FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ MAY 1994

THE OTHER NIH: THE LIFE AND WORK OF THE EXTRAMURAL STAFF AT NIH

by Seema Kumar

Ask any of the 2,800 extramural staffers at NIH whether their intramural colleagues know what extramural scientists at NIH do on a day-to-day basis, and all you may get is an inscrutable smile. They may be tactful enough not to broadcast the point, but many extramural administrators believe that the intramural community is not only clueless about what the extramural staff do here at NIH, but also totally unaware of and ill-prepared for a research life in the outside world. What's more, this opinion is not reserved for postdocs in the intramural program — it embraces even senior staff.

Health Scientist Administrator Judith Greenberg is the Director of the Genetics Program at NIGMS

"I doubt if intramural scientists even know about the existence of an extramural program [on campus]," says Judith Greenberg, a former intramural researcher who is now an extramural administrator at NIGMS. "I certainly didn't ... If you were to poll people walking around on campus and ask them, 'Is there an Extramural Program on campus?' a lot of people might wonder what you are talking about."

Greenberg is not alone in her suspicions. "I really think that the

continued on page 4.

THE REPORT OF THE EXTERNAL ADVISORY COMMITTEE ON THE NIH INTRAMURAL PROGRAM

More than a year ago, the House Appropriations Committee placed the Intramural Research Program squarely at a crossroads. In its fiscal year 1994 report, the committee insisted that NIH perform a critical evaluation of the quality, appropriateness, size, and cost of the IRP, in order to inform decisions on which way to take the program, how best to allocate resources between the IRP and extramural programs, and what to do about the deteriorating infrastructure of the Clinical Center. This evaluation took nine months and was performed by the External Advisory Committee of the NIH Director's Advisory Committee. Committee members, led by Paul Marks of Memorial Sloan Kettering Cancer Center and Gail Cassell of the University of Alabama at Birmingham, reviewed reams of data carefully assembled by a hardworking internal committee. The result was a 69-page draft report, now available from Lab/Section Chiefs and Scientific Directors. Below, we reprint the Executive Summary and major recommendations from the report, and on page 2, Michael Gottesman, Acting Deputy Director for Intramural Research, comments and invites responses to the report.

EXECUTIVE SUMMARY

The intramural research program (IRP) of the National Institutes of Health (NIH) has been among the most distinguished biomedical research establishments in the world. The research achievements and the record of "graduates" of the NIH intramural program are matched by few biomedical research institutions. The NIH Clinical Center, a 450-bed hospital, is one of the world's largest hospitals devoted solely to clinical research. It has been a unique and invaluable resource for the direct clinical application of new knowledge derived from basic research. Despite this distinguished past, changes in the national biomedical research environment have led Congress and others to question the quality, appropriateness, size, and cost of the NIH intramural program.

The IRP is one of two components of NIH. The other is the extramural research program (ERP), which supports research at universities and other research institutions throughout the country. The IRP accounts for about 11% of the total NIH budget.

The External Advisory Committee has concluded that unless addressed, problems identified in this report — and several previous reports — may condemn the NIH IRP to a mediocre future. Several factors are increasing the pressure on the NIH budget, both extramural and intramural. On the one hand are the rapidly expanding opportunities to significantly increase basic biomedical knowledge, accompanied by

continued on page 20.

CONTENTS

The Report of the External Advisory Committee on the NIH Intramural Program — How Will It Affect You?

Michael Gottesman

In August 1993, Ruth Kirschstein, then Acting Director of NIH, assembled an External Advisory Committee (EAC) to ponder a congressional mandate to evaluate the "role, size, and cost" of the NIH Intramural Program. Chaired by Paul Marks of Memorial Sloan Kettering Cancer Center and Gail Cassell of the University of Alabama, this distinguished committee labored nine months to produce a report that is now the subject of much excited discussion and commentary. This issue of *The Catalyst* contains an executive summary of the report, and people who are interested can get copies of the entire document from their Laboratory or Branch Chief or Scientific Director. What will this report mean for the scientists in the NIH Intramural Program?

First, the report strongly endorses the concepts of stable funding and retrospective review, which are the essence of the Intramural Research Program. For those of us who are planning a future in Bethesda, this comes as a great relief. The report reinforces our belief that NIH's pluralistic approach to research support, combining the intramural approach with prospective grant review for extramural research, is most likely to optimize returns on investment. History shows that this mix has been successful, and the future should be no different. The EAC report includes suggestions for determining the optimal balance of extramural and intramural funding. The report indicates that funding of the intramural programs in aggregate should not exceed 11.3% of the total NIH budget — the average for the past several years. This means that expenditures for intramural research would not rise faster than extramural expenditures, including any costs associated with new building. The President's FY 1995 budget calls for intramural expenditures of 10.8% of the total, and unless an unexpected crisis ensues, the suggested 11.3% cap should be achievable.

The EAC report states that as resources decline, we must jealously guard the quality of our intramural research programs and choose tenure-track and tenured scientists with great care. Currently, the Boards of Scientific Counselors (BSCs) responsible for reviewing programs quadrennially, and the Board of Scientific Directors has kept watch over the tenure system. The EAC Report recommends that there be more safeguards; the BSCs should be more clearly independent of the Scientific Directors and should review the Scientific Directors' performance regularly. I will be meeting with the chairpeople of the BSCs within two months to discuss how to make our review processes more uniform across the Institutes. Rigor and fairness are paramount issues, and I hope to establish standards for both that will protect our scientists and the independence of the review process.

The NIH leadership has actually anticipated the EAC report somewhat to expedite implementation of a new tenure system based on two of the strongest recommendations in the EAC report: the decision to create tenure-track positions should involve broad input from scientists in a Laboratory, Branch, or Institute, and national searches should be conducted for all new tenure-track positions to ensure quality and diversity. Will this mean that our own senior postdoctoral fellows will be locked out of positions at NIH? Absolutely not. You will soon be seeing many advertisements for tenure-track positions at NIH in major journals, with a synopsis biweekly in the DDIR's Bulletin Board on Gopher; NIH personnel can compete for these and may often be very well qualified for the jobs in their own or other institutes. This new openness will mean that opportunities for tenure-track positions at NIH will be increasing, not decreasing. Tenure decisions will be made within 6 years by a rigorous process involving recommendations by the Laboratory or Branch Chief, Scientific Director, Institute Director, Promotion and Tenure Committee, and a new Central NIH Tenure Committee consisting of outstanding NIH Intramural Clinical and Laboratory-based scientists. With limiting resources and the enormous investment in space, positions, and budget associated with tenure at NIH, we cannot afford to make mistakes in the tenure process, and the EAC duly noted this. The new tenure-track system (summarized on the DDIR's Bulletin Board and available through Gopher on NIH's Campus Information On-line Menu) is already in place but still awaits final approval by the Public Health Service. The new NIH Central Tenure Committee should be constituted within the next week or two. Watch for its membership in the DDIR's Bulletin Board.

The NIH intramural program is the largest biomedical postdoctoral training program in the world. We have about 2,500 postdoctoral fellows here, approximately 15% of all biomedical postdocs in the United States. The EAC report takes us to task for not paying more attention to the mentoring and education of our fellows. Look for better tracking of fellows from the time they enter NIH until 10 years or so after they have left. Expect seminars of broad general interest hosted by the Special Interest Groups. These can be easily identified given the new format of our "yellow sheet." If you are a postdoctoral fellow, you may be contacted by other fellows inviting you to join a trans-NIH Fellows Group. Dr. Varmus and I will be working with this group to improve the training environment at NIH.

Although the report touches on many other issues, including the role of CRADAs in the intramural program and administrative impediments to the conduct of research at NIH (to be addressed in our report on "Reinventing NIH"), let me conclude with some remarks about the Clinical Center. Forty percent of our on-campus laboratory space and a 450-bed research hospital are housed within Building 10. The EAC report acknowledges the poor physical condition of this facility and endorses a plan to begin building a replacement hospital with associated laboratories and renovation of the existing building phased in over the next 10 to 20 years. The EAC envisions a state-of-the-art 250-bed hospital with essential laboratories in the same building, with new space generated by the new building used to increase the average per capita space in Building 10 (much-needed breathing room!) and to begin to bring some important outlying scientific programs back to the campus.

Can we get by with a 250-bed hospital? Last year, we averaged 230 in-patients per day. Because 90% occupancy is probably not feasible in a research hospital, we may need to do some downsizing of our research activities to fit into the new facility or make increased use of additional day hospital beds. We will have several years to plan the best course for the Clinical Center and to adjust to downsizing in the clinical programs, but I expect that this will be a difficult transition.

Some readers will find the EAC report highly critical; others will see in it a thoughtful and constructive analysis of our strengths and weaknesses. As we move to implement its many ideas, I welcome your comments and suggestions. Fax them to *The NIH Catalyst* at 402-4303 (see page 24) or send them directly to me.

One final note. I hope you have been "tuning in" to the DDIR's Bulletin Board, posted every two weeks (usually on Monday) and available through Gopher. This will continue to be a source of up-to-date information about how the EAC reports recommendations are being implemented and about other important aspects of campus life.

Michael Gottesman
Acting Deputy Director for
Intramural Research

FAX-BACK Feedback

Below is a sample of the FAX-BACK comments we received for each topic raised in the March issue.

On creative suggestions for waste disposal

"Most disposable plastic ware used for tissue culture is about as infectious as empty yogurt pots. Universities allow it to be put into regular trash cans. Why not NIH?"— *A. George, NIDR.*

"Make sure all labs/clinics pay by weight or volume to create meaningful feedback for volume reduction." — *S. Leighton, NCRR.*

"I would like to raise my concern over general paper products waste. Everyday I see our waste receptacles being filled with junk mail, old journals and copies of reprints, and especially with "test" printouts and manuscripts which continuously stream from our laser printers. I have been collecting my share of this waste, and have inquired about recycling. Unfortunately no one has been able to give any constructive suggestions, but everyone shares the same exasperation about the lack of the centralized paper recycling service and the lack of generally accessible collection points for paper waste. Since we have a functioning aluminum recycling program here, I believe the same could and should be done for paper. Any suggestions about what to do with the paper I have collected for recycling?" — *R. Somogyi, NINDS.*

"I have been concerned for some time with the general problem of radioactive waste and more recently with incinerators. I would like to work with the task force in attempting to develop NIH policies that could ameliorate these problems." — *A. Minton, NIDDK.*

"NIH needs to get serious about ecologically sound, cost-efficient, and simple alternatives not only to current medical-pathological waste disposal problems, but also, and just as important, ecologically sound, cost-efficient, and simple alternatives to resource consumption and waste disposal in general. ... Of all Federal agencies, the National Institutes of *Health* should be particularly sensitive and responsible to environmental issues. While we are on the subject, I understand that the kind of paper *The Catalyst* is printed on cannot be recycled since it is glossy. At a minimum, it should be printed on paper like the *NIH Record* which is recyclable. *The Catalyst* staff additionally should locate printing stock which contains post-consumer recycled waste. C'mon NIH, I am not asking for a deep shade of green, just a light tinge would be nice." — *P.F. Torrence, NIDDK.*

Editor's Note: The recycled and recyclable paper stocks available to us were not able to reproduce technical photographs such as PET scans, MRIs, blots, or gels clearly. But we'll keep researching. If we find the appropriate stock, we'll switch immediately.

Consumer complaints or raves about scientific products (reagents, kits, equipment, instruments, etc.)

"What about an NIH computer bulletin board arranged by technique or by large equipment type or by reagent? Users could ask technical questions of other users of the same system or product; preview existing products on campus without the filter of the product's sales representative; and most importantly, borrow that little O-ring or 5 mg of whatever in an emergency from someone here on campus." — *Anonymous.*

"Product evaluations are a bit dangerous. This newsletter could really influence the purchase of products, and complaints could be deadly for a specific product. It is very important to be sure that the consumer has used a product exactly as specified before a complaint should appear. On the other hand, if a company admits to a problem with a specific product, this newsletter could be a great forum for distributing the information. For example, my lab had been using the Boehringer Mannheim CAT Elisa kit but gave up because of background. BM now admits the kits had some technical problems and we are receiving credit for our purchases." — *Howard Young, NCI-FCRDC.*

On techniques you would like to see covered in our Hot Methods Clinic and your tips and suggestions on the yeast two-hybrid system

"Clonetech sells a yeast two-hybrid system they call "Matchmaker" that comes with a lot of control plasmids. I'm currently using their kit and am about to do my first library transformation. Their technical service department is useless for help though." — *Connie Fisher, NCI-FCRDC.*

"I would really like to see an article on in vivo footprinting. Keiko Ozato's lab is actively using that technique. This section could also be very useful for describing techniques which have resulted in the elimination of radioisotope usage." — *Howard Young, NCI-FCRDC*

Your opinion on the new distribution system for *The NIH Catalyst*

"Wider distribution is great, but please keep mailing *The Catalyst*, as some of us virtually never use the cafeterias for gastronomic reasons. I am impressed by how topical and frank some of the articles and comments are — keep up the good work." — *K Yamada, NIDR.*

"Don't stop mailing — I love this publication but I'd never see it if I had to search it out in the cafeterias." — *J.A. Burris, NCRR.*

"Please don't discontinue mailings. We might never see *The Catalyst* in Frederick." — *Howard Young, NCI-FCRDC.*

"No! Do not discontinue mailing. You might consider making it available via mosaic on helix.nih.gov." — R. Mejia, NHLBI.

Editor's note: Starting in June, The NIH Catalyst *will be available, sans pictures and graphics, through the Campus Information menus on Gopher.*

Other topics

"I, as well as many of my colleagues, are concerned about the mandatory cutbacks, especially when the only thing that seems important is meeting a numbers goal. Everyone should be reminded that, as private citizens, we have the right to write our congressional representatives expressing our concerns about the cutbacks." — *Howard Young, NCI-FCRDC.*

"While I am not a particular friend of answering machines, I welcomed the introduction of the NIH voice mail system, believing that it would shield us somewhat from unwanted solicitations. How naive this assumption was, I realized when, instead of listening to my messages, I heard an advertisement of some event at Parklawn. Our (conventional) mail boxes are daily cluttered with junk mail and now NIH starts to do the same with our phone mail system. Will we soon have to listen to a string of memos and advertisements before we can retrieve our personal messages? The dissemination of general information via the personal phone mail is a blatant invasion of the privacy of NIH employees. It is a nuisance and waste of time that needs to be stopped before it becomes customary." — *B. E. Flucher and colleagues, NINDS.* ■

The Other NIH
continued from page 1.

intramural scientists think very little about us," says Joan McGowan, a program administrator in NIAMS' Bone Biology and Bone Research Branch. But she and Greenberg add that intramural scientists' obliviousness is an understandable instance of "out of sight, out of mind."

"They really don't see us that much ... If you are in a lab, you tend not to go to building 31 which is all extramural, and it is even more unlikely for you to go to the Westwood Building," says Greenberg. In fact, Greenberg quips that all that intramural scientists may know about their NIH extramural colleagues is that they belong to the cadre of people at NIH who dress in suits or jackets and ties.

But in a more serious vein, the extramural administrators also suspect intramural scientists' ignorance goes to the heart of their work, namely, the grants process.

"Most intramural scientists, even senior people, do not know how the grants process in the extramural program works until it is time to leave [NIH]," says Jerome Green, Director of the Division of Research Grants (DRG), the central office that receives all extramural grant applications.

Greenberg agrees. "Many senior intramural scientists have no concept of the grant process and therefore are not in a good position to advise their outgoing fellows or postdocs, either," says Greenberg, who is Program Director of the NIGMS Genetics Program. The extramural scientists say that the basis for this lack of knowledge lies in the way the intramural program is structured. Intramural scientists are, by design, freed of the burdens of grant writing so that they can devote their time and energies to conducting high-risk, innovative research. Given this atmosphere, say Greenberg, McGowan, and Green, it can be very easy to not know about the grants process or to lose touch with it. But the fact remains, they say, that when intramural researchers finally leave NIH, they may be at a disadvantage in the extramural world if they are not aware of the grants process of NIH, the primary support for biomedical research in the outside world.

Joan McGowan is the Chief of the Bone Biology and Bone Diseases Branch of NIAMS.

Intramural scientists may or may not agree with the extramural staffers' grim review, but could undoubtedly profit from a few pointers that the extramural experts have to offer, including how the NIH grants mechanisms operate, what to expect in terms of NIH funding, and how to write winning proposals.

To help bridge the intramural-extramural gap, we present a glimpse of extramural life at the NIH. We start in this issue of *The NIH Catalyst* with a day in the life of extramural NIH. In the next issue, we interview experts from the DRG and others who offer a popular course called "How to Write a Grant," and we conclude with a feature on mentoring and career development to prepare young scientists for tlife after NIH.

Jerome Green is the director of the Division of Research Grants, the central office that receives the nearly 100,000 grant applications each year.

A Day in the Life of Extramural NIH

Considering that the raison d'être of extramural NIH is to fund research, it won't come as a surprise that a day in the life of an extramural scientist typically revolves around some aspect of the NIH grant process — sifting through grant applications, considering which ones are worth funding, keeping up with grantees' progress, or counseling grantees throughout the process. But what may come as a surprise to intramural researchers, says Green, is the size, range, scope, and variety of the NIH extramural grants operation that administers and manages 85% of the NIH budget with a staff of 2,800.

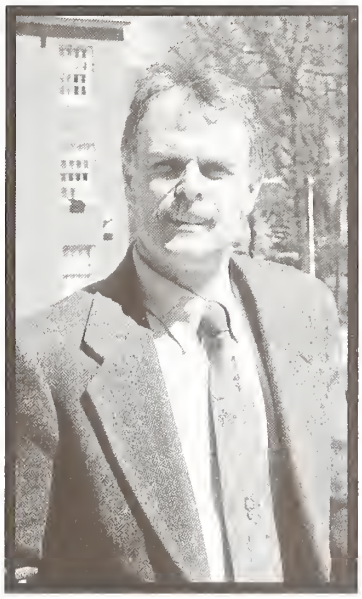
"We are not all alike," says McGowan, "There is quite a spectrum of individuals [in the extramural program]; some are involved more in science than in [grant] mechanisms, and some are more involved in mechanisms than in science."

Depending on the science-vs.-mechanism emphasis and on whether they work in contracts, programs, review, or management of grants, extramural researchers' work responsibilities might differ considerably, says Dennis Mangan, Director of the Periodontal Diseases Program at NIDR and a former intramural researcher at NIDR. "Grants managers often come from a business background and are number wizards, whereas contracts experts know both science and business and how to handle contracts with sensitivity and attention to detail," Mangan says. "Program folks come from a scientific background and are the contacts for extramural researchers who have questions. Review staff who come with a strong scientific background select and lead the study sections — the committees that reviews grant applications — and summarize the study section's review in logical, adequate terminology."

Although their backgrounds and day-to-day duties may vary, the extramural staff's work has a common goal: to facilitate the conduct of research that advances the science and health of the

country by using taxpayers' dollars. That, says Mangan, involves a lot of communication.

"We communicate with a lot of different people," says Mangan. "With our grantees, we communicate to address their concerns and needs. We also work with the DRG, our Grants Management Offices, and with other program officials, both within our institutes and at other institutes." This means that "every extramural administrator has to develop good communication skills and be comfortable working with, talking with, and writing people on a daily basis," says Mangan. They must have good organizational skills, diplomacy, and an ability to write and express themselves, says Greenberg. The program and review staff must, in addition, keep up with the scientific literature and the latest advances in their specific area of expertise and "know what the needs of our programs are. Are we short on grants in a particular area? Are there scientific "holes" in the program? And if there are, then those factors go into consideration for funding," says Greenberg.



Dennis Mangan is the Director of the Periodontal Diseases Program at NIDR.

Unfortunately for some, a day in the extramural life does not involve hands-on bench research or teaching. Some extramural NIHers miss that, but being an extramural administrator offers a wider perspective and new challenges and rewards. "You have to take a whole new attitude about science," says Mangan. "As an intramural scientist, I was focused on me, my lab, and my work in a small area of periodontal diseases. When I came over here, I had to take my blinders off and learn to look at the entire range of grants on periodontal diseases that were in my portfolio — from the most basic molecular science all the way across to the clinical sciences. And that was a challenge."

McGowan concurs. "You have to have a scientific perspective that is greater than your own area ... You are looking for opportunities to stand back from science and ... see if a particular application fits in well with what is needed in the program rather than whether this is the right clone to use," she says. "I find it very gratifying to be a facilitator of science. I thought I would miss teaching, but I use my teaching skills on scientists around the country."

Mangan found that "you start getting a thrill out of watching someone else achieve success in something that you might have wanted to do in your own lab ... But perhaps the most satisfaction we get now is in ... seeing the young scientists succeed and grow to become good mentors for the next generation of scientists."

What happens on a typical day in the extramural program is closely intertwined with the three funding cycles each year during which the DRG receives a total of nearly 40,000 grant applications. Grant applications are reviewed by 1 of 100 study sections, each comprising an average of 18 experts from around the country. These experts are identified and nominated by an NIH Scientific Review Administrator, who also coordinates the study section. The study sections review the applications, and then pass them on to the appropriate institutes with a summary report and a score. There, the applications undergo another level of review at institute-wide Council meetings. On the basis of the study section's scores, the reviewers' comments, the summary statement, the Council's review, and the institute's programmatic needs, the application is considered for funding. Each institute has a team of Health Scientist Administrators who help the Institute Director make final decisions on which applications will get funded. "Only 20 to 22% of all applications get funded, and the competition is very keen," says Green.

YOU HAVE TO HAVE A SCIENTIFIC PERSPECTIVE THAT IS GREATER THAN YOUR OWN AREA AND...SEE IF A PARTICULAR APPLICATION FITS IN WELL WITH WHAT IS NEEDED IN THE PROGRAM RATHER THAN WHETHER THIS IS THE RIGHT CLONE TO USE.

Maintaining accessibility in the system is a key part of extramural administrators' jobs. "We work with investigators before, during, and after their application process and answer their questions on what NIH or our institute is looking for in the grant and discuss the merits of their ideas and the type of grant they should apply for," says McGowan. Program directors also help scientists focus on their scientific areas and guide them through the application process. "For some investigators, the grant-writing process is a very scary and a very nebulous process. What we are here to do, a part of our jobs, is to help them through that process as best we can, given the resources we have and the time we have," says Mangan.

An important aspect of the extramural life revolves around the study sections. "We try to attend the study sections to listen to the reviews of the applications. We listen for any major concerns about the grants and the reaction of the study section with regard to whether they considered the project exciting, novel, necessary, timely, and state-of-the-art," says Mangan.

Personal notes, along with the study section's summary statement, help extramural program staff explain to investigators why their grant did not get funded and to advise them on resubmissions. "There is a fair bit of handholding in all of this," says Greenberg. "When people don't get a grant, they may feel that their job is at stake and they need encouragement, advice, or a reality check. So we spend that kind of time with the investigators as much as we do talking to them about their results."

continued on page 6

Extramural Scientists
continued from page 5.

Study section summary statements also help program staff prepare for Council — the second level of review held by the individual institutes. "We have to go into Council prepared to discuss the strengths and weaknesses of applications, what we intend to do about them, and strategies to solve any problems within an application," says Mangan.

Once the review is complete, the Health Scientist Administrators — most of whom have Ph.D.s — make recommendations about which grants should be funded. "When the institute makes decisions about what to fund, we don't have to take reviews in perfect numerical-priority-score order," says Greenberg. "We take into account various things such as whether an area is underrepresented, and once we make those funding decisions, we are responsible for administering them throughout their active phase — typically three to five years."

THERE IS A FAIR BIT OF HAND-HOLDING IN ALL OF THIS. WHEN PEOPLE DON'T GET A GRANT, THEY NEED ENCOURAGEMENT, ADVICE, OR A REALITY CHECK. SO WE SPEND THAT KIND OF TIME WITH INVESTIGATORS AS MUCH AS WE DO TALKING TO THEM ABOUT THEIR RESULTS.

Once the funding decision has been made, program staff members notify grantees of their awards, which often differ from their requested dollar amounts, and work with the Grants Management Office to send out official notices and pay memos — official memos that initiate payments on grants — to successful applicants.

(Look for Part II of this feature, a discussion on the NIH grant review process, how to write a grant, and mentoring and career development for young scientists, in the July issue of The NIH Catalyst.) ■

The Resources for Women Scientists File has been established in a file drawer in the reference area of the Building 10 library. The file contains notebooks with information on

1. Job and postdoctoral opportunities, including a description of how to search on-line for federal government jobs
2. Grant information and advice on how to write a grant
3. Articles on how to get a job and on mentoring
4. Meetings and conferences
5. Articles relevant to women in science
6. Information on how to deal with sexual harassment and discrimination, and on EEO resources at NIH
7. Association of Women in Science (AWIS) newsletters
8. A book from NSF on visiting professorships for women scientists

Clara Pelfrey (x60518) is in charge of the file. Anyone may use or xerox the materials, but the notebooks must remain in the library. ■

Cell Catalog Goes Online

Boot up, investigators! Another important resource is coming on line: the catalog of cell lines and DNA samples maintained by the NIGMS Human Genetic Mutant Cell Repository is now available by computer. Just a few keystrokes away are the most up-to-date and complete listings of cell lines and DNA samples. Cell lines are cross-referenced, so users can search by disease category for all associated fibroblast and lymphoblast cell lines and related DNA samples in the repository.

The on-line catalog is still in a prototype stage and does not have embedded graphics to display pedigrees and chromosome diagrams. However, a faster, second-generation form, expected to be available on World Wide Web next year, will have graphics capability and will replace the 900-page printed version that NIGMS now publishes. NIGMS will redirect their hard copy efforts to publishing a user's guide to the on-line catalog. The second-generation catalog will be hot-linked to other genetic databases, such as Online Mendelian Inheritance in Man, the Genome Data Base, and Genbank.

"The on-line service is an exciting opportunity to make the cell catalog more than just a catalog. It will be a comprehensive, user-friendly source of information about genetic diseases," says Judith Greenberg, the project officer for the NIGMS cell-repository contract with the Coriell Institute for Medical Research in Camden, NJ. NIGMS encourages potential users to check out the prototype on-line cell catalog and to send any questions or feedback to the cell-repository contractor at 1-800-752-3805.

To access the online catalog via Internet, use the following address: Telnet Coriell.umdnj.edu. Log in as: online. To access the catalog via a modem, call 609-757-9728. Long-distance telephone charges will apply to modem calls, but there is no additional charge for connect time. ■

More New Inter-Institute Interest Groups

A sixth major interest group has joined the ranks: the **Molecular Biology Biology/Biochemistry Interest Group** will inform the large and diverse community of molecular biologists and biochemists at NIH about the work of their colleagues and will provide a forum for distinguished investigators in molecular biology from outside NIH. To join this group, please mail the following information to **Cori De Graff** (Bldg. 5, Room 324) or fax it to her (496-0201): **Name, Laboratory/Section, Phone, Fax, Areas of research interest**. The other major interest groups on campus are: Cell Biology, Immunology, Genetics, Neurobiology, and Structural Biology.

In addition, five new narrower-focus interest groups are getting off the ground. Two long-established groups, the Yeast Club and the Lambda Lunch, are adding their names to *The Catalyst's* official list.

The NIH **Developmental Biology Interest Group** is seeking members. This group might act as an "umbrella organization" for the existing mouse, frog, and fly clubs, which deal mostly with developmental questions. The interest group would also hope to serve the communication needs of developmental biologists in other fields and provide an opportunity for postdocs and investigators with similar interests to meet and interact. The proposed activity for the group is a one-day meeting, once or twice a year. The first meeting is tentatively scheduled for mid-July, and the group plans to have one or two outside speakers, several NIH speakers, posters, and refreshments. If you are interested in joining the Developmental Biology Interest Group, please send your name, fax number, and mailing and e-mail address to either Igor Dawid (Bldg. 6B, Room 413; phone: 496-4448; fax: 496-0243; e-mail: IDA@NIHCU.BITNET) or Joram Piatigorsky (Bldg. 6, Room 201; phone: 496-9467; fax: 402-0781). Indicate whether you want to participate in the planned July meeting, and give a title if you wish to speak or give a poster. Planning for the July meeting is under way, and responses should be sent immediately.

The **NIH Apoptosis Interest Group (AIG)** meets once a month on a Monday afternoon at 4 p.m. in Building 30, Conference Room 117, to exchange ideas, frustrations, techniques, and protocols on research involving apoptosis, or programmed cell death. Currently, there are more than 50 individuals at NIH and in the Washington-Baltimore-Frederick area who directly or indirectly study apoptosis in specific cell, tissue, and organ systems; their collective knowledge represents a tremendous resource. To further expand this expertise, the AIG also sponsors occasional outside speakers. Meetings typically consist of presentations by two members followed by informal discussions and refreshments. The date of the next meeting will be posted on the DCRT NIH Centralized Bulletin Board System (listed as AIG) and in the "NIH Calendar of Events." For more information, call Dennis Mangan at 594-7641, Pierre Henkart at 496-1554, or Huber Warner at 496-6402.

The NIH Inter-Institute Hard Tissue Disorders Club has recently been formed by several investigators on the NIH campus with common interests in skeletal research. This group plans to meet on a regular basis to discuss basic and clinical aspects of hard tissue disorders, with occasional outside speakers who work in this area. For further information, contact Pamela Gehron Robey (phone: 496-6255 or fax: 480-2880).

A Nucleic Acids Biochemistry Interest Group, focusing on structure and mechanism, has formed to bring together scientists interested in various aspects of nucleic acid biochemistry, including structures, enzymology, and mechanism. Members can learn what others in the field are working on, make suggestions, and network. The group's monthly meetings feature short talks in an informal atmosphere followed by discussion and social interaction. Previous talks include "The use of Phosphorothiolate Oligonucleotides in Lambda Site-specific Recombination" and "A Kinetic Model Describing the Homology Search by RecA Protein." Anyone interested in joining should call Janet Yancey-Wrona (phone: 496-2038) or Alex Burgin (phone: 496-6934).

A Matrix Metalloproteinase Working Group has been formed to explore extracellular matrix turnover and remodeling. The purpose of this group is to enhance communication between intramural investigators at all levels, and to provide a forum for distinguished extramural and intramural speakers, Monthly meetings will be held in Building 30, first floor conference room. Next month's speaker: Henning Birkedal-Hansen, D.D.S., Ph.D.

To join, please fax or mail your name, affillation, NIH address, phone number, fax number and areas of research interest to W.G. Stetler-Stevenson, Building 10, Room 2A33; Fax: 2-2628; Phone: 6-2687.

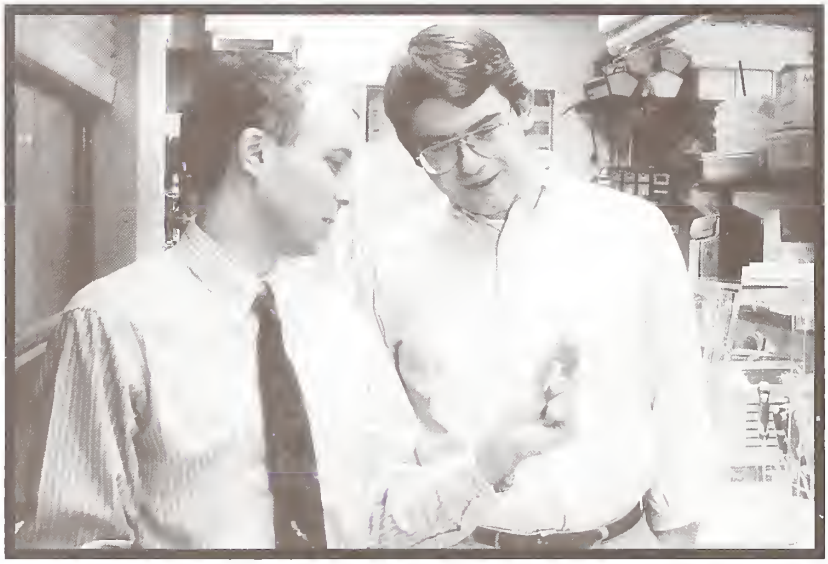
The NIH Yeast Club meets every two weeks on Friday from 4 p.m. to 5:30 p.m. at Bldg. 49, Conference Rm. A. This meeting is only for NIH yeast people, and is designed to be informal and to allow everyone a chance to present faily frequently. Interested people can contact Henry Levin (Bldg. 6B, Room 220; phone: 402-4281 or fax: 496-0243). For a list of the Washington area yeast meetings contact Reed B. Wickner (Bldg. 8, Room. 207; phone: 496-3452 or fax: 402-0240) or Alan Hinnebusch (Bldg. 6B, Room 309; phone: 496-4480).

Lambda Lunch, possibly the longest-running interest group on campus, meets weekly on Thursdays at 11:00 a.m., usually in Building 36, Room 1B13. Basic research on mechanisms of gene regulation, recombination, replication, and cell division in prokaryotic systems form the core interests of this group. The Lambda Lunch schedule is available via anonymous FTP. The computer address is FTP.CU.NIH.GOV and the directory is "LAMBDA_LUNCH." There are two files in this directory; "MAIL" contains the current schedule. ■

NIAMS Rheumatologists Come Across Gene for Kidney Disorder en route to Familial Mediterranean Fever Gene

by Seema Kumar

It was a clue that wasn't. When NIAMS rheumatologists, searching for the gene for familial mediterranean fever (FMF), found a family in which two of five siblings had both FMF and cystinuria — a genetic predisposition to kidney stones — they thought they had stumbled upon an important clue that the two diseases were genetically linked. If they found the location of the FMF gene, the researchers thought the linkage would also lead them to the gene for cystinuria. The apparent cosegregation of the two diseases in that family turned out to be a red herring; nevertheless, chasing down this clue led the researchers to successfully map the cystinuria gene on the short arm of chromosome 2. A team from Spain and Italy has found six mutations in this gene that account for cystinuria, proving that the gene causes cystinuria.



NIAMS' Elon Pras (left) and Daniel Kastner (right) examine a kidney stone from one of the patients in their panel of 17 families with cystinuria. Using linkage analysis, Pras and Kastner recently mapped the cystinuria gene to a narrow region of chromosome 2.

"It was a truly serendipitous situation," says Daniel Kastner, senior investigator at NIAMS, of his foray into cystinuria with Elon Pras, his postdoctoral fellow. "There is no connection whatsoever [between FMF and cystinuria]. Their occurrence in this family was just a clue that turned out to be wrong, but got us started on the pursuit of the cystinuria gene."

In fact, before they stumbled onto this family, the researchers had barely heard of cystinuria, says Kastner. "We had to first look it up [in the literature] just to find out what cystinuria was because [as] rheumatologists, we knew little beyond the fact that cystinuria causes cystine stones in the urine. That was about ... the extent of our knowledge."

The NIAMS researchers' actual objective was to find the gene for FMF, an inherited rheumatological disorder caused by a single recessive gene of unknown location and function. Stymied by the lack of families with FMF in the United States, Kastner began collaborating with the director of an FMF clinic near Tel Aviv, Israel, that follows some 2,500 FMF patients. Kastner spent the summer of 1989 in Israel collecting data from families with FMF, and his lab then began using positional cloning to map the gene for FMF. In August of 1991, Elon Pras, whose father is the director of the FMF clinic in Israel, joined Kastner's lab and "after about 10 probes, hit on the magic one on chromosome 16 that happened to be linked to the FMF gene," says Kastner. "Once we analyzed all the families, it turned out that they were all linked to chromosome 16."

In the course of collecting data on FMF, Kastner and Pras and their colleagues in Israel came across a Libyan Jewish family in which a man had married his niece and had five children, two of whom had both FMF and cystinuria. "That got us interested in this whole question of cystinuria," says Kastner.

"When you calculate the odds of cystinuria and FMF being in the same family, you get a result that suggests that the two diseases are on the same chromosome. We looked at it at that time as a good clue," says Pras.

The researchers' crash course on cystinuria taught them that the disease was one of the first disorders to be described as an inborn error of metabolism by Sir Archibald Garrod in 1908 and is, like FMF, an autosomal-recessive disease. Affected patients can develop kidney stones at any age from the first year of life; cystinuria is believed to be the most common cause of kidney stones in children and the most common single-gene hereditary cause of kidney stones. Although some patients remain asymptomatic, most have attacks of kidney stones, and in some patients, these attacks may occur as frequently as once or twice a year. The disease may run a very hard clinical course and is excruciatingly painful. Urinary obstruction can lead to colic, kidney infections, and even renal failure, and although there are treatments for the disease, cystinuria remains a significant cause of morbidity.

The researchers also learned that cystinuria is more prevalent than FMF; FMF is prevalent among North African Jews and Iraqi Jews, and among non-Jewish Armenians, but cystinuria is prevalent worldwide. Researchers estimate that 1 in 60 Americans is a carrier of the cystinuria gene and 1 in 15,000 Americans is affected by the disease. "It occurred to us ... once we had figured out the location of the FMF gene, that we could find more families with cystinuria and possibly confirm that the cystinuria gene would be near the FMF gene," says Kastner.

Soon after the team narrowed the FMF gene search to chromosome 16, Pras decided to go after the cystinuria gene while the rest of the lab continued to pursue the FMF gene. That summer, Pras found additional families with cystinuria while a new NIH clinical protocol also recruited some families with cystinuria. With a combined panel of

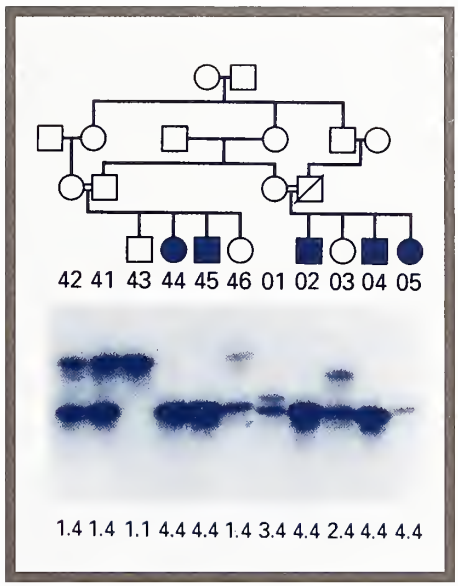
nine families, Pras began the hunt for the cystinuria gene by looking at markers on chromosome 16, fairly confident that he would soon confirm that the gene for cystinuria was there. To their dismay, the researchers found that "even after we had looked at both ends of chromosome 16 and everywhere in between ... there wasn't a consistent linkage on chromosome 16."

The first indication that they were barking up the wrong chromosome came last spring. Pras read a crucial paper in the *Journal of Clinical Investigation* reporting cloning and mapping of a human kidney gene on chromosome 2 responsible for the transport of amino acids.

"We immediately began looking at this gene as a possible candidate for the cystinuria gene," says Kastner. But when NIAMS scientists screened for chromosome 2 linkages in the nine families, they found that evidence of cystinuria linkages was only borderline: one of four affected children in one family did not fit the pattern.

Thinking that they had run into a false-positive result, the researchers decided to recruit more families. The researchers collected data from eight additional families, bringing the total number of individuals to 113, of whom 44 were affected. Results from the 17-family panel showed clearly that the gene was in a narrow area on the short arm of chromosome 2. Still, the one child did not fit the pattern.

"So we went back to that family — an American family with both parents available and nine children, four of whom were thought to have cystinuria — and found that the fourth affected child, a five-year-old-girl who did not fit the pattern, had been diagnosed as having cystinuria based on the fact that as an infant, she had an episode of urinary colic," says Kastner. "The cystine level in the girl's urine had never been checked, and two ultrasound tests to detect stones in her kidneys were inconclusive." The girl had been undergoing treatment, and the fact that she did not have any clinical problems was attributed to the treatment. Subsequent 24-hour urine-collection tests showed that the child did not have cystinuria. The researchers published their finding that cystinuria is linked to chromosome 2 in the April 1994 issue of *Nature Genetics*.



Family tree for an inbred family with two first-cousins marriages. Dark squares and circles show the affected males and females in the family. Genotypes of the family show that all affected members are homozygous for allele #4. None of the unaffected members are homozygous for this allele.

"What we have done strengthens the possibility that the transporter gene cloned by the U.S. and Spanish groups is the gene for cystinuria," says Pras. Proof that this gene does cause cystinuria came from a group of researchers from Spain and Italy who reported, in the same issue of *Nature Genetics*, six mutations in the gene that account for 30% of the cystinuria cases in the chromosomes they studied.

Kastner and Pras had also begun comparing the DNA sequences of the gene in affected and unaffected individuals in their panel of cystinuria families. Pras found mutations in one family that were different from the mutations found by the Spanish-Italian team.

The Spanish-Italian team also reported that the most common mutation, detected in three cystinuric siblings, blocked the amino acid-transport activity of the gene in *Xenopus* oocytes, establishing that the gene causes cystinuria. The gene normally encodes a transport protein that resorbs cystine from urine back into the blood; this process is interrupted in patients with cystinuria either because the protein is not expressed or is defective, and the excess excreted cystine precipitates, forming stones, says Pras.

"One family had gotten us interested in cystinuria, and that was just a coincidence," says Kastner. His lab has returned to the familiar territory of rheumatic diseases after this brief detour into the the realm of kidney disorders. ■

COMPUTER TECHNOLOGY ON TAP FOR NIH EMPLOYEES WITH DISABILITIES

DCRT is sponsoring a new program called the NIH TARGET Access Program (TAP) to help NIH employees with disabilities or special needs find the computer and electronic tools they need to be most productive. TAP allows NIH scientists and other employees to use the U.S. Department of Agriculture's (USDA's) TARGET Center, a state-of-the-art demonstration facility that features computer technologies for people with disabilities and special needs. Many TARGET Center technologies also offer benefits to the scientific community at large. One new product, for example, allows scientists to use a PC as the interface for microscopy operations. Scientists can project the microscope image, view it via televisions, store it, and access it from other computers. The system allows samples to be positioned, viewed and illuminated from the display screen. TAP was developed in cooperation with the NIH Office of Equal Opportunity.

The TARGET Center is located at the USDA Headquarters South Building in Washington, D.C., adjacent to the Smithsonian Metro station. The Center is fully accessible. To set up an appointment or for more information, call 301-594-DCRT (TTY 301-496-8294), or send e-mail to 4DCRT@nih.gov. ■

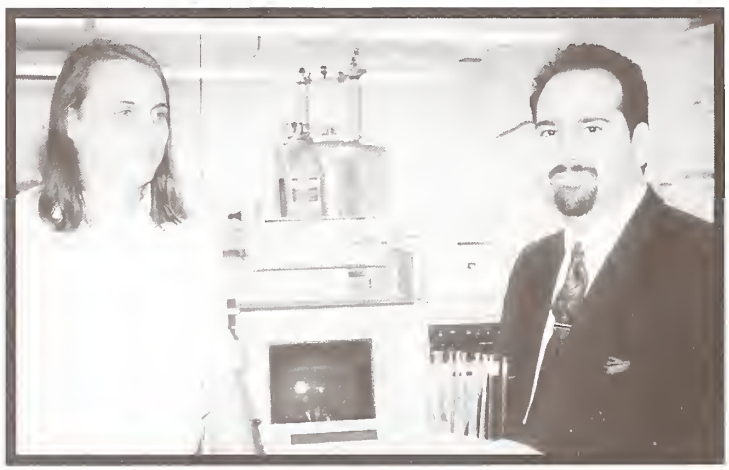
DCRT's CBEL: Improving Research Through Highly Parallel Computing

by Luella LeVee,
Freelance Writer, for DCRT

One of the brightest lights emerging from DCRT's recent reorganization is the Computational Bioscience and Engineering Laboratory (CBEL), whose major thrust, according to CBEL Chief Robert Martino, is "exploiting high-performance computer systems for biomedical applications." Much of the work of this new laboratory centers on a highly parallel supercomputer, the Intel iPSC/860, whose claim to fame is a fantastic computational speed that has helped more than 50 NIH scientists get research results in minutes instead of hours.

"It's exciting and a continuing challenge to have high-performance computing impact biomedical research and clinical practice, to observe applications in daily practice," says Martino.

CBEL's expertise in highly parallel computing is already speeding up some of NIH's work in image processing, structural biology, computational chemistry, and medical imaging. In addition, CBEL staff are providing NIH researchers with faster ways to visualize biological processes, search genetic databases, and conduct linkage and statistical analyses. "With our help, scientists are getting the latest up-to-date computing technology both in hardware and software and the best expertise to help them solve their problems using this technology," says Martino.



John Pfeifer (left), a CBEL computer engineer, is collaborating with Frank Delaglio (right), Laboratory of Chemical Physics (LCP), NIDDK, to adapt LCP's software to run on an Intel high performance parallel computer. Parallel computing will allow LCP to analyze larger proteins faster than by conventional computing.

The Benefits of Parallel Computing

Until recently, these scientific tasks were accomplished with conventional computers, where problems are solved sequentially, in a step-by-step fashion. In parallel computing, on the other hand, a problem is divided into several segments and each segment is sent to a different processor or node. The segments are then computed simultaneously, saving precious time for scientists.

"Everyday-workstation users can now access the parallel computer over the NIH network. For example, users can now access the parallel computer at their workstation to complete in less than 5 minutes, an imaging task that normally takes 6 hours on a workstation," says Martino. CBEL already has a few success stories to tell:

- With CBEL's help, NIAMS collaborators have a better understanding of the structure of the herpes simplex virus type 1. Parallel computing helped to determine the location of the major types of proteins that combine to form the virus's capsid.

- NIDDK has used a parallel-computing method to automate the spectral-assignment process in NMR spectroscopy, determining which signals in the multidimensional NMR spectra data belong to which atoms in the molecule under study. Using this method, the scientists assess the structure of calmodulin, a protein involved in a wide range of cellular-calcium-dependent signaling pathways.

- Another group of scientists from NIDDK used the parallel computer to simulate the kinetics of ultrafast chemical reactions in solution, such as the kinetics of nitric oxide rebinding to myoglobin following photodissociation. The method yielded insights into the chemical dynamics of ligand binding to myoglobin.

- NIMH investigators used parallel-image-registration techniques developed by CBEL staff and positron emission tomography (PET) images of the brain superimposed on computer tomography (CT) and magnetic resonance imaging (MRI) images to study the progression of Alzheimer's disease.

- High-performance computing has allowed NEI researchers to determine the onset time, the rate of information encoding, and the total amount of information encoded by neuronal responses to a visual stimulus in primates. This will help researchers to develop better models of the primate visual system.

"The most exciting part of my work is bringing technology to a significant biomedical problem. I really enjoy seeing the effect it has on biomedical applications," says Martino.

CBEL is also participating in Vice President Gore's High Performance Computing and Communications Initiative, an interagency program to bring high-performance computing to bear on "grand challenge" problems, such as predicting protein folding and designing drugs, and "national priorities," such as health-care reform.

CBEL's help is available to all NIH scientists, says Martino. "Our goal is not only to help scientists with computationally intensive problems taking a very long time on existing systems, but it's also getting scientists to think of new ways of approaching their problems that they haven't considered before because they didn't have the computational power. Sometimes, it is the software they are using, and we can assist through software engineering. Other times, a problem is appropriate for a parallel computer and we are able to help them out that way."

Dr. Martino invites NIH scientists with computationally intensive problems to call him at 496-1111. ■

SEMINAR HIGHLIGHTS

MEMBRANE TRAFFIC AND COMPARTMENTALIZATION WITHIN EUKARYOTIC CELLS

Jennifer Lippincott-Schwartz, NICHD, NIH Director's Seminar, April 19, 1994

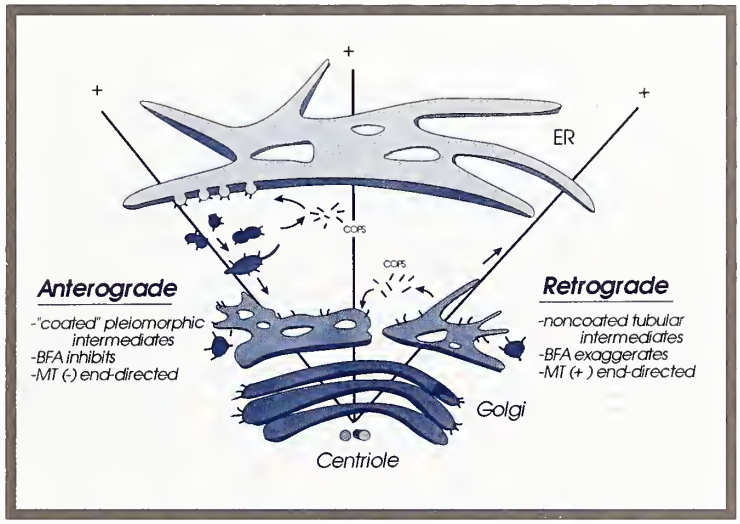
ABSTRACT

Many of the diverse functions of eukaryotic cells are carried out within discrete membrane-bound compartments or organelles that communicate with each other by membrane traffic pathways. My studies over the past few years, in collaboration with Richard Klausner and colleagues at NIH, have focused on the mechanisms by which cells maintain and remodel organelle structure and the role membrane traffic plays in this process. A key tool in our work has been the use of the fungal metabolite brefeldin A (BFA), which dramatically alters the distribution and flow of membrane through organelles. BFA reversibly blocks all forward, moving, or anterograde transport beyond the Golgi complex, causes the complete disassembly of Golgi stacks, and results in transport of Golgi components back to the endoplasmic reticulum (ER). These effects of BFA are not limited to Golgi membranes but extend to organelles of the endosomal system as well. Dissection of the biochemical basis of BFA's action has led to the finding that BFA blocks an essential activation step in the membrane interaction of a family of closely related proteins called ADP-ribosylation factors (ARFs), which are low-molecular-weight GTP-binding proteins distantly related to the *ras* proto-oncogene product. Blocking this activation step prevents assembly of a high-molecular-weight protein complex, called coatomer, onto the Golgi membrane. Because coatomer binding appears to be required both for the production of transport vesicles and for maintenance of Golgi structure, these findings suggest that dynamic processes of membrane traffic are integral to the existence and function of intracellular organelles.

QUESTIONS

Q: *What was your starting point in this research, and how have your questions evolved?*

A: The starting point in this research was the observation that although BFA efficiently blocks transport of proteins out of the ER, glycoproteins retained in the ER continue to be processed as if they were to be transported into the Golgi complex. A series of experiments employing diverse biochemical and morphological techniques showed that apparent maintenance of glycoprotein processing was due to redistribution of functional Golgi enzymes into the ER along a backward-moving, or retrograde, membrane-transport pathway. This finding prompted us to study the characteristics of this process, including how it occurred, its extent under normal conditions, and how BFA affected it. With this knowledge, we began using BFA as a new tool for investigating the biochemical basis for the regulation of membrane traffic and maintenance and the remodeling of organelle structure.



Bidirectional membrane traffic between the endoplasmic reticulum (ER) and Golgi complex. The membranes of the ER and Golgi complex communicate dynamically through distinct anterograde (ER-to-Golgi) and retrograde (Golgi-to-ER) pathways. Anterograde traffic involves discontinuous coated transport intermediates that move, minus-end-directed, along microtubules. The formation of these intermediates is inhibited by Brefeldin-A (BFA). Retrograde traffic, by contrast, appears to utilize uncoated tubular processes which move, plus-end-directed, along microtubules to the cell periphery. The formation of these intermediates is enhanced in the presence of BFA. Membrane transport along anterograde and retrograde pathways is thought to be controlled by a regulatory system governing assembly and disassembly of cytosolic complexes such as coatamer proteins, or COPS, on membranes. A controlled balance of membrane input and output through these pathways is crucial for the continued maintenance of Golgi structure, which functions as the conduit for proteins leaving the ER.

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

A: As we were investigating the phenotypic effects of BFA and the biochemical basis of BFA action, we made several surprising findings. First, we found that redistribution of Golgi components into the ER during BFA treatment occurs through a unique retrograde membrane-transport pathway: Golgi-derived membrane is carried on long membrane tubules that migrate along microtubules to the cell periphery. This pathway is also important for recycling membrane back to the ER. Transport of membrane components along the retrograde pathway is normally selective, but in the presence of BFA, it becomes nonselective.

A second surprising finding is that the Golgi completely disassembles through the retrograde pathway during BFA treatment. This is the first example of a drug causing an organelle to disappear rapidly within cells, and it indicated that organelles, such as the Golgi, are not static, unchanging structures. The observation that the Golgi can reassemble equally rapidly into a functional complex upon BFA washout further supports our view of dynamic organelles and provides a novel approach to investigating organelle biogenesis.

The third surprise was our finding that upon BFA treatment a discrete population of peripheral membrane proteins [including coatomer and (ARF)] rapidly

continued on page 23.

HOT METHODS CLINIC

FROM HOT STARTS AND FALSE STARTS TO SMART STARTS: IN SITU PCR

by Marta Corcoran, Ph.D., NCI, Mike Levin, M.D., NINDS, Steven Jacobson, Ph.D., NINDS, and Lance Liotta, M.D., Ph.D., NCI

The enticing promise of in situ polymerase chain reaction (ISPCR) is that by amplifying DNA within cells, the sensitivity of in situ hybridization (ISH) is elevated to permit detection of single copy DNA sequences or low copy mRNAs in individual cell preparations or tissue sections. This would allow researchers to visualize single cells bearing premalignant mutations or karyotypic alterations. In addition, genetic engineers and virologists might use ISPCR to demonstrate incorporation of transfected DNA, proviral sequences, or infectious agents.

How the Method Works:

The concept of ISPCR is simple. Specific nucleic acid sequences are amplified inside single cells to achieve copy numbers easily detectable by ISH. At first the procedure seems straight forward: simply place a slide containing a smear of cells or tissue section onto a thermocycler overlayed with the usual PCR reaction mixture. Following amplification, detection of product is evaluated by ISH. In practice, the simplicity is deceptive. Many NIH scientists experienced with PCR and ISH have been unable to get ISPCR to work reproducibly. For this reason, *The NIH Catalyst* has received a number of requests to feature ISPCR in this column.

Inspecting the details of ISPCR can provide insight into the sources of artifacts. As outlined in the figure, cells or tissues are first fixed, then treated with proteinases and permeabilizing agents. This treatment allows the primers access into the cell without damaging cell morphology. Ideally, the permeabilized cells should function as "amplification sacks" (1) with semipermeable membranes allowing primers, polymerases, and nucleotides to enter the cell, but trapping the amplified target sequence inside the cell. If DNA is the target sequence, direct PCR amplification is performed. However, if the target sequence is mRNA, a preliminary reverse transcription is required to generate a cDNA template which is subsequently amplified. To confirm sensitivity and specificity, these procedures require appropriate positive and negative controls. PCR amplification is conducted in either a thermocycling oven or a block cycler. The amplified intracellular sequences are visualized directly if digoxygenin (DIG)-UTP is incorporated into the amplification reaction or indirectly by post-PCR ISH using probes that recognize sequences flanked by the PCR primers (Fig. 1).

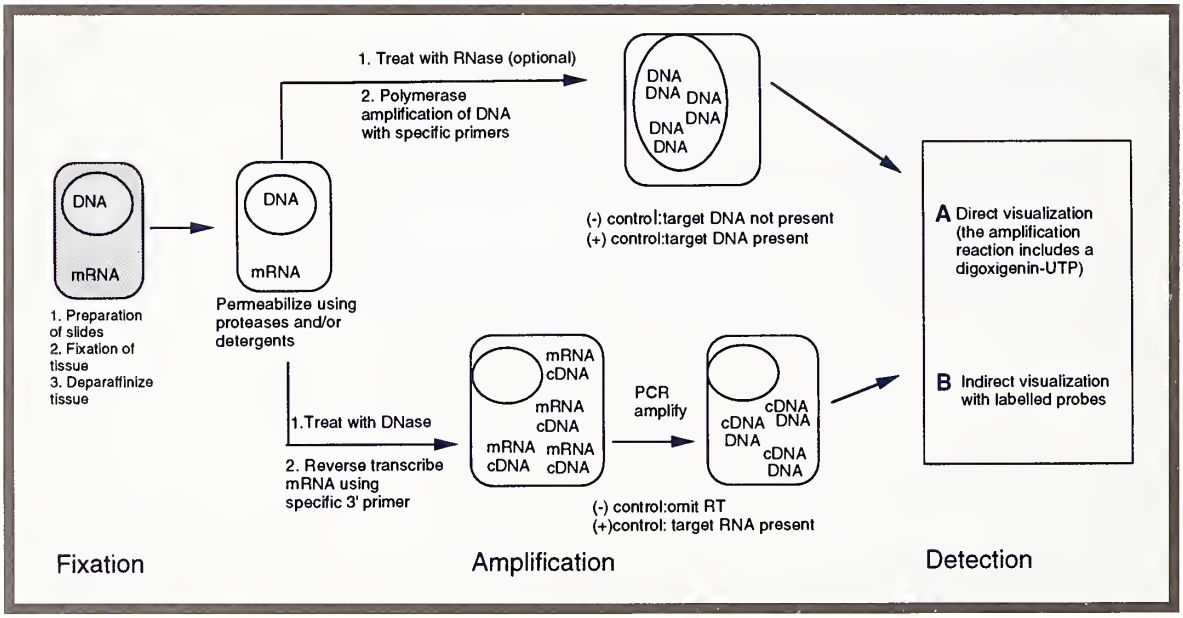


Fig. 1. Steps involved in in-situ PCR. The method can be used for direct or indirect visualization of DNA or mRNA, depending on the application.

After surveying various protocols and refinements utilized by researchers at the NIH, we are optimistic that the problems associated with ISPCR can be resolved. ISPCR can yield accurate results if the primers that yield appropriate PCR product are adapted to specific conditions so as to minimize artifacts due to diffusion of PCR products, non-specific priming and incorporation of nucleotides. A "hot start technique" for ISPCR has appeared in the literature that may reduce mispriming and primer oligomerization (2). Dr. Nuovo has published a protocol for RT in situ PCR which targets mRNA (3).

Two Protocols for In-Situ PCR

Since obtaining consistent results has been challenging, we have included two different ISPCR protocols in this article. The first, by Pierre Gresseus and John R. Martin of NINDS (4) directly incorporates DIG-labeled dNTP's into the amplified PCR product, and then detects the product with alkaline phosphatase. The second, by Michael Levin and Steve Jacobson of NINDS (5), utilizes overlapping multiple primer sets to retain amplified PCR products within the cell and detects the product with a specific ^{35}S labeled riboprobe to maximize specificity and specific activity. Mention of specific products does not constitute an endorsement.

In-Situ PCR with Direct Visualization

1. The treatment of slides, tissue fixation and de-paraffinization of tissues is similar to those used for ISH. De-paraffinize tissue using xylene and absolute ethanol followed by phosphate buffered saline (PBS) rehydration.
2. Digest the sample in 200µl of proteinase K for 5 min at 37°C. (For brain

sections, 10, 25, or 50 µg/ml proteinase was used. For trigeminal ganglia sections, 25µg/ml of the proteinase gave the best tissue preservation for subsequent amplification of *herpes simplex* virus.)

3. Wash with PBS three times for 5 min. Wash 5 min with distilled water.

4. For the "hot-start" technique preheat the slides, PCR mixture, coverslips and mineral oil to 82°C. The PCR mixture should include the following:

- 0.25µM primers
- 10µM each dATP, dCTP, and dGTP
- 3.5µM dTTP
- 6.5µM DIG dUTP
- 10% glycerol
- 2.5 units Stoffel Taq polymerase and 2.5mM $MgCl_2$ OR 2.5 units native Taq polymerase and 1.5mM $MgCl_2$

5. Apply PCR mixture to slide and cover with a coverslip which could be anchored with nail polish. Blanket with a layer of mineral oil and transfer to thermocycler oven in an aluminum foil boat.

6. Amplify 1 min at 96°C, 1 min 59°C and 1 min at 72°C. (For brain sections, amplify 15 cycles; for trigeminal ganglia use 30 cycles.)

7. Remove oil by dipping slide in xylene and absolute ethanol. Remove coverslip.

8. Wash three times for 5 min in 10mM Tris-HCl (pH 8.3) containing 1.5mM $MgCl_2$ and 0.001% gelatin. Wash three times for 10 min in 50% formamide-2X-SSC (0.3M sodium chloride and 0.03M sodium citrate) at 37°C. Wash twice in 2X SSC at room temperature.

9. Detect amplified sequences by alkaline phosphatase using anti-DIG antibody as described by Boehringer-Mannheim Genius Kit 3 (Nucleic Acid Detection kit).

10. Counterstain cell nuclei and mount with aqueous mounting medium.

Protocol for In-Situ PCR with Indirect Visualization (5,6,8)

1. For the amplification, all solutions are made in diethyl polycarbonate (DEPC)-treated water. Fixed cells are prepared as described by Fox et al. (6). Briefly, 5 X 10^6 cells are suspended in 100-200 µl of normal human serum. A cell suspension clot is formed by adding 200-300 µl of thrombin. Clots are fixed at room temperature with 4% paraformaldehyde followed by paraffin embedding. Five micron sections of the clot are placed on silinated slides.

2. Deparaffinize slides with xylene. Rinse in absolute ethanol and rehydrate in 0.1 M Tris HCl (pH 7.4) for 10 min. Permeabilize in 0.1M Tris HCl (pH 7.4) containing 0.3% Tween 20 and 0.3% NP 40 for 10 min.

3. Digest in proteinase K (10 µg/ml) for 10 min. at 37°C. Wash with 0.1 M Tris/HCl (pH 7.4) three times for 5 min. each.

4. Place in prehybridization buffer (this is the "PCR buffer" without Taq, primers, or dNTP's) for 30 min. Set thermocycling oven at 72°C. Warm mineral oil and PCR buffer to 72°C. PCR buffer contains:

- 1 µM each primer (4 overlapping primer sets)
- 200 µM dNTP's
- 1.5 mM $MgCl_2$
- 50 mM KCl
- 0.1 % gelatin
- 0.02 % NP-40

6.5 units Taq Polymerase per sample. A Taq-to-primer ratio of 0.0125 to 0.0275 is best for PCR/ISH. The following formula is helpful to calculate the ratio. Solve X for correct amount of Taq: ratio=(X µl Taq)(5 U Taq/µl)/ (total vol in µl) (total primer concentration in pmol/µl).

5. Apply 30 µl of warmed PCR buffer to each slide and add coverslip. Dry in oven for 30 seconds to evaporate excess PCR buffer, then surround cover slip with thick layer of nail polish. Place in oven to dry for 5-6 minutes.

6. Place slide in plastic bag containing 9 ml warmed mineral oil and seal bag with heat sealer.

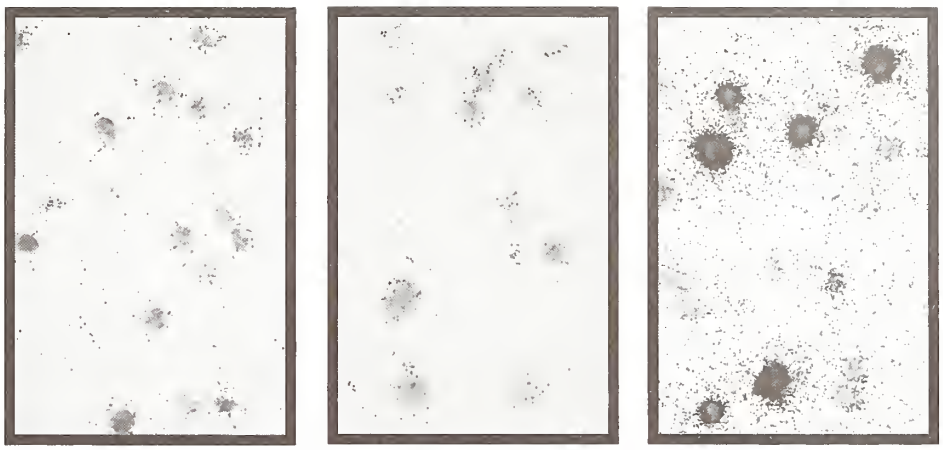
7. Place bag in the thermocycler oven and amplify for 40 cycles at: 92°C — 1min; 53°C — 1 min., 15 sec; 70°C — 2 min. To maximize efficiency, oven may be placed in cold room.

8. Remove oil with chloroform and remove coverslip. Rinse again in chloroform followed by absolute ethanol for 5 min. Wash twice with PBS for 10 min.

9. Dip in 2% gelatin for 30 sec. Post-fix in 10% glutaraldehyde for 20 min and rinse in 0.3M ammonium acetate in 95% ethanol for 5 min. Air dried sections are now ready for in situ hybridization and may be stored in sealed bags with Dry-rite for several days at 4°C. Following PCR amplification, the ISH protocol (6) was adapted to maximize the detection of viral DNA.

10. Allow slides to equilibrate to room temperature and rehydrate in PBS for 10 min. Place slide in 1X PBS containing 10 mM dithiothreitol (DTT) for 10 min. at 45°C. Place in 1X PBS containing 10 mM DTT, 10 mM iodoacetamide (IAA), 10 mM n-methyl maleimide (NEM) 10 min. Rinse twice in 1X PBS for 3 min.

continued on page 14.



Slides courtesy Mark Levin

Fig.2 Detection of HTLV-1 Tax *DNA in HTLV-infected human T-cells using:*

A. In-situ hybridization (ISH) *B. PCR/ISH without Taq (no amplification)* *C. PCR/ISH with Taq*

Hot Methods Clinic
continued from page 13.

11. Make 0.1M tri-ethanolamine hydrochloride (TEA) and raise pH to 8.0 with sodium hydroxide. Add 2.5 ml acetic anhydride to 500 ml of TEA. Drip this solution directly onto slides and rock for 10 min. Rinse in 2X SSC for 10 min.
12. Individually blot dry each slide and cover tissue section with 40 µl of prehybridization buffer for 1 hour. Prehybridization cocktail contains the following: 2X SSC, 1X Denhardt's, 50 mM phosphate buffer, 50 mM DTT, 500 µg/µl salmon sperm DNA, 250 µg/µl tRNA, 5 µg/ml poly d(A), 100 µg/ml poly (A), 0.05 pmole/ml randomer, 57% dextran/formamide (1:5=w:v).
13. In oven, denature slides at 80°C for 5 min and then place directly into ice-cold 2X SSC.
14. Individually blot dry each slide and add 40 µl of hybridization mixture. For the hybridization cocktail, 0.08 µl probe/µl cocktail is added (^{35}S-labeled riboprobe, specific activity of 2 X 10^6 dpm/µl). Cover with a coverslip and seal with rubber cement. Allow rubber cement to dry at room temperature for 20 to 30 min and place slides at 45°C overnight.
15. Remove cover slips and rinse three times in 2X SSC for 5 min. at room temperature. Incubate the slide in 0.25X SSC containing 1.2M DTT, 0.5M EDTA, and 76% deionized formamide for 30 min. at 37°C. Repeat incubation.
16. Rinse in 0.25X SSC containing 1.2M DTT, 0.5M EDTA, for 10 min. at 37°C. Place slide in RNase solution containing the following: 25 µg/ml RNase A, 25 µl RNase T1, 0.5M NaCl, 1.2M DTT in 0.01 M Tris/HCl (pH 7.4) for 40 min. at 37°C. Rinse in 2X SSC for 15 min. at room temp. Repeat rinse.
17. Dehydrate in 0.3M ammonium acetate in 70% ethanol for 5 min., followed by 0.3M ammonium acetate in 95% ethanol for 5 min. at room temperature and air dry.
18. Dip in NTB3 emulsion and incubate slides in dark at 4°C for 5 days. Develop with Kodak D-10 developer and regular fixer at 15°C.
19. Counter-stain with hematoxylin and eosin. Anchor coverslip with permount. Figure 2 demonstrates how a signal obtained from ISH can be specifically amplified by using this in situ PCR technique.

Troubleshooting Tips:

"Try the conditions on a cell cytospin first before tackling an intact tissue section. If these results are negative, examine the supernatant for products to see if primers and product may have diffused out of the fixed cell. Larger products can be strategically constructed by using concatamer primers or multiple-overlapping primers (2). The use of DIG-nucleotides have also been reported to help trap the product inside the cell.

"The tissue fixation technique and chemistry must be optimized for each application. Several chemicals are available, but buffered formalin is preferred. Protease concentration, treatment time, and the method utilized for fixation must be optimized for the tissue. The goal is to permeabilize enough for the probe to penetrate cellular barriers but not destroy cellular morphology. Proteases such as pepsinogen, pepsin, or chymotrypsin could be tested at different concentrations and for different times to determine the optimal conditions for the specific application. Nonionic detergents such as Triton-X 100 may also be used for permeabilization.

"The type of probe (RNA or DNA) and the detection method used should be optimized for each specific application. Several problems have been reported using direct incorporation of labeled nucleotides. The researcher needs to establish the best method to detect the target sequence."
— *Doug Kingma, NCI.*

"Perform solution-phase PCR on positive and negative areas of the tissue to confirm ISPCR results." — *John Martin and Pierre Gressens, NINDS .*

"The primers to be utilized as positive and negative controls must first be tested in soluble-phase PCR to optimize the conditions for ISPCR. Aspects that need to be evaluated are: product yield, specificity of priming, optimal number of cycles, and temperature conditions for each cycle (5). According to Gressens, amplification of products of approximately 100-base pairs is best. If the probe used for the ISH was inadequate or too large, using specific primers in ISPCR may boost the strength of the signal. In-situ PCR is one of the most powerful tools for molecular biology, but it is also very tricky...Pay close attention to false positives and negatives...Each cell should be considered a different reaction." — *Andrea Cara, NCI.*

In-Situ PCR Contacts

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RECENTLY TENURED

Ted Hackstadt *received his Ph.D. from Washington State University in 1980. He joined the Laboratory of Intracellular Parasites (LICP), NIAID, as an Expert in 1990 and is now Acting Chief of the Host-Parasite Interaction Section of the LICP.*

Researchers in my lab are interested in the basic biology of bacterial obligate intracellular parasites — in particular, *Chlamydia trachomatis*, one of the leading cause of sexually transmitted disease in this country and of infectious blindness worldwide.

Prokaryotic obligate intracellular parasites have evolved mechanisms that enable them to survive in extracellular environments during transit to susceptible host cells. These survival strategies generally involve a cessation of metabolic activity that can be reversed in response to environmental cues signalling arrival in an appropriate intracellular environment. Chlamydia have a complex life cycle that includes specialized cell types for extracellular survival and intracellular multiplication. During the extracellular stage of the life cycle, chlamydia take the form of small elementary bodies (EBs), with a core of condensed chromatin that disperses as the EBs differentiate into the larger, metabolically active reticulate bodies (RBs). In 1991, we found that modification of the DNA structure by histone-like proteins may be a central regulatory mechanism governing chlamydia's complex life cycle. Histone H1 homologs are rare among prokaryotes, but *C. trachomatis* possesses two proteins that have primary amino acid sequence homology to eukaryotic H1. These histone homologs, termed Hc1 and Hc2, are expressed only during the late stages of the chlamydial life cycle, during the reorganization of RBs into EBs, and they play a major role in establishing the nucleoid structure and controlling gene expression.

In *Escherichia coli*, Hc1 expression is self-limiting and produces a global termination of transcription, translation, and replication at concentrations equivalent to those found in chlamydial EBs. We have proposed that association of the chlamydial histones with DNA at levels below those causing condensation of the nucleoid may exert more specific regulatory effects through modification of DNA structure and topology, thereby influencing promoter activity and gene expression.

I am also interested in the intracellular compartmentalization of chlamydial replication. Chlamydia undergo their life cycles entirely within a vesicle that is not acidified and does not fuse with lysosomes. Lack of basic information on the physical and nutritional parameters within this vesicle, or chlamydial inclusion, severely limits attempts to identify environmental conditions that may serve to regulate the chlamydial developmental cycle. Using a variety of specific probes for various cellular organelles, in conjunction with conventional fluorescence and confocal microscopy, we have found that the Golgi apparatus may be involved in trafficking lipid to the chlamydial inclusion, implying that there is a direct interaction between the chlamydial inclusion and the Golgi network.

Christina Teng *received her Ph.D from University of Texas at Austin in 1969. She came to the Laboratory of Reproductive and Developmental Toxicology (LDRT) at NIEHS in 1983 from Baylor College of Medicine in Houston. She currently heads the Gene Regulation Group in the LDRT.*

I began my research at NIEHS 11 years ago with a simple goal: to isolate an estrogen-responsive marker from the mouse uterus in order to study gene regulation by estrogen at the molecular level. At that time, a suitable estrogen marker for the mouse uterus did not exist. Within a year, I succeeded in purifying a 70-kDa estrogen-responsive uterine secretory protein and raised polyclonal antibody against the protein. Two years later, we cloned the cDNA of this protein and identified it as lactoferrin. Due to the diverse roles of lactoferrin in milk, neutrophils, uterus, tears, saliva, and wet-surface mucosa, researchers studying nutrition, immunology, and the mammary gland have been interested in this protein for quite some time. My laboratory was the first to clone the cDNA for this biologically important protein, and we published the work in 1987. Since then, our laboratory and others have isolated the lactoferrin cDNA from humans, pigs, and cows.

Lactoferrin, transferrin, and melanoma antigen p97 belong to the same gene family. Research has established that lactoferrin plays antibacterial and antiviral roles and may function in immunity, cell growth, and differentiation. Lactoferrin is differentially regulated in various tissues. Both human and mouse lactoferrin promoter-enhancer regions contain regulatory elements typical of both housekeeping and inducible genes. We found that Chicken Ovalbumin Upstream Promoter (COUP)-transcription factor competes with estrogen receptor for binding to the estrogen-response element of the lactoferrin gene in mice, but not in humans. Recently, we found a cluster of sequence elements that respond to cyclic AMP, tissue plasminogen activator, and epidermal growth factor/transforming growth factor-α. These results, supported by in vivo findings, suggest that the lactoferrin gene is an interesting model to use in studies of gene regulation and cross-talk between different signaling pathways. ■

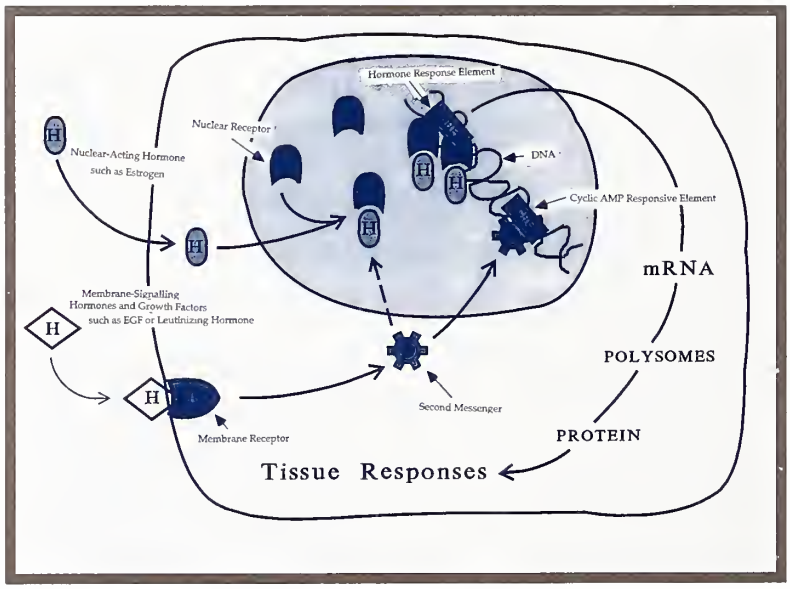
Commentary

Estrogen Receptor Knockout Yields Insights in Clinical and Basic Research Arenas

by Kenneth Korach, Chief, Receptor Biology Section, Laboratory of Reproductive and Developmental Toxicology, NIEHS

Since estrogen's discovery in the 1920s, scientists have believed that the hormone plays a crucial role in embryonic, fetal, and adult development, influencing female secondary sexual characteristics, reproductive cycle, fertility, and maintenance of pregnancy (1). In several target sites in the body — most notably, the reproductive tract, breasts, and neuroendocrine sites—estrogen's action is central to normal adult physiology and function (2). The dramatic lowering of estrogen concentrations that occurs during menopause has been implicated as one factor in osteoporosis and cardiovascular disease, but these effects are poorly understood, and it is unclear whether estrogen elicits a direct tissue action or indirect effects involving other regulators or signaling systems. Recently, in collaboration with the Developmental Endocrine Pharmacology Group at NIEHS, we found that other cellular signaling systems play a role in the mechanism of estrogen stimulation in reproductive tract tissue(3). The role of estrogen in men is even less well understood.

The demonstrated importance of estrogen in development and function, combined with uncertainties regarding its role, mechanisms, and sites of normal and pathological action, made knocking out the estrogen receptor (ER) gene to disrupt the expression of functional ER protein a highly desirable experimental goal, but also an endeavor that was unlikely to be successful or result in viable animals (4). However, we reasoned that if the knockout was lethal, we could finally determine the stage and possible sites during development at which estrogen becomes critical.



Examples of cellular mechanisms for hormonal stimulation. Steroids, thyroid hormones, and retinoids diffuse into cells where they interact with nuclear-receptor proteins, which function as ligand-activated transcription factors. The receptor-ligand complex dimerizes and binds to specific DNA sequences (HRE) upstream from genes regulated by the hormone. Regulation results in an increase in specific gene transcription that influences responses within target cells. Protein hormones and growth factors are examples of stimulants that interact with membrane receptors and elicit a cellular response mediated by an intracellular second-messenger signaling pathway.

Clinical evidence only increased our suspicion that an ER knockout would be futile, because no known conditions of estrogen insensitivity or ER gene mutations had been reported. In contrast, conditions of resistance to other hormones due to defects in other members of the hormone receptor gene family have been reported. Androgen insensitivity caused by disruptive mutations of the androgen-receptor protein results in abnormal male sexual differentiation and development (5). Thyroid (6) and glucocorticoid (7) resistance are other examples of clinical endocrine conditions that can result from receptor gene defects. Scientists attributed the lack of reported cases of estrogen insensitivity in humans and experimental animals to lethality during development (1) or to effects on embryo implantation. Blastocysts and two-cell embryos express estrogen receptor mRNA, supporting the possibility of an early developmental role (8).

Estrogens trigger their broad array of physiological responses, including tissue differentiation, growth, protein synthesis, and secretion (9, 10), by binding to a nuclear-receptor protein. Activation of the receptor induces tissue- and organ-specific responses to the hormone. The estrogen receptor is a ligand-inducible transcription factor that modulates target genes after it binds estrogen. Past findings had indicated that estrogen steroid hormones are required for tissue effects mediated by the receptor, but surprisingly, we demonstrated that specific growth factors, such as epidermal growth factor, could mimic estrogen in stimulating some biological responses. Mechanistically, this growth-factor action appears to operate via the estrogen receptor, providing a means of multiple signaling that converges and induces a tissue-specific response. Development of an animal model in which the two signaling systems were uncoupled—for instance, by eliminating a functional estrogen-receptor system—would allow the evaluation of the role of the dual signaling systems in physiological regulation.

Surprise One: A Viable Mouse

Defying our own skepticism, in 1990, we established a collaboration with Oliver Smithies' laboratory at the University of North Carolina in Chapel Hill and launched an attempt to produce a mouse homozygous for disrupted function of the estrogen receptor. We succeeded by inserting a sequence encoding neomycin resistance into exon 2 of the mouse estrogen-receptor gene. The neomycin insert includes a premature stop codon and polyadenylation sequences that inhibit proper transcription and translation of the ER gene, thereby functionally inhibiting its expression. Successful targeting of the sequence by homologous recombinations occurred in only two of 1,800 clones of embryonic stem cells in which we attempted to disrupt the ER gene. Several chimeric mice bearing the disrupted gene

continued on page 18.

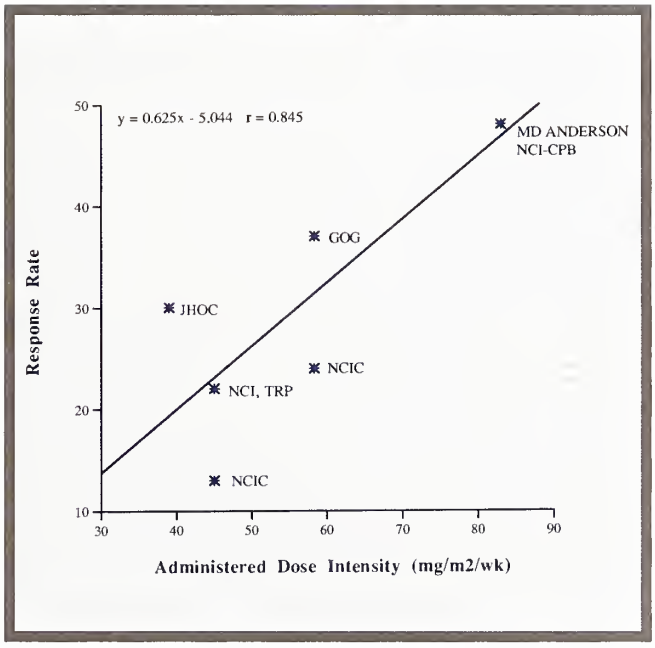
COMMENTARY

CONTROVERSIES IN THE TREATMENT OF ADVANCED OVARIAN CANCER

by Elise Kohn and Eddie Reed (EK, Unit Acting Chief, Laboratory of Pathology, NCI; ER, Acting Chief, Clinical Pharmacology Branch, NCI)

Identification and characterization of effective, novel treatment modalities is a critical mission for the Clinical Oncology Program (COP) of the National Cancer Institute. Within the Clinical Pharmacology Branch of COP, the Medical Ovarian Cancer Section has two arms: basic science research and clinical investigation. Our clinical focus has been on developing novel approaches to the treatment of epithelial ovarian cancer with emphasis on the treatment of newly diagnosed ovarian cancer patients and the subsequent treatment of patients with recurrent ovarian cancer or disease that is refractory to standard therapy. The unique integration of laboratory observations into clinical practice at NIH has allowed the development of novel treatment approaches for patients with newly diagnosed, advanced-stage epithelial ovarian cancer and patients with advanced and recurrent ovarian cancer. The new concepts in the use of Taxol identified in the Intramural Research Program have spurred further clinical investigation into 1) the use of dose-intense Taxol in an ongoing multi-institutional phase III study and 2) a three-drug combination for newly diagnosed patients in a phase II study.

Epithelial ovarian cancer, arising from the surface epithelial lining of the ovary, is the fourth leading cause of cancer deaths in women, accounting for approximately 15,000 deaths per year. Unfortunately, in over 80% of women, diagnosis is late, usually occurring after the cancer has spread throughout the abdominal cavity. The lack of early detection capability and the extensive disease at diagnosis result in a high mortality rate for this disease, with an average 5-year survival of 40%. The lack of successful screening techniques for the general population has heightened the importance of improved therapeutic and surgical interventions.



Analysis of response rate to Taxol as a function of administered dose intensity: The results from seven phase II trials are shown. The relationship is statistically significant, r=0.845. Institutions: JHOC, Johns Hopkins Oncology Center; NCIC, National Cancer Institute of Canada; NCI, National Cancer Institute; TRP, treatment referral program; CPB, Clinical Pharmacological Branch, M.D. Anderson Cancer Center.

The treatment approach to advanced-stage (abdominal-spread) ovarian cancer involves initial surgical debulking followed by chemotherapy. The addition of cisplatin to the therapeutic armamentarium and the advent of combination chemotherapy led to improvements in disease-free survival and overall survival for ovarian cancer patients. The standard chemotherapy treatment for newly diagnosed patients after surgery now consists of the combination of a platinum compound, cisplatin or carboplatin, with a DNA-alkylating agent such as cyclophosphamide. This combination has been shown to have synergy in vitro and to prolong disease-free survival and overall survival for ovarian cancer patients.

Over the past two decades, our ovarian cancer effort has defined the use and toxicities of platinum compounds and now has spearheaded the use of paclitaxel (Taxol) in high doses for patients with advanced ovarian cancer. The hypothesis under investigation is that more therapy, through either more intense doses and/or drug-combination therapy, might improve time to recurrent disease, disease-free survival, or overall survival.

Our first goal was the ability to intensify doses of therapeutic agents without unacceptable toxicity. In the 1980s, a dose-intense regimen of cisplatin was tested (1) This regimen, which doubled the administered dose of cisplatin, was profoundly toxic, resulting in significant and lasting neurotoxicity. With the identification of Taxol as a novel agent with a different spectrum of toxicity, we again addressed the possibility of increasing the administered drug dose to improve the therapeutic outcome.

William McGuire and colleagues at the Johns Hopkins Oncology Center in Baltimore generated excitement over Taxol's potential in 1989 with publication of their phase I/II study of continuous-infusion Taxol (2). This trial used a dose-escalation scheme, followed by expansion of a cohort that received the maximally tolerated dose that the researchers had identified in phase I. Because efficacy in patients with relapsed epithelial ovarian cancer was observed during the phase I component of the study, this disease was chosen for the phase II efficacy portion of the trial. The dose-limiting toxicity in this trial was myelosuppression-marked lowering of the white blood cell counts.

The advent of cytokine administration to obviate myelosuppression (3) suggested that it would be possible to increase the administered dose of Taxol with a white blood cell - stimulating agent, such as granulocyte colony-stimulating factor (G-CSF). We began our studies of dose-intense Taxol in 1990 with 18 patients in a Taxol dose-escalation study in which G-CSF was used to block bone marrow suppression. G-CSF administration was successful in preventing the dangerous myelosuppression, and sensory peripheral neuropathy emerged as the new dose-limiting toxicity in our trial (4). By using G-CSF, we were able to administer Taxol safely at a dose of nearly twice that recommended by the trial of McGuire and colleague (250 mg/m^2 vs. 135 mg/m^2 every 21 days).

continued on page 19.

ESTROGEN RECEPTOR
continued from page 16.

were produced, including one in which the mutation was transmitted through the germ line. Mating of this chimera produced heterozygous mice of both sexes. These mice, bearing one copy of the wild-type ER gene and one copy of the inactivated ER gene, were screened by Southern and polymerase chain reaction analyses to ensure heterozygosity. These heterozygotes were fertile and exhibited no remarkable phenotype associated with disruption of one ER gene. Crosses of the heterozygotes resulted in normal litter sizes of live offspring with a traditional Mendelian distribution of genotypes. An even sex ratio was seen in the mice homozygous for ER gene disruption, indicating that sex determination is not influenced by the absence of a functional estrogen receptor. Most importantly, these mice provided the first evidence that absence of an active estrogen receptor is not lethal. Attempts to breed the homozygous ER-negative mice showed they were infertile.

We are curently analyzing the tissues of heterozygotic- and homozgotic-recessive animals to check for alterations in phenotype associated with inactivation of the estrogen receptor. To characterize the transgenic line (ERKO), we are analyzing the molecular effects of different types of hormonally active compounds, such as diethylstilbestrol (DES) and Tamoxifen, to see whether any other, as-yet-undescribed proteins or receptors may be present that can mediate estrogenic activity.

The ERKO females may be the first physiological model for critically testing the role and action of estrogen in the ovary. Initial analyses of the first recessive females have shown that they contain reproductive tract structures but lack any of the uterine responses to estrogen treatment seen in animals with ERs. ERKO females also have hemorrhagic cystic ovaries, suggesting overstimulation by gonadotropins due to the lack of a functional negative-feedback mechanism. Ovarian histology shows no functional corpora lutea, even though the granulosa and thecal cells, which normally surround developing ova, are present. Folliculogenesis proceeds through primary and secondary stages but stops short of a terminal stage, with no ovulatory follicles present. Further analyses of the ovaries are being performed to evaluate biochemical indices of response to exogenous stimulants.

One of the most surprising findings was that ER-recessive male mice are infertile but appear to have anatomically normal male accessory sex organs. Histological analysis indicates that sperm are present in the testis and epididymis, but the sperm count is less than 10 percent of that in normal mice. ER-recessive male mice should be useful for evaluating the role of the ER in male reproductive biology.

Other observations on the ERKO mice are suggesting additional experiments. For example, adult female mice lacking the ER have undeveloped mammary glands. We are now attempting to cross an onco-mouse having an increased incidence of mammary cancer with ERKO heterozygotes to test whether the estrogen receptor is necessary for the development of breast cancer. Of particular interest to our own studies was the observation that the bone density of ERKO males and females is 20 - 25% lower than it is in wild-type mice. This suggests a direct role for estrogen-receptor action in bone physiology.

Once the ER-null mouse is characterized, it should be useful in understanding whether the effects of environmental chemicals associated with estrogenic-like effects operate through the classical estrogen-receptor signaling pathway. The mice could also be used to assess the activity of various drug preparations for possible estrogen-like activities. Similarly, groups of ERKO mice will be treated with DES to determine whether the same reproductive tract and other target-tissue cancers develop in these mice as develop in humans and wild-type animals.

ERKO transgenics are also being used as the background strain on which to reintroduce mutant estrogen-receptor protein (e.g., TAF-1 or TAF-2 deletion mutants) by classical gene-transfer technology. Previously, analyses of the expression and function of these mutant receptors could only be done by transfection studies in vitro. Now, animals can be produced that express only the mutant receptors, permitting analysis of tissue and gene regulatory specificity of the mutant receptor under physiological conditions in vivo. Reintroduction of ER protein using a tissue or cell-type-specific promoter can test for rescue of the recessive phenotypes.

Surprise Two: A Clinical Link

After we discovered that disruption of the estrogen-receptor gene was not lethal in our mice, we became curious about the absence of reports of parallel human syndromes or gene mutations. It has been pointed out that because of their genetic backgrounds, some experimental knockout mice may not totally reflect what would be expected for comparable conditions in humans (11). Thus, although our finding was exciting from an experimental standpoint, we were doubtful about its application and relevance to human physiology.

Our doubts were dispelled late last year when we were contacted by a colleague at the Children's Hospital in Cincinnati about a 28-year-old fully masculinized male patient who presented at the clinic for genu valgum (knock-knees) and upon examination was found to have unclosed epiphysis. The patient was insensitive to high-dose estrogen treatment, showing none of the expected side effects, such as gynecomastia. In light of our finding that ER gene disruption was not lethal, the attending physician considered the possibility that the patient had some inactivation of estrogen-receptor function and sent us blood samples for molecular genetic analyses.

Our analyses demonstrated that the patient, the offspring of a first-cousins marriage was homozygous for a mutation in exon 2 of the estrogen receptor gene. The mutation resulted in the creation of a premature stop codon, producing truncation of expression of a functional estrogen-receptor protein (E.P. Smith, J. Boyd, G.R. Grank, H. Takahashi, R.M. Cohen, B. Specker, et al., unpublished observations). This is

the first example in humans of a loss-of-function mutation in the estrogen-receptor gene producing an estrogen-insensitivity syndrome. In addition to nonclosure of the epiphysis, the patient has dramatically low bone density, a symptom similar to that observed in the ERKO mice. This patient has raised our confidence that the ERKO mice may be an acceptable model for a variety of human estrogen responses and accompanying mechanisms.

It is especially satisfying that this high-risk project, which initially seemed to have little likelihood of success, has yielded an animal model containing no functional estrogen receptor. This model is now giving us the ability and opportunity to evaluate the role of estrogen-hormone action in a variety of tissues at different developmental stages. Estrogen's importance in mediating physiological responsiveness and its role in cancer and other pathological conditions may finally be determined. We now hope to see this experimental system rapidly applied to answer clinical questions regarding osteoporosis, cardiovascular biology, and breast, endometrial, and ovarian cancers . ■

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OVARIAN CANCER

continued from page 17.

This observation led to our recent phase II efficacy trial of Taxol at this maximal dose for patients with chemotherapy-resistant ovarian cancer. The use of G-CSF allowed maintenance of intended dose intensity within 90% of the planned dose per cycle, of 250 mg/m^2. The frequency of response, defined as greater than 50% reduction in the measurable tumor mass in response to high-dose Taxol, was 48% (4). Notably, this response rate was observed in a cohort of patients who were resistant to platinum and, thus, were also resistant to other chemotherapeutic interventions (5). The result is also in striking contrast to the 24% response rate for platinum-resistant patients reported by McGuire and co-workers when they administered doses of Taxol in the range of 117 to 135 mg/m^2 (see figure 1). Our doubled response rate in heavily pretreated patients has been confirmed by Kavanaugh and colleagues at the M. D. Anderson Cancer Center (6) and collectively, the findings have sparked a controversy in Taxol-dosing recommendations.

A SECOND ISSUE IN THE TREATMENT OF EPITHELIAL OVARIAN CANCER IS WHAT THE OPTIMAL COMBINATION REGIMEN SHOULD BE.

The striking efficacy of dose-intense Taxol administration for patients with advanced relapsed and drug-refractory ovarian cancer was the basis for the development of a phase II randomized clinical trial of Taxol by the multi-institutional Gynecologic Oncology Group. The objective of this trial is to determine whether there is a dose-response relationship for the administered dose of Taxol. Patients will be assigned randomly to one of three groups to receive doses, per cycle, of 135 mg/m^2, 175 mg/m^2, or 250 mg/m^2. Shortly after initiation of the trial, the low-dose arm was terminated due to early evidence of a dose-response relationship between the lower two arms. The two higher-dose arms of the trial are now accruing patients and G-CSF is being used at the highest dose level of 250 mg/m^2 per cycle to ameliorate the myelosuppression we saw in our trial. The phase II clinical trial should definitively address the issue of Taxol-dosing recommendations for patients with advanced epithelial ovarian cancer.

A second issue in the treatment of epithelial ovarian cancer is what the optimal combination regimen should be for the initial treatment of newly diagnosed patients after debulking surgery. Previous clinical trials, many initiated in the Clinical Oncology Program, have identified the utility of combination chemotherapy in the treatment of such patients. The current standard regimen is the combination of cisplatin with cyclophosphamide; however, it is currently unclear whether this is the best therapy. Is the combination of cisplatin with cyclophosphamide is equivalent or superior to the combination of cisplatin with Taxol? Points of comparison include the response rate to initial therapy, durable responses to treatment (prolonged disease-free intervals or long-term disease-free status), and toxicity pattern. The combination of cisplatin with cyclophosphamide has a high response rate—in the range of 70 - 80% clinical complete responses—but only 20 - 25% of patients will have no evidence of disease at surgical restaging and half of those patients will relapse. In addition, the higher recommended doses of cisplatin and cyclophosphamide can have serious and lasting side effects.

On the basis of the preliminary report from a prospective, randomized study conducted and reported by the Gynecologic Oncology Group (7) cisplatin used in combination with Taxol may have clinical benefits over standard cisplatin with cyclophosphamide. This clinical benefit is small and has lessened with further follow-up. Thus, the next question became, if cisplatin with Taxol is similar to or slightly better than cisplatin with cyclophosphamide, is the combination of all three drugs potential-

continued on page 22.

Executive Summary of the Report of the External Advisory Committee

continued from page 1.

enhanced capabilities for translating such knowledge into clinical application. On the other hand are rising costs of biomedical research. These forces are leading to a new reality in the extramural research community. Research judged to be "good," "very good," or even "excellent" is no longer funded. Funding of new grants is at an all-time low of about 15 percent of submitted proposals.

The NIH IRP is also facing its own difficulties. Over the past decade, the IRP has experienced problems with recruitment and retention of senior scientists, expansion of a postdoctoral training program of uncertain and uneven quality, cumbersome administrative requirements, inadequately funded congressional and administrative mandates, and a deteriorating facility infrastructure — in particular, the Clinical Center.

Concerns about the health of the NIH IRP contributed, in part, to the establishment of the External Advisory Committee. Specifically, the fiscal year (FY) 1994 House Appropriations Committee Report directed the new Director of NIH "to review carefully the roles, size, and cost of the intramural program [IRP], and its relationship to the extramural research program, and indicated that NIH must put together a process "for allocating resources to and among its intramural programs based on a thoughtful analysis of these issues."

Recent congressional concern has focused on three issues with respect to the IRP: 1) whether the level of quality across the IRP continues to place it among the best institutions; 2) whether the allocation of resources to the IRP relative to the ERP can be justified based on rigorous considerations of quality and the importance of the research questions addressed in the IRP; and 3) given the high cost of the needed renewal of the physical facilities of NIH, particularly the Clinical Center, what new and renewed facilities are required to ensure high-quality research and productivity in the future.

The IRP has a fragmented federated structure with inadequate processes for oversight by NIH's Office of the Director. Each institute, center, and division has a different legislative history and mandate from Congress, and each institute's intramural program differs with respect to goals, scope, absolute size, and allocation of funding between extramural and intramural research. This complex structure for the administration and conduct of research has both strengths and weaknesses. Although it has contributed to a research establishment of great diversity and vitality, it has led to an administrative structure that in the present environment of constrained resources, frequently hinders effective management of the IRP. This Balkanization of the IRP has contributed to unevenness in quality, quality control, and productivity.

At least three previous advisory committees have made recommendations for improving the IRP, some of which have been implemented but many of which have been ignored. This may be attributed in part to systemic problems that transcend NIH and require major administrative or legislative remedies and in part to resistance to change within a large institution.

The IRP possesses several unique characteristics that set it apart from the extramural research program. These include relatively long-term and stable funding of research programs, availability of the Clinical Center's patients and facilities, few or no distractions from research for scientists, and a primarily retrospective rather than prospective review process for determining scientific quality and the funding of research. It must be emphasized that a strong ERP requires a strong IRP, and quality — not necessarily uniqueness — should be of the highest priority in determining support for the intramural research program. Those with the responsibility to make decisions must use a rigorous approach to evaluate quality in terms of personnel, training, management, and priority of the research program.

Periodic, objective, unbiased peer review is crucial to the long-term excellence of all scientific institutions, including NIH's IRP. Science progresses, and scientists must respond. The review process can be positive when it calls attention to deficiencies in time for them to be corrected. When improvement is not adequate, a review provides reliable justification for shifting resources from unproductive to more productive scientists. Every effort must be made to put in place personnel systems that facilitate recruitment of outstanding people and provide for termination of individuals whose research programs are of inadequate quality or are not sufficiently productive.

The challenge of "reinventing" the IRP requires that NIH rethink some of its practices regarding 1) appointing and promoting scientists NIH-wide, 2) recruiting and retaining outstanding scientists, 3) invigorating postdoctoral training programs that transcend institute lines, 4) using patient and research facilities in the Clinical Center, 5) instituting efficient management and review practices that are more responsive to the needs of the research enterprise, and 6) exploring opportunities for increased collaboration with the extramural community, including industrial and academic laboratories.

The recommendations contained in this report aim to create more uniform and consistent processes for setting priorities and ensuring quality across the NIH IRP. Although each institute should retain a level of autonomy in its research programs, more centralized control of the process for ensuring quality is desperately needed.

To enhance quality control, the External Advisory Committee makes several recommendations related to review of quality and productivity of scientists, scientific directors, and training programs. It is unlikely that the NIH intramural budget will increase significantly beyond the cost of inflation in the foreseeable future. The need to renovate the Clinical Center is also likely to drain funds from the operating budget of the intramural research program. One way to make room for new investigators will be to reclaim resources from those investigators whose research is no longer productive. This report outlines mechanisms to use in achieving the goal of redirecting intramural research resources to the most productive programs, thereby improving accountability and freeing resources for new recruitment and new initiatives and for renewing the Clinical Center. ■

EXECUTIVE SUMMARY OF THE REPORT OF THE EXTERNAL ADVISORY COMMITTEE,
continued

Major Recommendations

The Extramural Advisory Committee makes the following major recommendations. Additional recommendations and justification and methods for implementing the recommendations are presented in the body of the report.

1. To improve the processes by which Senior Scientists and Scientific Directors are reviewed, the External Advisory Committee recommends that a standing Advisory Committee to the Deputy Director for Intramural Research be formed that would be composed mainly of the Chairs of the external Boards of Scientific Counselors of each institute, center, and division. This committee should be charged to provide ongoing review of the processes of quality control across NIH. The committee should be chaired by the Deputy Director for Intramural Research (DDIR).

2. To improve quality review, the committee recommends that the selection and appointment process be altered for the Boards of Scientific Counselors to ensure expert, arm-length membership; that the process by which Boards of Scientific Counselors review the programs of intramural scientists be made more explicit; and that the criteria used to evaluate Scientific Directors be made more rigorous.

3. To ensure a strong tenure system that provides the intramural research program with creative and productive scientists, an NIH-wide Tenure Committee, advisory to the DDIR and composed of 12 to 16 tenured scientists serving staggered terms, should be established to review and recommend for approval (or rejection) all potential appointments to tenured and tenure-track positions. Recommendations for appointments to the tenure track should be made by each institute, center, and division through its existing processes, then forwarded to the Tenure Committee with all appropriate documentary support. Once the Tenure Committee is in place, it should no longer be necessary for the NIH Board of Scientific Directors to review or approve tenure decisions.

4. To improve the intramural training program, the independence and career development of trainees should be emphasized. Trainees should be encouraged to seek positions outside NIH after a two- to four-year program so that space and resources are continuously provided for recruitment of new trainees.

5. To provide ethnic diversity in the intramural training programs, there should be better linkage with NIH-funded extramural programs, including the NIH Minority Access to Research Careers and the Minority Biomedical Research Support undergraduate programs, and with the Short-Term Training Program for physicians. The intramural program should also increase the number of physician scientists from underrepresented minority groups by increasing research experiences for minority medical students.

6. An annual, prospective planning process should be conducted by each institute, center, and division to determine the allocation of resources to the intramural and extramural programs. The process should be outlined in a written document and reviewed, approved, and monitored by the NIH Director and the NIH Advisory Committee to the Director. Extensive consultation with the extramural research community should be part of this process. The overall NIH scientific mission should be assessed and allocation decisions should be made on the basis of scientific excellence and opportunity. The total IRP budget for institutes, centers, and divisions (ICDs) should not exceed the current rate of 11.3 percent of the total NIH budget. This percentage should be reviewed and appropriately adjusted through the prospective planning process, following full implementation of the recommendations that emerge from the quality review of the intramural program as outlined in recommendation number 1. It is anticipated that implementation of this process of quality assurance may require 3 to 4 years.

7. The procedures for procurement and staff travel should be streamlined and improved, as should the procedures for appointing technical and scientific staff as part of the process of "reinventing government." NIH could serve as a model for developing and testing novel procedures to make the procurement process efficient and responsive to research needs while simultaneously ensuring the integrity of federal expenditures.

8. To ensure that the NIH intramural program is fulfilling its mandate to facilitate technology transfer, NIH should broadly communicate in a clear and precise manner the scope, purpose, definition, and processes of implementing and monitoring Cooperative Research and Development Agreements (CRADAs).

9. To renew the Clinical Center, there should be a phased program starting with a 250-bed Clinical Center Hospital and followed by a modular approach to construction and renovation of research laboratories. Funds recovered from phasing out weaker intramural research programs should be used to the extent possible to fund renewal of the Clinical Center. However, recognizing the likelihood that these funds will not be adequate to meet the costs of renewal of the Clinical Center, the External Advisory Committee recommends that additional funds be allocated by Congress for this purpose. Funds must not be diverted from the ERP to the IRP for renewal of the Clinical Center.

10. If, on renewal of the Clinical Center, inpatient nursing units and laboratory research space become available in excess of the needs of the ongoing programs of the Clinical Center, then establishing priority for the use of such space should be at the discretion of the Director of NIH, with the understanding that priority should be given to programs currently housed off the Bethesda campus (both clinical facilities and research laboratories). Such consolidation of NIH intramural programs should facilitate quality control and could reduce costs.

11. Recognizing that it is not within the authority of the Director of NIH to change the current classification of the intramural research program as an administrative expense, the committee strongly believes that it should not be classified in this manner. Such a classification leads to budgetary procedures that are not rationally related to the scientific process and that do not support the goal of achieving the highest quality and productivity of the intramural research program. ■

OVARIAN CANCER
continued from page 19.

ly better than either combination alone? Several bodies of data collectively suggest that the combination of all three drugs may be preferable.

Our clinical team has been conducting a trial of the combination of Taxol and cyclophosphamide in patients with recurrent, platinum-refractory epithelial ovarian cancer. In preliminary analysis presented in April, we reported that this two-drug combination is better than either drug alone (8), with improved disease-response rate, frequency of complete clinical remissions, and median duration of response. These data suggest that, clinically, in tumor cells that are refractory to platinum-based therapy in a cohort of patients for whom effective choices are very limited, Taxol in combination with a bifunctional DNA alkylating agent may be very effective.

Recent basic research on DNA repair may shed light on the possible molecular mechanisms that underlie these clinical observations. The mechanism of antitumor activity of the platinum family drugs is the formation of platinum-DNA cross-links or adducts. In mammalian cells, platinum-DNA adduct repairs are made via the nucleotide-excision-repair pathway (9, 10). Taxol inhibits the repair of platinum-DNA adducts by up to 90%, and this is associated with markedly enhanced cell kill that is specific to the sequence of drug administration (10). The leading hypothesis for this effect of Taxol is that Taxol dissociates high-energy phosphates from energy-requiring reactions during the process of nucleotide-excision repair (9).

The DNA damage of bifunctional alkylator-DNA binding, on the other hand, is probably repaired by the mismatch-DNA-repair system (11), also an energy-requiring system. We hypothesize that the enhanced effect of Taxol with cyclophosphamide is related to inhibition of alkylator-DNA adduct repair, similar to that demonstrated for cisplatin.

The reliance on different cellular-DNA-repair pathways by cisplatin and cyclophosphamide would explain why the administration of the combination of cisplatin and cyclophosphamide has been shown to be better than either drug alone, and the ability of Taxol to inhibit both DNA-repair pathways supports the hypothesis that the combination of all three agents should result in superior clinical efficacy. This hypothesis is now being tested in a dose-escalation phase I clinical trial in the Medical Ovarian Cancer Section.

We have just completed a study of the sequential use of cyclophosphamide, Taxol, and cisplatin, used with G-CSF for bone marrow support for newly diagnosed, advanced-stage epithelial ovarian cancer patients. Our preliminary results, reported last year, have shown that these agents can be administered together safely (12). Optimal doses result in acceptable neurologic toxicity, minimal bone marrow toxicity, and antitumor activity that is greater than seen with other regimens. A multi-institutional phase II study of this regimen is planned.

Because the presence of platinum-DNA adducts in the circulating white blood cells of patients accurately mirrors the drug effect on tumor cells, these studies offer an opportunity to observe clinical-laboratory correlations. We have initiated laboratory studies of the levels of DNA-adduct formation in the white blood cells of patients undergoing treatment with the three-drug combination of cisplatin, cyclophosphamide, and Taxol. This will allow testing of the hypothesis that Taxol is favorably altering DNA repair and will help to establish a basic science foundation for understanding the interactions of these drugs.

Patients or referring physicians seeking information regarding these trials may contact the Medical Ovarian Cancer Section at (301) 402-1357 or the PDQ database available through the National Library of Medicine. ■

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MARYLAND'S OUTSTANDING YOUNG SCIENTIST

NICHD's Alan Hinnebusch recently received the 1994 Maryland's Outstanding Young Scientist award for his pioneering studies on the control of protein synthesis in yeast. He received an Allan C. Davis medal at a ceremony at the Maryland Science Center on April 25. ■

DIRECTOR'S SEMINAR
continued from page 11.

dissociate from Golgi membrane, before the Golgi membrane is redistributed into the ER. This observation indicated that BFA has a specific site of action on membrane, and this has set the stage for investigations of the biochemical basis for BFA effects and the regulation of membrane structure and traffic.

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

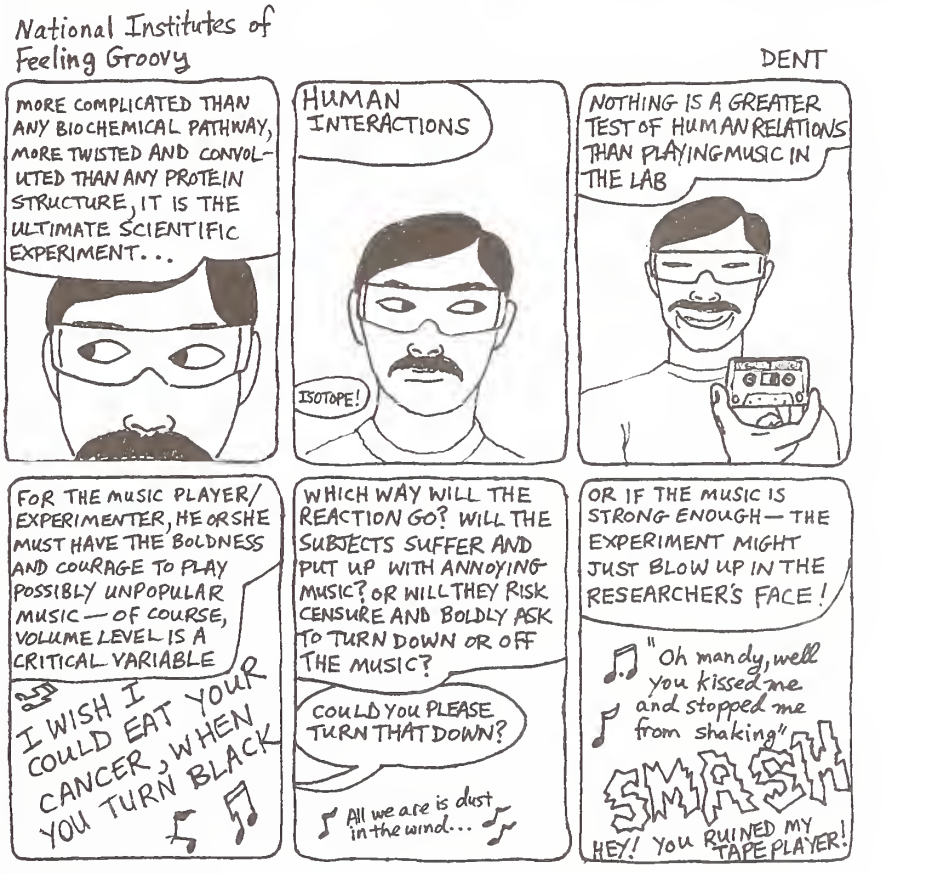
A: Our efforts at understanding the basis of BFA's effects on organelle structure and membrane traffic were not without stumbling blocks. We were quite fortunate, however, to have a wide variety of reagents to use in identifying organelles and membrane-transport intermediates in morphological studies. These were provided in large part by numerous very generous outside investigators. Also crucial for this work was our ability to adapt existing biochemical protocols and in vitro reconstitution assays to identify and characterize the peripheral membrane proteins whose association with Golgi membrane is affected by BFA.

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

A: Our finding that the membrane traffic pathways and the very existence of some organelles can be reversibly regulated by a simple fungal metabolite, BFA, opens the possibility of selectively blocking intracellular traffic patterns by this class of drug. This offers tremendous therapeutic potential in the treatment of intracellular parasites and toxic and infectious agents, since virtually all these agents rely on host membrane traffic routes that are sensitive to BFA. Thus, for example, BFA can inhibit viral gene expression on the surface of cells and is, therefore, a potentially potent antiviral agent. In addition, BFA prevents various bacterial toxins, including botulinum toxin, from entering into cells. BFA can also block the presentation of antigens that initiate an immune response and thus may improve the course of autoimmune disease and graft rejection. Finally, BFA has been shown to have extraordinary selectivity in its effects on the growth of different populations of cells, suggesting potential as an anticancer agent. Studies with BFA, therefore, have refined our understanding of the control mechanisms underlying membrane traffic, thereby providing clinical and basic investigators with unparalleled power to manipulate the transport of molecules through the membrane systems of the cell.

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

A: My current work is focused on understanding how cells control the organization and distribution of organelles through membrane trafficking pathways and the role this plays in developmental and disease processes. For this, we are examining both simple eukaryotic cells including *Toxoplasma gondii*, where we hope to define the minimal requirements for secretory transport, and more complex cellular systems including mammalian tissues and embryos, where we hope to find new relationships between membrane organization and function. These studies will require an understanding of the role of microtubules, microtubule motor proteins, and cytosolic organelle-associated proteins in organelle localization and transport. ■



FAX-BACK

In this issue we are asking for your feedback in four areas: the External Advisory Committee's report on the intramural program; improving intramural scientists' knowledge of the NIH grant process; the Hot Methods Clinic; and improving mentorship at NIH. **Fax your responses to 402-4303** or mail it to us at Building 1, Room 134.

In Future Issues. . .

- OAR's New Director
- Extramural NIH, Part II: NIH Grant Process and How to Write a Grant
- Hot Methods Clinic: 5'-RACE
- IRP Scientists Rate the Research Journals

1) What comments do you have on the External Advisory Committee's report on the Intramural Research Program and what suggestions would you make for implementing the report's recommendations?

2) Are extramural scientists correct in surmising that intramural scientists are ill-prepared for research life in the outside world? How can intramural scientists increase their knowledge about the NIH extramural grant process and better prepare themselves for the extramural world?

3) Do you have any suggestions or comments about the in-situ PCR featured in this issue's Hot Methods Clinic? Can you provide any tips for our next Hot Methods Clinic feature: 5'-RACE: a technique for extending a cDNA in 5' direction? What techniques would you like to see covered in future issues?

4) In our next issue we plan to discuss mentoring and career development of young scientists at NIH. What have been your experiences with mentoring? What suggestions do you have to improve mentoring and education of young scientists?

The NIH Catalyst is published bimonthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 134, NIH, Bethesda, MD 20892. Ph: (301) 402-1449.

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