golgi stacks with inhibition of protein secretion (10), and loss of fluid-phase endocytosis (10). These data indicate that Arf proteins have dynamic roles in the maintenance of the integrity of several organelles, particularly the Golgi and ER.

Arfs' Role in Membrane Traffic

Although more sites of Arf action have been discovered over the past 10 years, details of the molecular actions of Arf proteins remain poorly understood. Some insights have come from the recognition of the roles of coat proteins in membrane traffic in general and in Arf action in particular. Arf proteins have been found on both clathrin-coated (11) and non-clathrin-coated, or Golgi–derived (12), vesicles. The major constituent of nonclathrin-coated vesicles is a protein complex, termed coatomer. Coatomer has been purified from cytosol and is made up of seven coat proteins (designated a through z COP), which appear to exist as a stable heptamer, cycling between soluble and particulate pools (13). A role for Arf proteins as regulators of the reversible binding of coatomer to Golgi-enriched membranes is implied by the demonstration that when cytosol is depleted of Arf proteins it fails to support coatomer binding, whereas addition of purified recombinant Arf1 is sufficient to restore GTPgS-stimulated coatomer association with membranes (14). In addition to the observation that in vitro binding of coatomer to membranes appears to require Arf, the Arf itself has been shown to bind reversibly to phospholipid micelles and membranes in a GTP-dependent manner (15). Thus, regulated binding of guanine

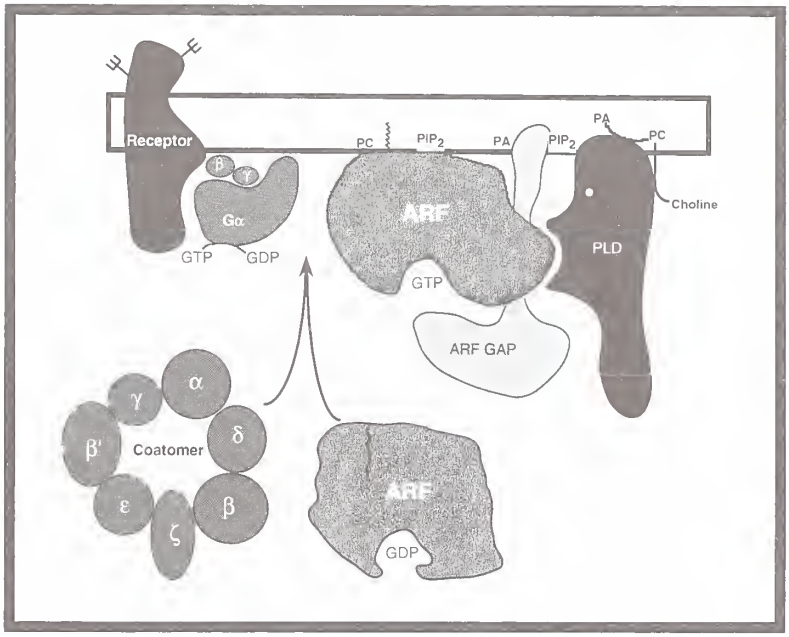
by Richard A. Kahn, Laboratory of Biological Chemistry, NCI

nucleotides to Arf is proposed to control the association of coat proteins with membranes.

Arf proteins have three biochemical properties that distinguish them from all other GTP–binding proteins: 1) GTP binding is highly dependent on phospholipids (16), 2) the activated protein, Arf•GTP, stably associates with phospholipid micelles or membranes whereas Arf•GDP is soluble (15), and 3), in the absence of GTPase activiting Protein (GAP), purified Arf proteins have no detectable intrinsic GTPase activity (17). These observations support the idea that the reversible interaction with membrane phospholipids and the binding of guanine nucleotides are highly integrated.

Our understanding of Arf action changed dramatically last year with the demonstration that Arf is an activator of phospholipase D (PLD), the membrane–bound enzyme that catalyzes the hydrolysis of phosphatidylcholine (PC) to phosphatidic acid (PA) and choline (18,19). PLD has been implicated as an effector of several growth factors, though the roles of phospholipases C and D are somewhat confused as a result of the cell's ability to interconvert the products of the two lipases (20). Brown et al. (18) found that phosphatidylinositol-4,5-bisphosphate (PIP_2) is required for Arf stimulation of PLD activity and that Arf-dependence of PLD activity was maintained through several steps of purification, suggesting a direct interaction between Arf and PLD. The activation of a specific enzymatic activity by Arf provides the first enzymatic effector for Arf actions and contrasts with the view of Arf as a structural protein constituent of organelle or vesicle coats.

Further evidence of a complicated interrelationship between Arf action and membrane phospholipids is provided by research on Arf GAP. GAP activates the latent GTPase activity, which leads to the termination of the activated state of Arf. For at least one other GTP-binding protein, Gq-stimulated PLC–b, the effector was also shown to act as a GAP (21). The initial characterization of a specific Arf GAP was recently reported by Randazzo and Kahn (17). Arf GAP activity is highly dependent on PIP_2, can be further stimulated by PA, the product of PLD, and is inhibited by PC, the substrate of PLD.



Molecules important in Arf signaling: a heterotrimeric protein, Gs; Arf GAP, phospholipase D (PLD), and a heptameric complex, coatomer. Translocation of the Arf and coatomer from cytosol to membrane-bound forms is indicated by the arrows. Interactions of Arf, Arf GAP, and PLD with specific phospholipids are also indicated within the boxed area, which represents a membrane bilayer. (PA, phosphatidic acid; PC, phosphatidylcholine; PIP_2, phosphatidylinositol-4,5-bisphosphate)

The requirement for PIP_2 in two different Arf-dependent assays — for PLD and Arf GAP — suggested a potential common site of action for this acid phospholipid. Direct effects of PIP_2 on Arf proteins are described in Terui et al. (22) and include an increase greater than 100-fold in the rate of GDP dissociation and, paradoxically, loss of GTP binding. PIP_2 promotes the dissociation of GDP and binds to and stabilizes the nucleotide-free form of the protein. These effects of PIP_2 on Arf are homologous to the actions of the guanine nucleotide exchange factor, Cdc25, on Ras (23). The specificity and magnitude of the effects of phospholipids on Arf properties in vitro make it very likely that these same interactions are important in vivo and raise the possibility that PIP_2 may play the role of a nucleotide-exchange factor for Arf in cells.

Structural and mutagenesis studies have begun to reveal details of several specific Arf interactions. Arf has at least two distinct protein-binding domains, one that requires an intact amino terminus that is required for Gs and Arf GAP binding and one that is less sensitive to deletion of the amino terminus and to which cholera toxin binds (24). In addition, Arf has two distinct phospholipid-binding sites. One is a relatively non-specific site that requires an intact amino terminus, confers lipid dependence on nucleotide binding, and helps to stabilize the active form of the protein. The other is a highly specific PIP_2 site whose occupancy promotes stability of the apoprotein (22). Comparisons of the crystal structures of Ras and Arf are now possible because the Arf•GDP structure has been solved (25). This model will allow directed studies of the residues involved in Arf's interactions and will provide a structural framework on which to build further models to explain all of Arf's activities.

The physiological relevance of our observations on Arf remain to be established, but it seems reasonable to suggest that several of the key regulators and effectors of Arf actions are those identified by the in vitro studies. When the actions of any Arf protein are being consided, the proximate membrane and its specific lipid composition must now also be

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Tissue Microdissection: Ultraclean Cell Samples for Genetic Analysis

by Michael Emmert-Buck, M.D., Ph.D.; NCI, Lance Liotta, M.D., Ph.D., NCI; and Zhengping Zhuang, M.D., Ph.D, NCI

Our laboratory recently developed a simple, precise system for isolating and genetically microanalyzing extremely small tissue samples. The method allows for the reproducible amplification of DNA or mRNA from individual cells selected microscopically — from a patient biopsy, for example.The dissection can be carried out on very small samples that are easy to obtain, prepare, and store, such as single 10-µ sections of frozen or paraffin-embedded, formalin-fixed archival material. We believe that this could be a fundamental gateway technique allowing basic and clinical biomedical researchers in a wide range of subfields to dramatically increase the precision of localizing normal and pathological entities, processes and changes.

In our lab, the application of this technique ranges from the discovery of new tumor-suppressor loci involved in cancer susceptibility and progression to genetic diagnosis of cancer and infectious diseases at the microscopic level. This approach could potentially be extended to other genetic analyses in which localization to minute cell clusters is desirable, such as the examination of tissue obtained from transgenic animals or from patients undergoing experimental gene therapy. Other possible applications include pinpointing latent viral infection or tracking the biochemical features of specific cells within heterogeneous tissues.

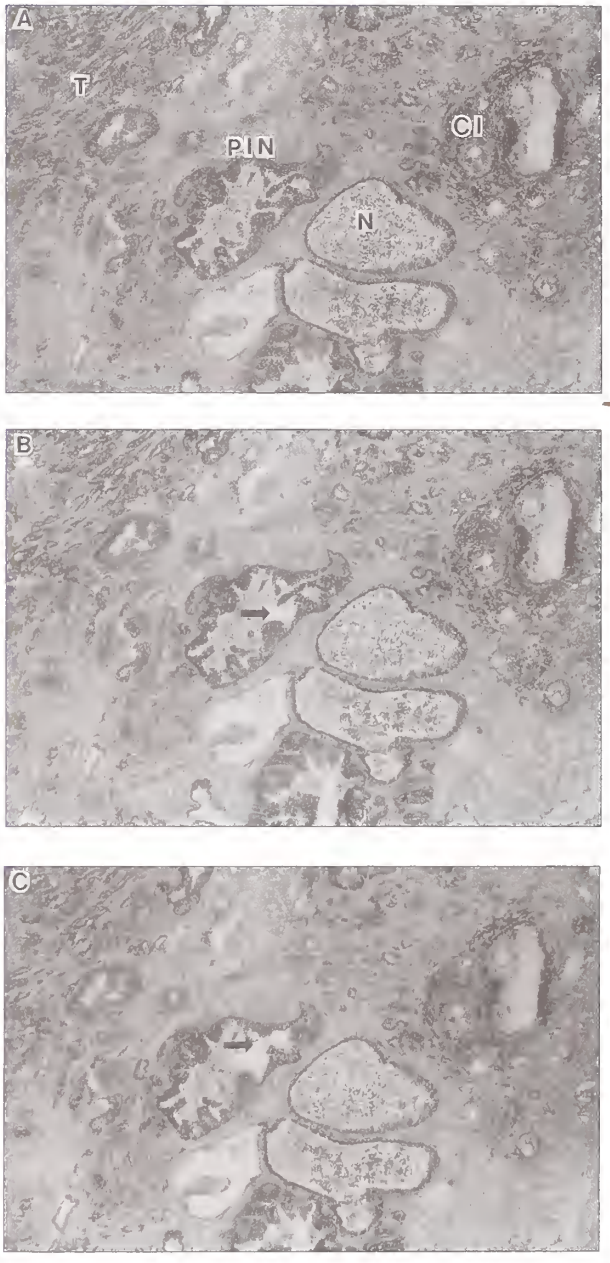

Figure 1. Microdissection of prostate carcinoma. *Histologic field of invasive prostate carcinoma (panel A). Invasive tumor (T), normal prostate epithelium (N), and a focus of chronic inflammation (CI) are present. The large structure in the middle of the section is a focus of prostatic intraepithelial neoplasia (PIN), which appears to be progressing to invasive cancer. Two separate papillary proliferations are procured for analysis (panels B and C, arrows). Recent studies in collaboration with Paul Duray of the Laboratory of Pathology, and Marston Linehan's group (Rudy Pozzatti, Cathy Vocke, Scott Jennings, and Charles Florence) of the NCI surgical branch indicate the putative in situ tumors are genetically similar to invasive prostate cancer, suggesting that they are indeed precursor lesions.*

The Method and How It Works

In the past, tissue heterogeneity has posed a significant problem for investigators conducting genetic analysis of pathologic lesions surgically removed from patients or experimental animals. Tissue sections typically contain multiple cell types. For example, a breast cancer biopsy contains normal epithelial cells, myoepithelial cells, stromal cells, endothelial cells, inflammatory cells and fat cells, as well as cells from muscle and nerve. The actual cancer cells may constitute significantly less than 50 percent of the cells in the tissue sample. Consequently, if the tissue is homogenized, the recovered DNA or mRNA will reflect an average from many cell types and not the specific DNA or mRNA of interest. This problem is compounded further in the genetic analysis of the progressive stages of cancer, in which the cells of interest can only be located with high-power microscopic visualization. Normal and possibly premalignant contaminating host cells pose a significant research impediment to the analysis of chromosome loss of heterozygosity (LOH) because the contaminating cells, with two copies of the allele, will mask the loss of an allele in the tumor cells. This is particularly problematic in assays that use the polymerase chain reaction (PCR), in which an allele from contaminating normal cells can become significantly amplified. Studies using mRNA from microdissected cells, particularly studies screening for mRNA differences between two or more cell populations, e.g., differential display patterns in normal epithelium vs. dysplastic epithelium or in situ carcinoma vs. invasive carcinoma, absolutely require finely microdissected samples that select only the particular cells of interest. Any high-copy mRNA from contaminating cells will interfere with these experiments.

Our method of tissue microdissection differs from previously published methods for tissue microdissection used to study human tumors. Earlier microdissection methods required the procurement of a large piece of tissue from a histologic slide of a tumor with an abundance of malignant cells. Selection of an optimal quadrant enriches the malignant cell content in the specimen but does not provide the sample purity necessary for many PCR-based assays, particularly those using mRNA.

To overcome the drawbacks of these previous approaches, our microdissection method amplifies DNA or mRNA from much smaller, purer samples, that is, cells that are singled out and removed from histologic tissue sections under high-power microscopy. The individual cells or groups of cells, which have been stained with eosin, are excised by electrostatic attraction with a 30-gauge needle and placed in a single-step extraction buffer, which provides the starting point for PCR amplification.

In our laboratory's research on genes associated with breast cancer, this microdissection method enables investigators to sample the DNA from pure populations of normal epithelium, in situ carcinoma, and invasive carcinoma, all in the same 10-μ section of a patient's biopsy. Studies in collaboration with Maria Merino and Rodrigo Chuaqui, also of the Laboratory of Pathology, NCI, show a novel allelic loss on chromosome 11q13 in 70% of the cases of human breast carcinoma studied (n = 70). We observed the allelic loss in both in situ and invasive components of the tumors. In all cases, the identical allele was lost, providing the first molecular evidence to support the long-held hypothesis that in situ breast cancer is a precursor to invasive cancer.

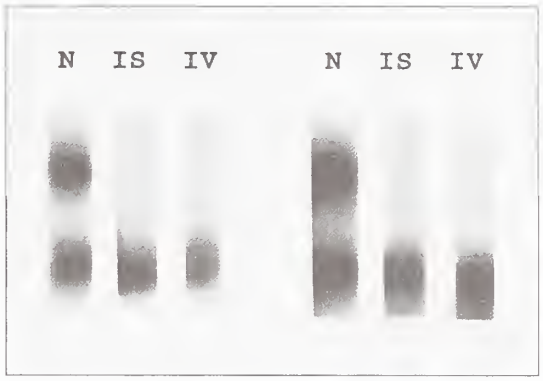

Figure 2. Detection of allelic loss of chromosome 11q13 in in situ and invasive breast lesions. *Analysis of chromosome 11 loss of heterozygosity by PCR amplification using specific primers for PYGM, a polymorphic DNA marker on 11q13. Microdissection of these two biopsies of breast cancer show normal epithelium (N), in situ tumor (IS), and invasive tumor (IV). PYGM analysis revealed loss of heterozygosity in the in situ and invasive lesions.*

The polymorphic DNA marker used in this study was PYGM, located on chromosome 11q13. Reactions were cycled in a Perkin Elmer Cetus thermal cycler as follows: 94°C for 1.5 m, 55°C for 1 m, 72°C for 1 m, for a total of 35 cycles. PCR was performed in 10 μL volumes and contained 1 μL 10X PCR buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM MgCl2; 0.1% (weight-to-volume) gelatin; 2 μL of DNA extraction buffer; 50 pM of each primer; 20 nM each of dCTP, dGTP, dTTP, and dATP; 0.2 μl [32P] dCTP (6000 Ci/mM); and 0.1 unit Taq DNA polymerase. Labeled, amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide; 20 mM EDTA; 0.05% bromophenol blue; and 0.05% xylene cyanol). The samples were denatured for 5 m at 95°C and loaded onto a gel consisting of 6% acrylamide (49:1 acrylamide:bis). Samples underwent electrophoresis at 1800 volts for 2 to 4 hours. Gels were transferred to 3mM Whatman paper, dried, and autoradiographed using Kodak X-OMAT film. The criterion for loss of heterozygosity from the microdissected in situ and invasive breast samples was complete absence of an allele.

Protocol

DNA Analysis from Tissue Samples

1) 10-μ sections of formalin-fixed, paraffin-embedded tissue, or of frozen tissue are prepared on a glass slide per normal surgical pathology protocol (4). mRNA is recovered more efficiently from frozen tissue.
2) Slides with paraffin sections are de-paraffinized by two 5-minute baths in xylene, followed by similar pairs of 2-minute baths in 95% ethanol, 50% ethanol, and distilled water. The slides are then air dried.
3) Slides with frozen or de-paraffinized sections are stained briefly in eosin (1% eosin in 80% ethanol) and air dried.
4) An adjacent hematoxylin- and eosin-stained section is used to scout out the tissue section for optimally clean sites for microdissection—for example, areas in which specific small cell populations of interest are comparatively isolated and free of significant inflammation or other contaminating cells.
5) Microdissection of selected populations of cells is performed under direct light-microscope visualization using an inverted microscope and a sterile, 30-gauge needle. Experienced microdissectors can reliably recover pure cell populations of five or fewer cells, as well as cells arranged as a single layer, such as normal epithelium or the epithelial lining of cystic lesions.
6) Cells of interest are detached from the slide by gentle scraping and will adhere to the tip of the needle via electrostatic attraction.
7) Those cells are immediately suspended in a pH 8.0 solution containing 0.01 M Tris-HCl, 0.1 M ethylenediamine tetraacetic acid (EDTA), 1% Tween 20, 0.1 mg/mL proteinase K, and then incubated overnight at 37°C. For optimal PCR amplification we

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MAKING THE GRADS AT NIH: JOINT GENETICS PROGRAM ENTERS SECOND YEAR

by Janet Joy

Even though NIH isn't a university, more graduate students are working in the life sciences here than at many universities. Michael Fordis, Director of the Office of Education, estimates that there are about 170 graduate students on the NIH campus. For most of these students, graduate training at NIH is independently arranged on an ad hoc basis. For the graduate students in the two-year-old NIH-George Washington University (GW) Graduate Program in Genetics, however, the situation is very different.

Under this program, developed by the Office of Education, GW provides a tuition waiver and students receive a predoctoral Intramural Research Training Award (IRTA) fellowship from NIH for support during their graduate training. During their first six to eight months, students work in up to three different laboratories before choosing a thesis adviser. Over the next two years, they do research and complete their course work at GW. The program is small, limited to five students per entering class.

Students in the first class, which entered in the fall of 1993, selected laboratories in NCI, NIAID, NIAMS, and NINDS for their thesis research. Graduate students in the class that entered last fall will be doing rotations in NCI, NCHGR, NHLBI, and NINDS.

Andrea Kamage, an NIH-GW graduate student working on tumor vaccine projects with Judith Kantor of NCI, says she chose the program because "I got the university setting plus I got the NIH setting. At every other university I looked at, there was this tiny little group of students and that's all you'd ever see." Susan Zullo, who is doing her graduate work on developing a novel viral vector for gene transfer into the central nervous system with Joseph Higgins of NINDS, emphasizes the tremendous diversity of research opportunities. "You can work on just about anything you want here," she says. "The flexibility of the Genetics Program allows you to custom make your program."

> "I GOT THE UNIVERSITY SETTING PLUS I GOT THE NIH SETTING. AT EVERY OTHER UNIVERSITY I LOOKED AT, THERE WAS THIS TINY LITTLE GROUP OF STUDENTS AND THAT'S ALL YOU'D EVER SEE."
> —ANDREA KAMAGE, NIH-GW STUDENT.

NIH offers tremendous opportunities for graduate students, but it may prove daunting for students accustomed to campus life and student culture. "It's best for someone who is mature, self-motivated, and organized," Kamage says "Definitely, you have to be very independent because you're not going to have many people in your lab in the same situation as you are. More than likely, you'll be the only graduate student in the lab."

Klaus Strebel of NIAID cautions that the first year or two can be trying for both advisers and graduate students because the students must take a fairly heavy course load and are only in the lab part time. This means that the adviser essentially gives up a full-time position to a part-time worker. However, Strebel was very supportive of his student Mary Karczewski, saying that she has made important contributions to his lab's work and will be a co-author on several publications. In an attempt to understand how the virus infectivity factor (vif) protein of the human immunodeficiency virus-type 1 (HIV-1) is involved in infectivity, Strebel and Karczewski are trying to identify the subcellular distribution and to determine whether vif interacts with any specific structures in the cell and whether it directly affects any other viral proteins.

Students interested in the NIH-GW Genetics Program may obtain an application from either GW or the NIH Office of Education. The applicants are initially screened by GW and then by NIH, and the top candidates are invited to NIH for interviews with interested faculty. Final selection is based on the decision of each Institute, Center, or Division to support the student.

Candidates for the next class of students will be interviewing at NIH in the late winter and early spring. The Office of Education assists the students in identifying appropriate supervisors and helps arrange interviews. Once a student enters the program, the Office of Education is available as a central facility for graduate student services. All NIH investigators who work in the broadly defined area of genetics are eligible to serve as research advisers to students in the NIH-GW program. For more information on the program call the Office of Education at 496-2427. ■

Changing of the Guard

The NIH Catalyst would like to thank its founding Managing Editor, Seema Kumar, who is leaving after two years to become Science Editor at the Whitehead Institute for Biomedical Research in Cambridge, Mass. The new Managing Editor is Rebecca Kolberg, a science journalist who has written on biomedical topics for *Journal of NIH Research*, *Science*, *New Scientist*, and various consumer publications. Her latest position was Editorial Producer at Medical News Network, an interactive television venture for physicians. ■

RECENTLY TENURED

Jeffrey Cohen *joined the Medical Virology Section of the Laboratory of Clinical Investigation, NIAID, in 1991 and is now a Senior Investigator there. He received his M.D. from Johns Hopkins in 1981.*

My laboratory focuses on the molecular pathogenesis of human herpes viruses. The major approach has been to probe viral-gene functions by deleting or mutating target sequences within the genomes. I developed a system to produce mutations in the Epstein-Barr virus (EBV) genome by transfecting cells with cosmid DNAs to obtain recombinant viruses. Using this system, I showed that the EBV nuclear antigen-2 (EBNA-2) protein is required for B-cell transformation by the virus. Further experiments identified the specific domain of the EBNA-2 protein that is responsible for activating transcription. By deleting this domain or replacing the domain with a transactivating region from herpes simplex virus VP16 protein, we demonstrated that this domain is required for transformation of B cells by

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The Lab Behind the Leader, New NINDS Director

Zach Hall, who recently assumed the helm of NINDS, has outlined his leadership goals elsewhere. But it was the scientific interests and background of the new Director of NINDS that most intrigued* The NIH Catalyst. *Hall, who has made fundamental contributions to the understanding of the neuromuscular junction, came to NIH from the University of California in San Francisco, where he was Chair of the Department of Physiology and head of the Biological Science Training Program. The founding editor of* Neuron, *a leading journal of cellular and molecular neurobiology, Hall offers this description of his research activities.

Our laboratory studies the neuromuscular junction as a model of synaptic structure and function in the nervous system. Our recent work has focused on two areas: the mechanisms of assembly of the acetylcholine receptor (AChR) and the role in synaptogenesis of agrin, a neurally-secreted protein that causes AChRs to cluster.

The AChR is an ion channel composed of four different highly homologous polypeptide subunits ($\alpha_2\beta\gamma\delta$) that surround a central aqueous pore. We have investigated the assembly of the subunits in muscle cells and in COS cells and have found that assembly occurs according to a defined pathway in which the first step is the formation of the two heterodimers, $\alpha\delta$ and $\alpha\gamma$. Each subunit has a stereotyped transmembrane orientation with a long, extracellular *N*-terminal domain, four transmembrane domains, and a large cytoplasmic loop between the third and fourth transmembrane regions. Experiments with dominant-negatives and with chimeric subunits indicate that the identify of each subunit during assembly is determined by the *N*-terminal domain in the lumen of the endoplasmic reticulum (ER), where AChR assembly takes place. These domains mediate the interactions leading to heterodimer formation; interactions in later steps in the pathway appear to also involve amino acid sequences in the long, cytoplasmic loops of the subunits. Before the subunits assemble, their lumenal domains must fold. Although we have found that the unfolded subunits are associated with BiP, a chaperone protein resident in the ER, the kinetics of formation of the complex that we detect is incompatible with the protein having a role in subunit folding. We have recently begun to study the folding process in an in-vitro translation system.

Zach Hall

An early event during the formation of the neuromuscular junction is the clustering of AChRs in the postsynaptic membrane underneath the nerve terminal. The clustering occurs, at least in part, in response to a protein, agrin, that is released from motor nerve terminals. Neurons and muscle cells express different forms of agrin, which are generated by alternative RNA splicing. How agrin causes AChR clustering is unknown. We have investigated the action of agrin on cultured muscle cells in several ways. First, In collaboration with the laboratory of Richard Scheller of Stanford University, we have examined the ability of the various forms of agrin to cluster the AChRs of cultured muscle cells. The most active forms, found exclusively in neurons, are those containing an eight-amino acid insert at one of the splicing sites. Other forms of agrin, found in muscle and other tissues, are significantly less active. Second, we found that a variant of the C2 muscle cell line that is defective in the synthesis of proteoglycans is much less sensitive to agrin than is the parental line, suggesting that proteoglycans are involved in the action of agrin. Finally, we have looked for agrin-binding proteins in muscle that might mediate the action of agrin in muscle cells. Our results and those from several other labs show that the major agrin-binding protein in muscle cells is α-dystroglycan, an extracellular matrix protein previously identified as part of a complex of proteins associated with dystrophin. Because dystrophin is associated with actin and α-dystroglycan binds laminin, the complex is thought to form a transmembrane linker between the extracellular matrix and cytoskeleton. We are currently investigating whether the interaction of agrin with α-dystroglycan is responsible for the formation of AChR clusters, or whether it plays some other role. ■

CELEBRITY SCIENTISTS
continued from page 1.

the result of a dream (bad?). But I quickly realized that it was true because, according to my wife, I became strangely passive after friends from Sweden called to congratulate me. I believe this passivity came as a result of being told almost every year for more than a decade that I would get the Nobel Prize. It became so engrained in my mind that I decided, a few years ago, not to think about it. The thought was buried somewhere in my neuronal network, only to be aroused by a bell, something like the warning of an e-mail message emanating from the computer.

Calm and composed, I went through the first day as if it were just another day ... except that I had to meet the press. There they were, packed like sardines in a small auditorium with flashing lights and hordes of camera-bearing slaves accompanying the reporters. Suddenly, I seemed important. Out of my mouth spewed forth a stream of words, many of which certainly had been stored for several years for that special moment when someone, somewhere, might listen to them. I couldn't stop. Reporters seemed to take down everything as if I were Moses handing them the Ten Commandments. They even applauded at the end, suggesting that they were just as crazy as I. We were all celebrities at that moment! We were all crazy, and in my mind, that is truly what it means to be a celebrity. Andy Warhol had it right — every person should have 15 minutes as a celebrity. I just hope that the experience disappears just as quickly.

Futreal: It was actually rather surreal. We have been working hard for four years, and to finally get to the goal we were chasing was immediately exciting and accompanied by a sense of relief. As for the overnight celebrity aspect, that, too, was rather disconcerting in some aspects. It was nice for us to be recognized as being successful and, more importantly, as having been a key component in making a very important step in this crucial area.

Q: *What impact do you expect this fame to have on your research, your career, and your relationships with colleagues?*

Rodbell: This fame will certainly change my life. I'm already being treated as if all of the brilliance in the world is embodied in Marty Rodbell. Invitations to speak at Rotary Clubs have the highest priority. Since retirement, I have been giving lectures before senior citizen groups in the Research Triangle [Park, N.C.] area, and I am slated to talk before high school students in Chapel Hill. I have a small lab at NIEHS and intend to keep it within the limits of our meager budget and space. I doubt that this situation will change, given the budgetary and other problems at the Institute. I intend to use my new-found fame in a constructive manner by speaking out to any forum about my feelings on science policy and the role of science in society. As for my relationships with colleagues, they will remain as warm and interactive as ever. My personality will never change, certainly not because of the prize and all the hoopla ... Hopefully, that will blow over quickly, and I can return to practicing my piano, playing tennis, writing, and dreaming — a wholesome, fruitful time for me.

NIEHS administrators and researchers celebrate the isolation of the BRCA1 *gene. Left to right, Kenneth Olden, Director of NIEHS; Carl Barrett, Acting Director of Environmental Carcinogenesis Program; Roger Wiseman, Senior Staff Fellow at Laboratory of Molecular Carcinogenesis (LMC); and Andrew Futreal, postdoc at LMC.*

Futreal: Obviously, I hope this will have the effect of providing the opportunity to pursue my own research ideas in a lead-independent fashion. The cloning of *BRCA1* was just the first step. A more difficult task lies in determining function and biology for the gene and, ultimately, translating this knowledge into useful clinical information, either through *BRCA1* itself or through knowledge of its partners and pathways. Careerwise, it has certainly made being able to pursue and obtain a desirable position very feasible. My relationship with colleagues has remained productive. I don't feel that those core relationships have really been altered in any significant way. Of course, the opportunities to interact with a more diverse group of investigators have increased, an aspect I really enjoy.

Q: *What's been your experience in dealing with the press? Are media inquiries encroaching on your day-to-day work? How would you rate the news coverage of your research? What advice would you offer a colleague who's suddenly forced to face the press?*

Rodbell: As for dealing with the press, I found them to be very interested in the type of science that deals with communication, which is my field. We quickly found a common language. However, I am amazed at the variety of interpretations they came up with. Accurate? No. Interesting? Yes. More importantly, they seem to accomplish their role in "transducing" my thoughts to the public. What more can one ask? Advice: talk to the press as if you were talking to your colleagues. One must act natural and

talk freely about your thoughts. Don't be a pompous ass!

Futreal: On the whole, I really think that my interactions with the press have been very positive. However, there are always things that are taken out of context, and there are instances in which you wish you had been more articulate. The press interfered somewhat with day-to-day work, which was to be expected, given the media interest in this gene. It's been more a problem of getting large blocks of time to do experiments. Overall, I think the press has done a good job in putting the cloning of *BRCA1* in its proper cautionary perspective, relative to what it means in both immediate and long-term time frames. As for advice on "meeting the press," that's a tough question, partly because my media experience is still a blur and because news coverage probably varies from case to case. Just make sure that your shirt is tucked into your shorts! ■

RECENTLY TENURED
continued from page 13.

EBV. In more recent experiments, we inoculated mice with severe combined immunodeficiency with a panel of EBV mutants that differ in their EBNA-2 sequences. The pattern of EBV-induced tumor formation in the mice paralleled the ability of the mutants to transform cells in vitro. Currently, my research focuses on EBV genes that help the virus to evade host immune responses.

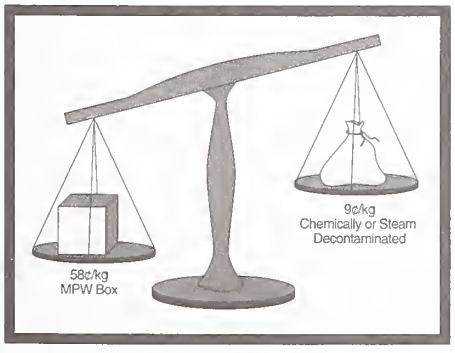
Last year we reported on an in vitro system for producing a recombinant form of the varicella-zoster virus (VZV), which causes chicken pox. We constructed a set of four cosmids that encompass the 125-kb VZV genome. Transfection of cells in culture with these four cosmids results in recombination and production of infectious VZV. Using this system, we introduced mutations into the viruses, which are being tested in a guinea pig model of VZV infection. In addition, a herpes simplex virus glycoprotein gene was inserted into the VZV genome, and the recombinant VZV functioned as a vaccine to protect guinea pigs from challenge with herpes simplex. The VZV strain under study is the live, attenuated vaccine virus that may be approved for universal immunization of children in the United States. This vaccine strain becomes latent in the body and, unfortunately, can reactivate to produce herpes zoster (shingles). We are trying to define which viral gene(s) may be responsible for development of latency or reactivation. By inducing mutations in these latency-associated genes, we may be able to produce a safer vaccine that cannot reactivate and cause shingles. ■

SCIENTISTS TENURED AUGUST 1994 TO DATE

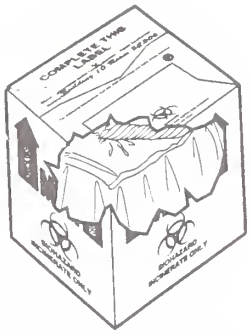
David Bodine, NCHGR
John Cidlowski, NIEHS
Seong-Jin Kim, NCI
Stephen Wank, NIDDK
Lois Travis, NCI

WASTE DISPOSAL
continued from page 1.

dar, contains more detailed information on waste-decontamination packaging and disposal. The bright yellow Waste Calendars were delivered to NIH labs late last year. For a copy of the Waste Calendar or additional information on how you can help reduce waste at NIH, call your Institute's, Center's, or Division's Occupational Safety and Health Specialist at 6-2346. ■



Comparative Costs of Disposal. MPW Boxes vs. Chemically or Steam Decontaminated Biological Waste

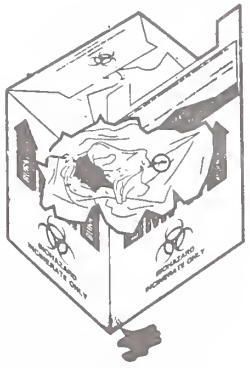


Proper MPW Packaging:

- Put only MPW into MPW burn box
- Use TWO bags for MPW contents
- Seal each bag separately
- Seal sharps containers
- Complete box label
- Keep weight less than 40 lbs
- Minimized MPW volume

Avoid MPW Packing Mistakes:

- Failing to complete label on box top
- Including non-MPW, i.e., soda cans, newspapers, office paper, food scraps, etc. - increasing the volume of material to be handled as infectious waste.
- Packing too much in box, flaps won't close easily and box may open in transit
- Packing sharps objects which can puncture bags or box side - permitting contents to seep out of box
- Failing to use TWO bags and/or failing to seal separately - allowing fluids to leak out of box during transport



SEMINAR HIGHLIGHTS
continued from page 5.

other gene, the mannose-6-phosphate receptor, Denise Barlow and her colleagues in Vienna took advantage of a spontaneous mutation in the form of a large deletion. But for *H19,* there was no useful mutation available and, therefore, we used intraspecific and interspecific F1 hybrids between species of mice that had been separated over an evolutionary time scale — 3 to 6 million years. This was enough time for point mutations to arise in the *H19* gene, and these mutations could be detected by a sensitive RNA-protection assay in which even single base differences between species could be detected. This method, as well as polymerase chain reaction - based methods that exploit the same heterogeneity, are now the standard methods in the field for following the specific expression of the maternal and paternal alleles of a gene in a wild-type animal.

Q: *Do you see any potential areas where this research might provide insight to clinical scientists?*

A: A great deal of important work has implicated defects in imprinted genes in human disorders. The best studied of these are the Prader-Willi and Angelmann syndromes, which are tightly linked on human chromosome 15. What is fascinating about these disorders is that Prader-Willi is inherited paternally, whereas Angelmann is inherited from mothers; thus, they behave as another pair of reciprocally imprinted genes. Recently, Ute Francke's group at Stanford in Stanford, Calif. and Arthur Beaudet's group at Baylor in Houston have identified nonprotein-coding RNAs in the region, implying that many of the features that we have discovered at *Igf2/H19* may also exist at this locus. Furthermore, the *Igf2/H19* region on human chromosome 11 has been implicated in Beckwith-Weidemann syndrome. Both the sporadic and inherited forms of this syndrome display parental bias, suggesting the gain-of-function of a growth-promoting gene, possibly *Igf2.*

Q: *How are you following up on this work, and what questions would you ultimately like to answer?*

A: The most important immediate question that our work raises is whether noncoding genes such as *H19* function through a purely transcriptional mechanism, as implied in Figure 1, or whether the product of the gene plays a role in this or any other biological process. Excitement about *H19* itself was increased with the discovery of another nonprotein-coding RNA, *Xist,* which maps to the X-chromosome-inactivation center. This site is required in *cis* for an X-chromosome to be inactivated. Unlike any other gene on the X chromosome, *Xist* is exclusively transcribed from the inactive X chromosome, and this has led to much speculation about its role in X-chromosome-inactivation.

The long-term puzzle I would like to solve is the function of imprinting. From a genetic point of view, imprinting is a dangerous process because it renders the organism functionally hemizygous for the imprinted gene. One has to assume that there is a compensatory benefit. The most interesting model for what this might be was proposed by David Haig, an evolutionary biologist and currently a Fellow at Harvard University in Cambridge, Mass. He suggested that imprinting is the consequence of a tug-of-war between the male and female genomes for the preferential inheritance of their genes in the next generation. In nonmonogamous species in which the embryo is consuming maternal resources, the male's interest is best served by having his immediate progeny consume those resources at the expense of the mother, with whom he will not likely mate again. The mother, on the other hand, must conserve her resources in any one litter if she is to reproduce again. We are testing this model by examining the evolution of imprinting within mammals, concentrating on marsupials and monotremes, which have very different reproductive strategies from each other. ■

FELLOWS WORKING FOR FELLOWS
continued from page 3.

foreign or visiting fellows, are eligible for the award. The award winners, who will be announced Feb. 15, are selected through a review of submitted abstracts by a committee composed of postdoctoral fellows and tenured investigators. For more information on the Award for Research Excellence, contact Kathy Partin (phone: 496-9347; e-mail: PARTIN@HELIX.NIH.GO).

In FY 1995, the Fellows Committee will sponsor a full-day symposium on current research into a basic biological process. The aim of the program is to provide a coherent review of a topic of general interest to the NIH community, as well as to stimulate interaction among fellows. The symposium, tentatively scheduled for October, will feature leading figures in the field from within and outside NIH, and its sessions will be chaired by postdoctoral fellows. Fellows who are interested in working on the symposium organization committee should contact Thomas Kristie (phone: 496-3854; fax: 480-1560; e-mail: THOMAS_KRISTIE@NIH.GOV).

The Fellows Committee was formed by expanding the Clinical Associates Committee to include both basic science and clinical representatives of each ICD at NIH. Current members are members of the Clinical Associates Committee, appointees named by the Scientific Directors, volunteers, or members of a fellows group organized by NIH Director Harold Varmus. In the future, to increase the representative nature of the panel, one basic science and one clinical representative will be nominated by the fellows of each ICD and will be appointed by the Scientific Director of each ICD. Representatives serve one-year terms, with a maximum of two terms of service.

Committee meetings, which are open to all fellows, are held at 4 p.m. on the first Thursday of every month. For more information, contact Jay Pearson (fax: (410) 558-8393; e-mail: J.D.Pearson@NIH.GOV) or LaRoy Penix (fax: (301) 295-0863). ■

HEARING RESEARCH
continued from page 7.

Clinical Implications

A common disorder of hearing is "ringing of the ears," or tinnitus, which can range in effects from being a minor annoyance to being a cause of suicide. The biological source of tinnitus remains widely debated, but many scientists agree with the idea that spontaneous vibrations of outer hair cells may account for at least some types.

Although only 5% of the afferent fibers of the cochlear nerve terminate on outer hair cells, their essential contribution to hearing is apparent from studies with aminoglycoside antibiotics, such as gentomycin, that can selectively destroy outer hair cells. Clinical and experimental studies indicate that without outer hair cells, our hearing would be 100 times less sensitive. What sound we could hear would be significantly distorted and fuzzy. A better understanding of the motor function of the outer hair cell, so critical to hearing, could eventually help many individuals suffering from hearing impairment.

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ARF PROTEINS
continued from page 9.

considered. Specific interactions between a single protein and lipid components of biological membranes as well as cellular proteins are not limited to members of the Arf family. By combining structural studies with the many functional assays of Arf activity now available, we hope to be able to describe in detail a molecular mechanism that may serve as a model for other proteins that interact reversibly with membranes. Such information may ultimately prove valuable in studies of the etiology and potential repair of defects in cellular physiology associated with a variety of human diseases.

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The Electronic Catalyst

The NIH Catalyst is now available electronically, as is the latest news from Building 1 on the DDIR's Bulletin Board. Current and back issues of both electronic publications can be accessed through the Campus Information Menu on Gopher or Mosaic. ■

HIV/AIDS IN THE WORKPLACE: A TRAINING PROGRAM TAILORED TO SCIENTISTS

As of early January, more than 2,000 intramural scientists still hadn't gotten around to registering for a new HIV/AIDS training program that is mandated by the President.

The streamlined course, which only lasts an hour, is specifically designed for researchers who already have extensive background on the transmission of HIV. Mimi Kravetz of the Division of Workforce Development says that when the HIV/AIDS training program was initiated in March 1994, both scientists and support staff were required to attend a 2 1/2 hour course.

However, a special, shorter curriculum was developed for researchers after complaints that the longer course covered much information that most biomedical scientists already know. "This new course was done for them (intramural researchers) at their request," Kravetz says.

The scientist-oriented course focuses primarily on "policy-type" information, with special attention being paid to the regulatory and supervisory aspects of dealing with HIV/AIDS issues in the workplace, Kravetz says. In addition to listening to presentations, participants receive informational brochures to take back to their labs and offices

Although training on HIV/AIDS in the workplace is presidentially mandated, Kravetz says no specific penalties have been set forth for federal workers who refuse to take part. At NIH, penalties for failing to attend the HIV/AIDS training program are established by each Institute, Kravetz says. Plans are not yet established on whether researchers will be required to undergo yearly refresher courses.

The new—and possibly final—sessions will be held in Lipsett Auditorium, Building 10, on Feb. 6, 13, 21, 23 and 27; and March 13 and 20. Participants must sign up for the course in advance and have a confirmed reservation. The $10 cost of the course will be covered by each researcher's ICD.

Registration information is available through Mimi Kravetz, Division of Workforce Development, phone 402-3392; administrative offices; or HIV/AIDS training coordinators. ■

HIV/AIDS Training Coordinators

ICD	Name	Bldg/Rm	Phone — Fax
CC	Stacey Bauman	10/1N252	6-1618—2-3601
DCRT	Stacy Vandor	12A/3031	6-6951—2-0007
DRG	Carol Striker	WW/438	4-7279—4-7384
FIC	Sharon Nieberding	31/B2C29	6-4625—2-1135
NCHGR	Deirdre Davis	31/3B31	2-4833—2-4831
NCI OD/ MAB,GAB, RCB, PMP,MISB, ASB, EFDB, ICIC and ADAM	Dee Brieske	EPS/550	2-4628—2-2188
NCI/DCE	Claudette Amoyt	31/11A11	6-6556—6-1297
NCI DCT	Andrea Gabossy	31/3A44	6-5964—6-0826
NCI DEA	Jean Arwood	EPN/16	6-7867—6-7911
NCI DCBDC	Mary Stinson	31/3A11	6-3381—2-0612
NCI DCPC	Adjoa Greenridge	31/10A50	6-9606—6-9931
NCI OD/OLCA EEO, OIA OLAS, and OTD	Debbie Pierce	31/11A33	6-5801—6-6005
NCI OD/OPOP ADO, and OCC	Teni Lyles	31/11A34	6-6002—6-6005
NCRR	Sonia Gaskin	12A/4055	6-1989—2-1774
NEI	Barbara DiSimone	31/6A18	6-4274—6-3958
NHLBI	Ruth Fritz	31/5A10	6-3245—2-4131
NIA Beth	Susan Haas	31/2C02	6-5347—2-3442
NIA Balt	Terri Neibuhr	GRC	410 558-8116 410 558-8322
NIAAA	Barbara Lindstadt	Wilco 406	3-0281—3-6076
NIAID	Michael Crumley	31/7A04	6-1521—6-7838
NIAMS	Karen Garrett	31/4C21	6-0436—2-4948
NICHD	Sherrie Davis	31/2A25	6-3365—6-4757
NIDA Balt.	Lena Eads	ARC	410 550-1509 410 550-2745
NIDCD	Chris Clements	31/3c11	2-0508—2-1591
NIDA Pkln	Maryann Pafitis	Pkln 10 21	3-9593—3-9127
NIDCD	Diane Foltin	31/9A30	6-4231—6-3951
NIDR	Faye Harbrant	31/2C23	6-6971—2-4088
NIEHS	Jennifer Anderson	101	919 541-2361 919 541-3026
NIGMS	Kellee Miller	45/3A5 13	4-2749—4-7730
NIMH Pkln	Margot Darby	Pkln 7C15	3-9094—3-1401
NIMH IRP	Carloyn Nichols	10/4C101	6-5337—2-0858
NINDS	Marjorie Kuhn	31/8A23	6-6334—2-2818
NINR	Debra Minor	31/5B03	2-2631—0-4969
NLM	Marilyn Chaikin	38/2N05	6-3661—0-4971
OD OA	Brenda Keagan	1/331	6-2511—2-1229
ORMH	Chris Spates	1/258	2-2515—2-0420
OIR	Eugenie Lackey	1/331	2-4166—2-0027
ORWH	Terri Kendrix	1/213	2-1770—2-1798
OEO	Evadne Hammett	31/2B40	6-6301—2-0994
ODP	Pam Clatterbuck	FED/618	2-2900—0-5158
OTT	Carmen Holmes	6011 Exec./ Blvd., 325	6-7736—2-0220
OER	Alice Murphy	1/158	6-1413—6 0232
OAR	Darlene Blocker	31/4B62	2 -357—2 3360
OSPTT	Edie Smith	1/332	6-0842—2-1759
OC	Judy Fouche	1/344	6-5548—6 0017
DFM	Kathy Adams	1/B122	6-9370—0-1850
OFM	Chenaceitta Taylor	31/B1C12	6-9498—2-0368
DP	Irene Douglas	EPS/850	6-9355—2-2144
OHRM	Sharon Mathsen	1/B160	6-2424—2-0345
DCG	Merle Tigert	6100 Exec. / Blvd., 6D01A	6-6431—6-8018
DL	Melissa McKerrow	EPS/750	6-0158—2-0577
OMA	Debra Jenkins	31/1B03	6-1873—2-0548
ORS	Carrie Tyrus	31/2B13	2-1528—2-1057

No Quiescence in Sight For Cell Cycle Group

As the NIH Cell Cycle Interest Group is finding out, rapid growth is not a phenomenon confined to aggregations of cells. Because attendance at its first two gatherings exceeded the capacity of the seminar room in Building 37, the interest group is moving. The next meeting, on Feb. 7, which will focus on apoptosis, is tentatively scheduled to be held at a more spacious site, Room 142, Building 60 (The Cloisters). The Cell Cycle Interest Group was formed to facilitate communication between scientists working at the NIH campus and nearby institutions who are interested in the cell-cycle and related problems. For more information on the group or scheduled events, send your name, phone and fax numbers, and mailing or e-mail address to Patrick O'Conner (Building 37, Room 5C19; phone: 496-3269; fax: 402-0752; e-mail: OConnorP@dc37a.nci.nih.gov) or Mary Dasso (Building 18, Room 101; phone: 402-1555; fax: 402-0078; e-mail: mdasso@HELIX.NIH.GOV).

Cell Cycle Interest Group Calendar

Feb. 7
Yves Pommier, LMP-NCI
"Topoisomerase active drugs and apoptosis."

David Cohen, NIAID
"Cell cycle disregulation and cell death during HIV infection of T-cells."

March 7
Doug Ferris, BRMP/PRI DynCorp, Frederick, Md.
"Identification and characterization of a human mitotic polo-like kinase."

Roxanne Duan, CBMB, NICHD
"The Kidney Cancer Tumor Suppressor gene product, VHL-1."

April 19
Ed Harlow (Distinguished Speaker), Massachusetts General Hospital
Title to be announced. ■

HOT METHODS CLINIC
continued from page 11

recommend procuring a minimum of 20 cells per µL; however, we have detected loss of heterozygosity (LOH) starting with as few as five cells.
8) The mixture is heated at 95 ° C for 5 minutes to inactivate the proteinase K, and 2 µL are then used for standard PCR analysis (5). A sample of the PCR protocol is described in the legend to Figure 2.

Tissue-Microdissection Contact
Michael Emmert-Buck, M.D., Ph.D, NCI
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e-mail: mbuck@helix.nih.gov

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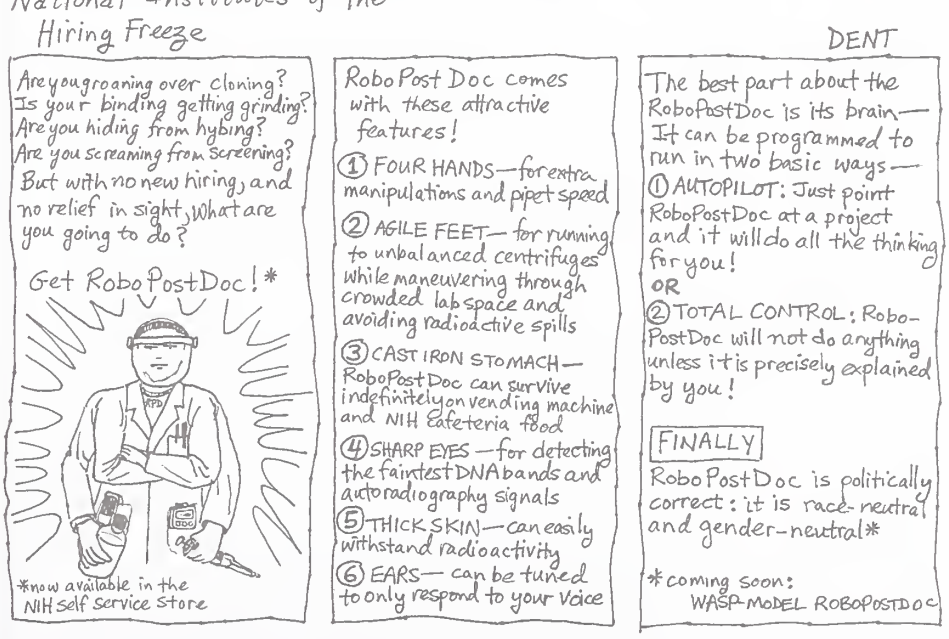
Electronic Swap Meet Up and Running

Amid all the hustle and bustle of the holiday season, intramural scientists may have overlooked one goody tucked away in NIH's maze of online resources. The Research Materials Exchange Bulletin Board sprang to life Dec. 16 in the "Intramural Research News" section of the "NIH Campus Info" menu. It appears that intramural researchers are more eager to give than to receive, with about twice as many offering extra supplies or equipment as those seeking research material.

Items listed under the "available" category varied widely, ranging from legal-size hanging folders to a Beckman LS2800 scintillation counter and an LKB 1275 Minigamma counter. Topping the "in search of category" was a request for a microwave that has not been used for ethidium bromide, along with an urgent plea for a loan of an optical disk drive.

To post an available or sought item, send a one-sentence description of the material, your name, campus address, campus phone and/or e-mail to Mike Lenardo (e-mail: Lenardo@nih.gov or fax: 402-8530). ■



FAX-BACK

In this issue we are asking for your feedback on: scientific ethics, laboratory waste disposal, tips for our Hot Methods Clinic, and intramural researchers who are couples. **Fax your responses or comments on other intramural research concerns to 402-4303** or mail them to us at Building 1, Room 334.

In Future Issues...

- Clinical Research Horizons, Part II
- Office of Technology Transfer: Issues and Changes
- SLPI Defense Against HIV-1
- Love and the Lab: Scientific Couples and Intramural Bliss?

1) What do you think of the NIH intramural program's guidelines for investigating allegations of scientific misconduct? What additions or revisions do you suggest?

2) Do you have any questions about the procedures for medical pathological waste disposal (MPW) outlined in this issue? Can you offer any other advice to labs trying to cut down on MPW bulk?

3) Do you have any suggestions or comments about the tissue-microdissection techniques featured in this issue's Hot Methods Clinic? What updates can you provide on previous Hot Methods? What techniques would you like to see covered in future issues?

4) In a future issue, we plan to talk with couples in which both partners are intramural researchers. We would like tips on couples to interview, as well as suggestions on specific topics to cover.

The NIH Catalyst is published bimonthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 334, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; e-mail: Rebecca_Kolberg or Celia_Hooper %NIHOD1E.BITNET@CU.NIH.GOV.

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