FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

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

FROM PROBLEMS AND POLITICS TO THE COMMUNAL EXPERIENCE IN SCIENCE:

AN INTERVIEW WITH HAROLD VARMUS

In mid-December, The NIH Catalyst *spoke with NIH's new Director, Harold Varmus. Dr. Varmus was unanimously confirmed as Director on Nov. 20 and sworn in by HHS Secretary Donna Shalala on Nov. 23.*

Q: What have you learned in the meetings that you have had with groups of scientists on campus?

A: In the past two-and-a-half months, I've met with almost 1,000 people, covering all the institutes except NIEHS. I've heard a lot of complaints about the problems of crowding, both on and off campus, and that is troubling. A lot of things could be better: the interaction among laboratories; the training, recruitment, and retention of people.... I don't want to get too specific here because we have an ongoing review of the IRP [see "From the DDIR, page 2]. The Extramural Advisory Committee to the Director will produce a very insightful report, and I expect to put a lot of their recommendations into place.

As a researcher with a lab here, I care a lot about intramural problems—the FTE restrictions, space, seminar programs, getting graduate students at NIH, improving our postdoctoral fellows, access to researchers in multiple disciplines...I would like to create an environment that is as rich as possible. Right now, I am not convinced that it is as good to be a trainee here as it is at the best academic research universities. The interaction among laboratories is not as good, the seminar programs are not as distinguished, and they are not addressed to broad audiences.... You don't see the crowds at seminars that we should see. I don't want to overstate the problem, but

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The brief article that follows discusses an issue that is under active review, namely, the recent FIAU drug trial that resulted in five deaths. The article also represents a snapshot of issues as they appear at this point in time; details of the FIAU research and clinical protocols are the subject of several independent investigations which should shed more light on what happened during the FIAU trials. Although the conclusion of these investigations are not in yet, The NIH Catalyst *felt that it was urgent and critically important to clinical research to encourage reflection and open discussion of these issues within the intramural community. On the FAX-BACK page, we ask for your feedback on the implications of the FIAU trial for clinical research.* ■

FIAU STUDY MAY HAVE REPERCUSSIONS FOR CLINICAL RESEARCH

by Seema Kumar

It is every clinical investigator's worst fear: an investigational new drug that shows promise in animal studies and preliminary clinical trials with few apparent side effects suddenly turns out to be toxic to the patients in whom it is being tested. The scenario is always a remote possibility — but rarely a reality — in human trials of new drugs. Add to this another twist — that the drug apparently brings about its devastating effects months after patients have stopped taking it — and you have the makings of a terrible ordeal.

IN SEPTEMBER, THE ORDEAL TOOK ANOTHER TURN: THE REVIEW OF HOW AN INSTITUTION PROTECTS THE SAFETY OF RESEARCH SUBJECTS.

Intramural investigators Jay Hoofnagle, Stephen Straus, and their colleagues have been living amidst this ordeal since June 25, when the first of five patients was hospitalized and subsequently died from the toxic effects of fialuridine, or FIAU, a nucleoside analog that held promise of becoming the most effective drug in a limited arsenal of agents for treating chronic hepatitis B virus infection. Some 300 million people worldwide suffer from chronic hepatitis B infection, which affects about 50,000-100,000 people in the United States. The infection can lead to cirrhosis of the liver, liver cancer, and death. The FIAU clinical trial was stopped on June 26, and for the following two months, the investigators were consumed by crisis management: taking care of the other 14 patients in the trial, four more of whom died and two of whom underwent liver transplants. They also had to handle the barrage of media attention and prepare for an FDA hearing and several other investigations.

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HOT METHODS CLINIC: DIFFERENTIAL DISPLAY

by Lance Liotta

This article introduces what we hope will be a recurring feature in The Catalyst. *The feature will serve as a "clinic" in which we discuss and diagnose problems with new scientific methods that are hot.*

The Hot Methods Clinic will take a troubleshooting approach — starting with a potentially powerful new method that a few scientists on campus are having success with, and relaying their tips to others who are struggling or just gearing up to try the technique.

The new Hot Methods Clinic will have the following sections:

1. An explanation of the new method
2. A consensus protocol with the latest unpublished refinements, if possible
3. Trouble-shooting tips and quotes from successful users
4. A list of names and phone numbers of scientists throughout NIH who can provide advice, help, and collaboration.

We hope that the Hot Methods Clinic will foster inter-laboratory and inter-institute communication and collaboration, help young scientists who are setting up new projects, and provide information that could save researchers time, money, and resources.

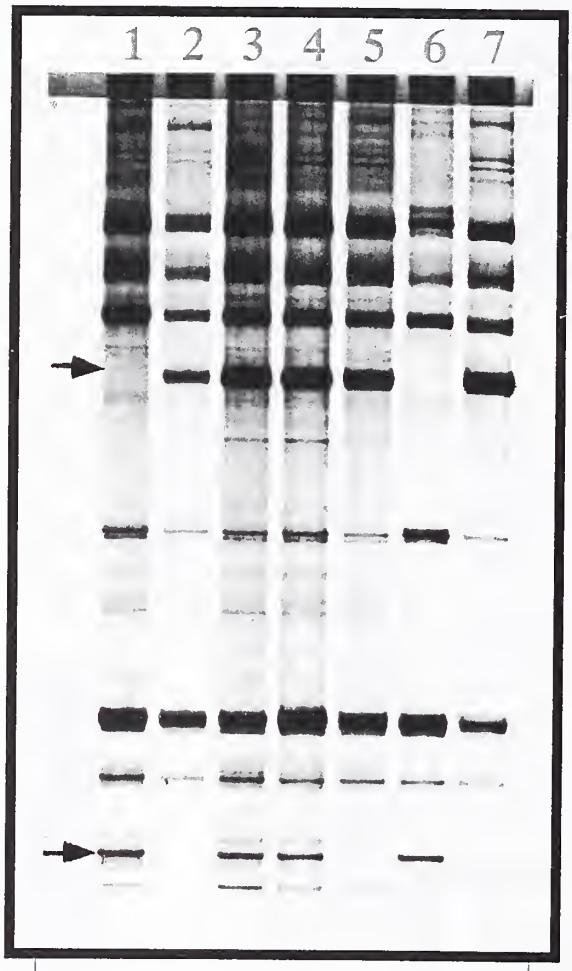
The first hot method to be discussed is **Differential Display:** *a new, direct way to isolate and sequence differentially expressed genes.*

How the method works

Differential Display was first described by Peng Liang and Arthur Pardee (1) as a way to identify and clone eukaryotic genes that are differentially expressed under different conditions, in different cell types, or under different pathologic states. The basic concept is to amplify a spectrum of messenger RNAs and distribute their 3' termini on a denaturing polyacrylamide gel. This allows for direct side-by-side comparison of a large series of mRNAs between a pair of related cells or conditions. Once an mRNA species band is identified that is differentially expressed, the band can be cut out of the gel and sequenced. The sequence information can be used to develop probes for further characterization and cloning.

You start with RNA samples isolated from cells growing under different conditions or from different cells. The source cells should be as similar as possible for a given research question. First, reverse transcription is conducted with a primer designed to bind to the 5' boundary of a polyA tail. This is followed by polymerase chain reaction (PCR) in the presence of a second 10-mer, arbitrary in sequence. The series of amplified cDNA subpopulations of 3' termini of mRNAs are then run out on a DNA sequencing gel. Theoretically, by changing primer combinations, up to 15,000 individual mRNA species may be displayed.

Liang et al (2) have recently described refinements in the original method and showed how it could be used to identify novel genes related to differences between normal mammary epithelial cells and breast cancer cells, and between normal and ras/p53 mutant transformed cells.



Differential display products obtained from normal prostate epithelium (lanes 2 and 5) and prostate carcinomas (lanes 1, 3, 4, 6, and 7) analyzed on a 6% sequencing gel. Arrows mark the position of bands corresponding to transcripts that are down-regulated in two tumor samples (top) and in the two normal samples (bottom).

Protocol for Differential Display Technique

(Prepared by Rudy Pozzatti—402-1963. This protocol was developed largely from the procedure published by Liang and Pardee [1]. Mention of specific products does not constitute an endorsement.)

First Strand Synthesis

Follow protocol described in Stratagene first strand synthesis kit (catalog #200420). For one reaction, use 5 to 10 µg of total cellular RNA. In a 1.5 ml microfuge tube, add RNA and diethyl pyrocarbonate (DEPC)-treated water to bring volume to 33 µl. Add 3 µl (300 ng) of "downstream" olgio dT-containing primer. Incubate at 65° C for 5 minutes, then place at room temperature for 10 min to allow sample to cool gradually.

Add the following reagents (in order) from the Stratagene kit:

5 µl of 10X first strand buffer
5 µl of 0.1 M DTT
1 µl RNase Block I
2 µl 25 m*M* deoxyribonucleoside triphosphates (dNTPs)
1 µl M-MuLV reverse transcriptase (20 units/ml).

Mix gently and incubate at 37° for 1 hour, then place on ice. The protocol for first strand synthesis warns that excess salt can inhibit Taq polymerase. Therefore, we have always de-salted the samples by passing over G50 sephadex spin columns from Boehringer Mannheim. The columns should be pre-washed with DEPC-treated water twice before loading sample. After the sample is passed over the spin column, measure the volume of recovered material.

The PCR Reaction

1) cDNA. Under the conditions listed below, titration experiments showed no significant benefit to adding more cDNA than is obtained from 1 µg of RNA in the first-strand-synthesis reaction. It is convenient to use 10 µg of RNA in the first-strand-synthesis, then divide the sample into 10 aliquots and perform 10 PCR reactions with 10 separate "upstream" primers and the identical "downstream" primer that was used in the first-strand synthesis reaction. We use the following downstream primers and upstream oligos:

Downstream Primers		Upstream Oligos	
1)	5' TTT TTT TTT TTT CA	11)	5' CTG ATC CAT G
2)	5' TTT TTT TTT TTT GC	12)	5' GAC TCA CTG G
3)	5' TTT TTT TTT TTT AG	13)	5' CCA GTA CAG
4)	5' TTT TTT TTT TTT GG	14)	5' GTG AGC TCC
5)	5' TTT TTT TTT TTT AA	15)	5' GGA TTC GTA C
6)	5' TTT TTT TTT TTT AC	16)	5' CAC GAC CTG A
7)	5' TTT TTT TTT TTT CC	17)	5' ACT CTG GTG T
8)	5' TTT TTT TTT TTT CG	18)	5' TGC GAT CAA C
9)	5' TTT TTT TTT TTT CT	19)	5' TAG CGA CTG T
10)	5' TTT TTT TTT TTT AT	20)	5' CTA GTG AGT C
10.1)	5' TTT TTT TTT TTT GA	21)	5' AGG CTC AGT CG
10.2)	5' TTT TTT TTT TTT GT	22)	5' TCG GTG GAC CA

2) For optimal separation of bands, we label with ^{35}S-dATP (New England Nuclear, NEG034H). The final concentration of ^{35}S-dATP in the PCR reaction is 1µM, corresponding to approximately 17µCi of isotope per reaction.

3) dNTP concentrations: We use 20 µM concentration of each of the four dNTPs. Our titration experiments showed that if we used lower concentrations, shorter PCR products predominated over longer ones. Higher concentrations of dNTPS resulted in fainter labelling of product molecules.

4) "Upstream" Primers: We have used oligos nine- and ten-nucleotides in length. Our experience was that far fewer product bands were obtained in PCR reactions when nine-mers were used rather than 10-mers. We did not look at the effects of titrating the concentration of either the upstream or downstream oligos, and we routinely use 2µM concentration for both in the PCR reaction. The PCR reaction is done in a total volume of 12.5 µl composed of the following:

- 1.25 µl of 10X reaction buffer from Perkin Elmer (1.5 mM $MgCl_2$).
- 1.25 µl of 200 uM stock of cold dNTPs
- 1.4 µl of 35S-dATP from New England Nuclear
- 0.2 µl (1 unit) of Perkin Elmer Taq polymerase
- 0.5 µl of each primer from 50 pmol/µl stock solutions (2.0 mM final con.)
- 5-6 µl of cDNA from first strand synthesis reaction (corresponding to the amount of DNA made from 1 µg of RNA).
- Water to make up a final volume of 12.5 µl.

Thermocycling is done in a Perkin Elmer model 480 unit:

95° C for 40 seconds
40° C for 60 seconds
72° C for 30 seconds

This pattern should be run for 35 cycles followed by a 10 minute, 72° C incubation.

Gel Electrophoresis

Mix 4 µl of the PCR reaction mix with 7 µl of deionized formamide and 1 µl of BPB/XC dye. Boil 3 minutes and quench on ice. Load 4 µl of this mix on a 6% sequencing gel (we use Sequagel-6 gel mix from National Diagnostics, 0.4 mm thick gel and a rectangular shaped tooth comb on a BRL S2 apparatus.) Run the gel at 60 watts constant power until XC reaches the bottom (3.5 hours).

Fix gel with acetic acid and methanol. Soak with Amplify solution (Amersham) for 15 min. Dry gel and expose:
24 hours at -80° C with Amplify
2 to 3 days without Amplify.

Cloning of fragments from the sequencing gel

Carefully align the autoradiogram over the dried sequencing gel and use a 21-gauge needle to puncture the film and mark the dried gel. Slice out the band of interest with a razor blade using the needle holes as a guide. Place the gel slice in an Eppendorf tube and rehydrate the gel slice in distilled water. Change the water several times over a period of several hours to wash out residual urea, gel fixing solution, and Amplify solution.

Using a razor blade, peel off as much of the backing paper as possible from the gel slice. Place hydrated gel slice in a fresh Eppendorf tube, crush the gel slice with a pipette tip and add 50 µl of water. Incubate at 37°C, with shaking, overnight. Centrifuge the sample to eliminate gel fragments and remove 5 µl of the supernatant to prime a cold PCR reaction that contains the same upstream and downstream oligos that were used in the original hot PCR reaction.

PCR conditions:
100 µl volume
200 µM NTPs
100 pmoles of each oligo.
1 unit of Taq polymerase
40 cycles using the same conditions as before: 95° C for 40s; 40° C for 60s; 72° C for 30s.

Run 20 µl of the PCR reaction on an 8% mini-PAGE. In some cases, a product band is not visible. In that event, use 5 µl of the 40-cycle reaction to prime a second PCR reaction run under the same conditions for only 20 cycles, and repeat the PAGE analysis. Product bands can now be cloned in the T/A cloning vector available from Invitrogen.

Troubleshooting Tips

"You have to thoroughly DNAase treat the original RNA sample to remove all traces of contaminating DNA. Otherwise you will get smeared or false bands. Use a control without RT."
—Sharon Savage, Howard Hughes Medical Institute Research Scholar, NCI

"Total RNA works much better than PolyA selected RNA." *—Rudy Pozzatti, NCI*

"Be careful. The Differential Display you see may not reflect the in vivo situation when you go back to verify the expression by Northern analysis." *—Steve Zeichner, NCI*

"The method can be too broad and too sensitive. You may have to sort through a lot of possible genes before you find anything interesting. Verify any new sequences by Northern analysis as early as possible. " *—Bob McIsaac, NIDDK* ■

Differential Display Contacts

Rudy Pozzatti,	NCI	402-1963
Steve Zeichner,	NCI	402-3697
Bob Mc Isaac,	NIDDK	496-6145

References

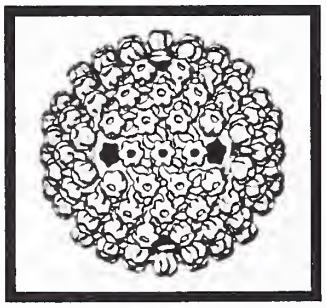
1. P.Liang and A. B. Pardee, "Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction." *Science* **257**, 967 - 971 (1992).

2. P. Liang, L. Averboukh, and A. B. Pardee, "Distribution and cloning of eukaryotic mRNAs by means of differential display: refinements and optimization." *Nucleic Acids Res.* **21**, 3269 - 3275 (1993).

INTEREST GROUPS SPARK COLLABORATION AND SCIENTIFIC EXCHANGE ACROSS NIH

by Seema Kumar

No one really knows how many of them there are at NIH, but name one biomedical discipline and you will likely find an actual or potential interest group for that discipline here on campus, says Jay Knutson of NHLBI. Whether they span broad areas, such as structural biology, or focus on specific areas, such as the superfamily of hormone-regulated DNA-binding proteins, discipline-based interest groups form an important part of scientific exchange and collaboration at NIH, say intramural investigators. Such interest groups also have the endorsement of NIH Director Harold Varmus, who is pleased to see "some of the interinstitute activities ... coming into bloom." Varmus sees the emergence of these discipline-based groups as a "very good sign," heralding improved interactions among the NIH laboratories.



Herpes simplex virus capsid is being studied by SBIG collaborators.

Leaders of the interest groups that *The NIH Catalyst* heard from in response to a FAX-BACK question in the August issue agree. They say that many such groups already exist at NIH, but admit that, apart from a few biggies such as the Structural Biology Interest Group (SBIG), most interest groups have not been aggressive or successful in advertising their activities to colleagues on campus. But with interest groups becoming fashionable, many of the groups expect to get more exposure for their activities and plan to come out of the woodwork.

"It is only natural" that there be many interest groups at NIH, says Vittorio Gallo of NICHD who heads the Glia Club. "NIH is an institution organized by disease-specific institutes, whereas scientific disciplines crosscut these areas." In such a setting, and given the high concentration of biomedical researchers at NIH, chances are that without such interinstitute interactions, researchers would find it difficult to know about others working on similar areas or analogous systems, says Alasdair Steven of NIAMS, who chairs the SBIG. Stories about scientists struggling with a technique or a system only to find out later that the world's expert on that technique or system is across the street or down the hall are not unheard of, say Steven and his SBIG and NIAMS colleague Craig Hyde.

Many interest groups sprang up on campus primarily to avoid this situation. "That was one of the reasons for starting the Mouse Club," says Kathy Mahon, who belongs to the club headed by Heiner Westphal of NICHD. "In my experience, you would not even know until you went to a meeting ... that [someone at NIH] was working on an area of interest to you."

"Without groups like this, you just don't get the full advantage or even close to the advantage of having people with similar interests being so close by — they might as well be in another institution," says Tom Sargent, who heads the Frog Club. "That is the main reason we started the club," and inevitably, scientific exchange and collaboration follow, says Sargent.

"This is the easiest way to bring together people who have similar interests," says Knutson. "In the past, I have given seminars to different groups on campus and have invited scientists to give seminars here. But, that is a hit-or-miss type of thing." Having faculty groups that are interested in similar areas "offers a regularity — a way to build a cadre of interested people who would kick around ideas together and share techniques," he says. Knutson adds that faculty groups give wider audiences a view of the individual disciplines and techniques across campus by "expanding the number of mouths" spreading the word.

Although almost all of the groups' meetings have the seminar-followed-by-questions-and-discussion format, "interest group meetings are more than just seminar series," says Sargent. Faculty group meetings, he says, tend to be more informal, creating a comfortable setting in which to discuss preliminary findings and get feedback from the community, to kick around ideas, to find out about new techniques that might help solve a research problem, to share information about resources; and to pick up some tips and tricks and, perhaps, some gossip about publications, politics, and policies.

Knutson recently heard a rumor that he found encouraging. "People have said that this campus [will be] becoming more campus-like with even more interest groups and ... the kind of collegiality and interactions that universities have, and that is a pleasant thing to [look forward to]."

An Interest-Group Sampler

The Structural Biology Interest Group

The Structural Biology Interest Group, one of the biggest and best-known interest groups on campus with more than 300 members, formed as an ad hoc committee a little over a year ago. Coincidentally, then-NIH Director Bernadine Healy had chosen structural biology as one of the critical areas in her strategic plan. When Steven and Hyde began discussing the possibility of an interest group with their colleagues, their idea was met with so much enthusiasm "that we realized we had struck a sympathetic chord" with our intramural colleagues.

"The original focus of the group was networking. To do this, the group launched an extensive seminar series last year in which the various subdisciplines of structural biology — X-ray crystallographers, NMR spectroscopists, electron microscopists, and computational biologists — introduced themselves. Based on these meetings, a group-by-group directory of structural biology at NIH has now been compiled," says Hyde. Since then, the SBIG has expanded its goal. "In the long run ... the group can ... also act as an information clearinghouse for what is going on [at NIH] in structural biology, what is available, who is the expert on a specific area, or who is publishing a paper in a journal," says Steven. In this year's seminar series on Receptor-Ligand Interactions, SBIG is pairing researchers who study the structure of a molecule with researchers who study the molecule's function. The SBIG also has another important goal: to attract the best and brightest to the NIH program. The SBIG has developed a brochure designed to help this recruitment effort. To get on the SBIG mailing list, contact Craig Hyde or Alasdair Steven at 496-0132.

The Genetics Focus Group:

NIH Director Harold Varmus has asked the NCHGR to help organize a campus-wide focus group for intramural genetics researchers. Robert Nussbaum of NCHGR has planned an open meeting on January 18, at 5:30 p.m. in the first floor conference rooms A and B of Building 49. The purpose of this meeting is to discuss how such a focus group might best serve the needs of the intramural genetics research community. Come with suggestions and recommendations. "We are looking for creative ideas that will enhance and enrich inter-Institute networking and collaboration in genetics research," says Nussbaum. Questions? Call Nussbaum at 402-2039; fax: 402-2120; e-mail rlnuss@helix.nih.gov. ■

The Fluorescence Interest Group

Jay Knutson started the Fluorescence Group three and a half years ago "largely as a convenience to our own lab, almost serving the function of data seminars that a lot of labs have." But Knutson says that almost immediately he realized that extending the idea to include their dozen or so collaborators on campus and at local outside institutions would serve a more useful purpose: "Those who are already collaborators tell us about the problem they want to study using fluorescence and the rest of the group makes suggestions on how best to do that. For new members, the seminars give exposure to what we can do here and how it has been used to solve other problems." Knutson says that the fluorescence club "is the sort of match-making process" designed to result in fruitful marriages between experts on fluorescence technology and researchers who can use techniques.

The interest group has helped spread the word about NIH's state-of-the art facility in fluorescence technology. The group meets every Friday in a conference room down the hall from Knutson's office. To get on their mailing list and to receive the group's fluorescent orange agenda, call Jay Knutson at 496-2557.

The Mouse Club

The year-old mouse club, which meets once a month, is open to all investigators who use the mouse as a model system to study development, oncogenesis, or mouse genetics, but "focuses mostly on ... new technologies like transgenics and embryonic stem-cell technology to knock out genes," says Kathy Mahon of NICHD. Mahon says the club is a place for scientists to exchange ideas and present data, but "it is also a place where people working on similar systems at NIH can identify ways to use existing NIH services such as the animal facility or the veterinary program" to find out the best and most efficient ways to get "the services that we all need or will need at NIH."

Xenopus laevis

The 35-member club meets on Wednesday. For more information on the mouse club, call Westphal at 496-1855.

The Frog Club

"The Frog Club," assures Tom Sargent, albeit subjectively, "is one of the most fun and interesting clubs on campus." An interest group of researchers who work on a variety of areas such as embryology, electrophysiology, endocrinology, and molecular biology, Frog Club members are united by their experimental animal — the South African clawed frog, *Xenopus laevis*.

In their third year, the monthly meetings of the 40-member club are "well-attended" and begin with two 20-minute talks followed by questions and a "lively discussion." As the evening wears on, meetings really get hopping, with informal discussion of policy, politics, movies, and baseball, says Sargent. The group meets on the last Friday of every month and is looking for new members. If you are interested in joining, call Sargent at 496-0369. ■

A Gaggle of Interest Groups

• ***The Superfamily of Hormone-Regulated DNA-Binding Proteins Group*** meets on the first Thursday of every month. It is usually active from October to December and February to June. Activities include a seminar series that is not listed on the yellow sheet. To obtain a schedule of upcoming seminars and/or to become a member, call Stoney Simons, Jr., at 496-6796.

• ***The Glia Club is*** run by Vittorio Gallo of the Laboratory of Cellular and Molecular Neurophysiology, NICHD, Bldg. 49, Room 5A76; phone: 402-4776; fax: 402-7777.

• ***The NGF Club,*** for those interested in nerve-growth factor and other neurotrophic factors, is run by Gordon Guroff, Deputy Scientific Director, NICHD, Chief Molecular Genetics Section, NINDS, Building 9, Room 1W115; phone: 496-4049; fax: 402-0117.

• ***The NIH Drosophila Group*** meets on the third Tuesday of each month and is open to anyone interested in fruit-fly research. Club members take turns talking about their work. A schedule of upcoming meetings can be obtained from Sue Haynes at 496-7879; fax 496-0243; e-mail shaynes%lmgrax.dnet@dxi.nih.gov.

• ***The NIH-Wide Image Processing Group*** sponsors seminars on roughly a monthly basis. It prepares and distributes the *Directory of NIH Image Processing Facilities*. The booklet is currently available from the DCRT Information Office, Bldg. 12A, Room 3025; phone: 496-6203; fax: 402-0007, e-mail: FlemingR@nih.gov. The DCRT information office plans to update this guide over the next few months. If you wish to contribute, contact Benes Trus, Bldg. 12A, Room 2033; phone: 496-2250; fax: 402-2867; e-mail: Trus@helix.nih.gov. If you would like to be on the group's mailing list, contact DCRT's Bonnie Douglas, Bldg. 12A, Room 2017; phone: 496-2847; fax: 402-0007; e-mail: DouglasB@magic.dcrt.nih.gov.

• ***The Protein Folding Journal Club:*** is moderated by Byungkook Lee, Laboratory of Molecular Biology, NCI; phone: 496-6580; e-mail: bkl@helix.nih.gov. The group also sponsors occasional seminars.

• ***The Neural Nets Journal Club*** is moderated by George Hutchinson of DCRT; Bldg. 12A, Room 2041 or 6100 Executive Boulevard, Room 5C01; phone: 402-1940; fax: 402-1946; e-mail: gah@helix.nih.gov. It meets every other week on Fridays, 12 noon - 1p.m., Bldg. 12A, Room 3026.

• ***The Cell Biology Interest Group*** recently formed to provide a framework for communication and interaction among the many NIH scientists working in the diverse fields of cell biology. The group's monthly activities will include organizing a workshop to be held in Wilson Hall from 3 to 5 p.m. one Wednesday each month, arranging lectures in cell biology by scientists from outside NIH, and producing a *Directory for Cell Biology at NIH*. If you would like to be included in Cell Biology Interest Group activities, send your name, laboratory and institute, two sentences describing your research interests, your telephone number, fax number, e-mail address, and mailing address to Rick Klausner, Bldg. 18, Room 101; fax: 402-0078.

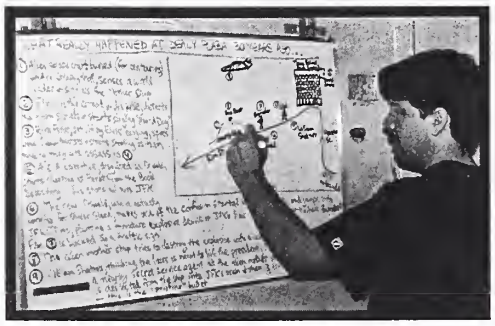
• ***The RNA Club*** may be NIH's newest faculty group. It was established to foster increased communication among researchers who share a common interest in RNA and RNA-binding proteins. The group meets on the first Tuesday of each month at 4 p.m. in Bldg. 41, Room C509. For more information contact Carl Baker at 496-2078 or Susan Haynes at 496-7879. ■

The Seeds of Mirth: Stress, Creativity, and Coping at the National Institutes of Humor

by Celia Hooper

The blackboard in the hall outside Room 4N114 has become a Clinical Center landmark despite the fact — or perhaps even because of the fact — that it has no redeeming scientific value whatsoever. It is a verbal playground, a center for silliness. But although the board may do nothing to push back the frontiers of science, like other small flashes of humor on the NIH campus, it brightens the days, sparks talk and *esprit de corps*, and may even serve such serious functions as releasing stress and stimulating creativity.

"I do it for amusement, to liven things up, and to vent my frustrations from long hours in the lab," says Alex Dent, the board's first author and a postdoc working with Louis Staudt in NCI's Metabolism Branch. Dent started posting light-hearted tidbits, lists, drawings, poems, questions, and other contributions on the board about eight months ago. Many of his quasi-daily creations invite contributions from readers, and a set of erasable markers is conveniently stashed next to the board.



NCI's Alex Dent and his recent Rube Goldbergesque account of the JFK assassination.

Dent says that when he first came to Staudt's lab in 1991, he found the staff a bit "uptight" compared with the more jovial, laid-back crew in the lab where he was a graduate student at the University of California at San Diego. "I wanted to loosen things up a bit," Dent says. "Things have changed for the better" in the meantime, and the board may have played some small part.

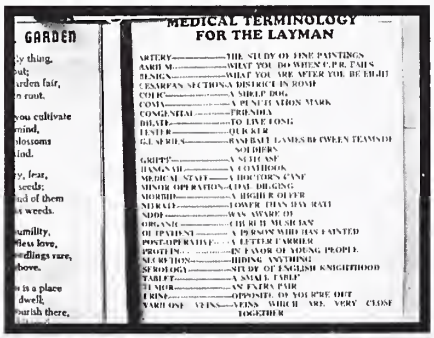
Technician Randy Maile, one of Dent's chief collaborators on the board, says, "As much as we may fool around with the board, this lab works really hard — incredible hours. We are serious about what we do." (Researchers in Staudt's lab study genes that govern the immune system and that may become dysregulated in lymphomas and leukemias. They are now combing subtractive cDNA libraries for genes that are active specifically in B cells.) Staudt concurs, "I would say that our lab has some of the hardest workers on this hallway [4N].... There are people here many weekends and nights. If you are going to spend a lot to time in the lab — maybe more time than you spend at home — it has got to be an enjoyable place to come into. This [board] was completely a grass-roots movement. I had nothing to do with it, but I'm glad it's happening." Staudt says the board's boost to morale transcends the boundaries of his lab. "What is amazing to me is to see Scientific Directors of the institutes stopping by and spending time to read the board."

The popularity of the board would come as no surprise to anyone who was lucky enough to have attended a lecture given in early September by physician Steven Allen, Jr., son of comedian Steve Allen. Allen's lecture, "Juggling Life's Stress," drew a capacity crowd to Masur Auditorium and an overflow room. Those managing to get in the doors were issued three silk scarves and subsequently induced to get goofy while purportedly learning to juggle. Scientists and secretaries, administrators and press officers stuffed the scarves in their ears and up their noses, draped themselves like Scheherazade, and eventually attempted to juggle the scarves . . . or at least fake it with flare. "We need to give ourselves permission to be silly," Allen said. "We can unhook stress with playfulness."

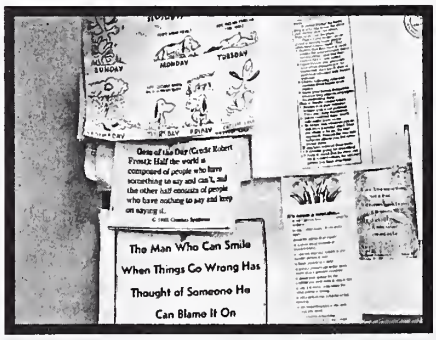
Allen said the benefits of humor may extend beyond the release of stress. "There is some tantilizing early-stage research indicating that subjects [in an experiment] showed an enhancement of creativity after engaging in light-hearted activity," Allen said. He says his successful formula for preparing for the medical boards was to cram for 45 minutes and then play Frisbee for 30 minutes. Allen also mentioned results of studies that might help administrators testifying before Congress or otherwise acting as custodians of the public's trust: observers judge people who smile as being more trustworthy than people who do not smile.

Introducing Allen, NIH Deputy Director Ruth Kirschstein said, "If you want to get serious about the subject of humor as therapy, both the NIH library and the NLM have literally hundreds of medical journal articles, books, and videos on the subject of 'wit and humor,' 'creativeness,' and 'play.'" Kirschstein invited Allen to assist Vice President Gore's project to reinvent government. "Help us reinvent the NIH as the National Institutes of Humor," she said.

Phlebotomists at the Clinical Center seem to have learned about the therapeutic value of humor some time ago. Ryvette Smith, who heads the phlebotomy unit, says the walls of the cubicles where her staff draws blood from patients have been adorned with jokes, cartoons, and humorous definitions and sayings as long as she can remember. Many items have been contributed by the patients, some of whom must have their blood drawn dozens of times in the course of their treatments. Smith rates 98 percent of her staff as "very humorous" people. "I think it definitely helps take some of the stress off" — for both patients and staff. She says a sense of humor is an essential self-defense that helps phlebotomists cope with the abuse and wrath of very ill or ill-tempered patients. "We couldn't survive any other way."



Humor in the Clinical Center's phlebotomy unit draws out the best in patients and staff.



Philip Gold, NIH's resident stress expert and chief of NIMH's

Clinical Neuroendocrinology Branch, says humor can serve as a pressure valve for releasing stress — but only up to a point. "I think where humor helps discharge anxiety is in situations that are not overwhelmingly stressful . . . where laughter is a way of stepping back, diffusing the danger . . . seeing some sides of the situation that are escapable, or mocking the situation and diminishing its danger or gravity," says Gold. "It's a way of getting some distance, so the self realizes that, even if loss occurs, you can survive.... But if the stressful situation is completely overwhelming, humor is not going to be helpful." This explains why people laugh at jokes that flirt with issues that are "somewhat dangerous," Gold says.

Consider, for example, the politically dangerous ice that HHS Secretary Donna Shalala skated across when she spoke at NIH on Women's Equality Day on August 26 and told a joke that went something like this:

> Once, not very long ago, there was a biology department whose faculty had long consisted exclusively of white men. Under pressure to diversify the staff, the department head successfully recruited a top-flight women scientist. But when the woman joined the staff, the chairman feared the rest of the staff would not interact well with the new faculty member. After consulting with campus authorities on interpersonal relations, the chairman decided that the best way to integrate the new scientist and make her feel welcome was to sponsor a group activity that would facilitate mutual bonding. After thinking about what this activity should be, he finally settled on what he thought was a great idea: a faculty fishing expedition.
>
> The chairman hired a large fishing boat, and the faculty group set out to sea. Half a mile offshore, the woman realized with a start that she had a problem: She had forgotten her purse. Just as other members of the fishing party were starting to grumble at the prospect of a trip back to shore, the woman hopped overboard — and proceeded back to land by walking across the water. As he watched the woman make her way to shore, one senior faculty member nudged a colleague. "*See*," he hissed. "I *told* you she couldn't swim!"

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Cool

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The front cover shows a way cool picture, dude, of a chromosome stained with a very cool new dye that shows almost nothing, so it's easy to pick out the really cool stuff. If you still can't see it now, then you're not cool enough for *Cool*. For details, see article by Dworkin and Wilson in a recent issue.

This parody of a leading scientific journal was widely circulated among NIH scientists in 1990.

RECENTLY TENURED

Serge Beaucage *is a Staff Scientist in the Division of Hematologic Products of the Center for Biologics Evaluation and Research. He received his Ph.D. in 1979 from McGill University and came to CBER in 1988 from Standford University School of Medicine.*

My laboratory is involved in the design, synthesis, and application of "antisense" oligonucleotide analogues in the control of gene expression. The hybridization of an antisense oligonucleotide with a complementary messenger RNA (the "sense" molecule) prevents the translation of the mRNA into protein, presumably by impairing ribosomal assembly, as a consequence of the degradation of the DNA-RNA heteroduplex by RNase H, or both. Several research groups have shown that oligonucleoside phosphorothioates are useful as antisense molecules, and that they inhibit the replication of HIV-1 in chronically infected cells. My laboratory recently discovered 3*H*-1,2-benzodithiol-3-one 1,1-dioxide, a sulfur-transfer reagent for rapid and efficient solid-phase synthesis of oligonucleoside phosphorothioates.

Two years ago, my research group synthesized α,ß-oligonucleotides with alternating (3' → 3')- and (5' → 5')-phosphodiester linkages as a potential new class of antisense molecules. These oligonucleotides exhibited improved resistance to nucleases and good hybridizing properties and were easily synthesized by the efficient phosphoramidite methodology, which Marvin Caruthers and I developed in 1981. On the basis of molecular modeling and molecular dynamic-simulation studies, my laboratory is currently designing a second generation of α,ß-oligonucleotide analogues to further improve the base-pairing abilities and nuclease resistance.

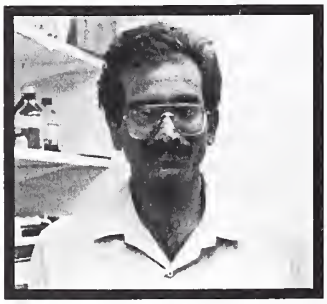
The effectiveness of oligonucleotide analogues as potential therapeutics also depends on the ability of these biomolecules to permeate cells. We are committed to the design and synthesis of oligonucleotides conjugated to lipophilic carriers to facilitate their entry into live cells. And should antisense oligonucleotides become potent therapeutics against infectious diseases and cancer, large quantities of the analogues will be necessary. In anticipation of that possibility, we plan to refine the phosphitylation reaction effected by phosphoramidite derivatives to allow an even more efficient synthesis of DNA and RNA oligonucleotides. ■

SCIENTISTS TENURED NOVEMBER 1993 TO DATE

Martha S. Linet, NCI

Alan Breier, NIMH

Murali K. Cherukuri *came to NIH from India as a Visiting Fellow in 1985. He is currently a Senior Investigator in the Radiation Biology Branch, Division of Cancer Treatment, NCI. Cherukuri received his Ph.D. in 1984 from the Indian Institute of Technology in Madras, India.*



Researchers in our laboratory study free radical - mediated oxidative biologic damage, a process associated with a wide variety of diseases, including postischemic reperfusion injury, carcinogenesis, and amyotrophic lateral sclerosis. Free radicals such as superoxide, hydroxyl, carbon-, nitrogen- and oxygen-centered radicals are produced in the normal course of metabolism, but if they are not properly detoxified, they can induce biological damage. These free radicals are highly reactive and, hence, have very short biological half-lives. Detecting, characterizing, quantifying, and modulating the concentrations of these free radicals is important in understanding their role in biology and in protecting against their deleterious effects.

Radiation chemists and biologists quantitatively study the effects of free radicals in biological systems, both in vitro and in vivo, to understand the effects of ionizing radiation on biological matter and to protect normal tissue and sensitize tumor cells. These efforts have resulted in the development of chemical radioprotectors, which can protect normal tissue, and of chemical radiosensitizers — agents that can sensitize hypoxic cells, postulated to exist in solid tumors.

Recent studies in our laboratory have identified a class of agents called nitroxides, which are stable free radicals effective in providing whole-body radioprotection in mice. Chemically, we have shown that these agents possess many antioxidant properties, which include mimicking the enzyme superoxide dismutase (SOD), and can detoxify superoxide radicals. In addition, we find that these agents are effective in inhibiting transition-metal - mediated oxidative damage. Electron paramagnetic resonance spectroscopy (EPR) also shows that, unlike native SOD, nitroxides can permeate cells and are effective in intercepting intracellular oxidative damage. We have also shown that these compounds can be used in assessing free radical modes of action in many chemotherapeutic agents.

We are also developing a spectroscopic device to detect free radical intermediates at very low concentrations. Currently, researchers use conventional EPR spectroscopy for direct detection of free radical intermediates produced in biochemical pathways. However, this technique can only be used on very small sample volumes (<200 µl), because of the absorption of incident microwave radiation by aqueous biological samples at the frequencies used (9000 MHz). We are currently developing pulsed Fourier-transform EPR spectrometry that can be performed at low frequences (~300 MHz), thus permitting detection of free radicals in larger biological samples. The loss in sensitivity inherent in reducing frequency can be overcome by taking advantage of the faster spin dynamics of the electron, which permits averaging of several spectral acquisitions per second (~105/s). Once this system is operational, it might be possible to detect extremely low concentrations of endogenous free radicals present in cells and tissue as a normal consequence of metabolism. In addition, using exogenous free radical probes, it might be possible to follow specific free radical pathways in cells and tissue. ■

Edward Chu *received his M.D. from Brown University in 1983. Chu came to NIH in 1987 as a Medical Oncology Fellow in NCI's Clinical Oncology Program. He is now a*

Senior Investigator in the Biology and Therapeutics of Solid Tumors Section of the NCI-Navy Medical Oncology Branch.

Our research efforts have focused on elucidating the mechanisms of cytotoxcity and resistance for the antimetabolite class of antineoplastic agents. Specifically, we have focused our attention on the fluoropyrimidine, 5-fluorouracil (5-FU), the most effective single agent to treat human colon cancer and a commonly used treatment for breast cancer. Using a human colon cancer cell line (H630), we observed that short-term exposure to 5-FU increased the level of thymidylate synthase (TS) protein expression in these cells and that this induction of TS was associated with the development of drug resistance. Repression of this 5-FU-mediated induction of TS by the cytokine interferon-gamma led to a 20-fold enhanced sensitivity of these cells to 5-FU. Further studies revealed that the 5-FU-mediated induction of TS was regulated at the translational level. Using two different in vitro methods — an in vitro rabbit reticulocyte translation system and an RNA gel mobility shift assay — we showed that human recombinant TS protein specifically inhibited translation of TS mRNA by directly binding to its corresponding mRNA. These studies demonstrated that the expression of TS is controlled in a negative autoregulatory-feedback manner, the first of its kind to be described in a eukaryotic system.

Using an immunoprecipitation:RT-PCR technique, we recently showed that TS protein is complexed to its own TS mRNA in human colon cancer cells. Our research efforts are currently focused on precisely identifying the critical *cis*-acting elements on the TS mRNA that mediate this RNA-protein interaction. One important binding site includes the translational start site in a putative stem-loop structure. In addition, we are attempting to identify the specific RNA-binding domain(s) on TS protein by using TS mutant proteins whose X-ray-crystal structures are known and by isolating the specific TS peptide sequence that attaches to the mRNA.

While we were investigating the functional role of these novel RNA-protein interactions, we found that a TS ribonucleoprotein complex exists between TS protein and the mRNA of the nuclear oncogene *c-myc*. These findings suggest that TS protein may be involved in the coordinate regulation of several genes other than its own. A more detailed understanding of these RNA-protein interactions involving TS protein may provide insights leading to novel treatment approaches for human colon and breast cancer. ■

Kathleen Clouse *received her Ph.D. from the University of Minnesota in 1985. She came to Georgetown University in 1986, working with the NIH-NIAID AIDS program, and joined CBER in 1989. She is now a staff scientist in the Laboratory of Cytokine Research.*

The primary area of research in our laboratory is the role of cytokines in the regulation of viral activation and the immune response to viral infection, particularly in HIV infection. We initially reported that bacterial lipopolysaccharide (LPS) stimulates normal human monocytes to secrete cytokines capable of inducing HIV-1 expression in chronically infected human cell lines. We then determined that the cytokine that induced this response is tumor necrosis factor - α (TNF-α). Our subsequent investigations showed that select viral antigens could also cause the secretion of HIV-inducing TNF-α. This suggested that pathogens could act indirectly as cofactors in AIDS by inducing TNF-α, which secondarily augments HIV expression.

We found that specific viral proteins are responsible for stimulating TNF-α secretion. We also clarified the existing controversy regarding the ability of the HIV envelope protein, gp120, to induce cytokine secretion by showing that both the primary amino acid sequence and the posttranslational modifications of gp120 can change the protein's effects on cytokine production. Ongoing studies are aimed at determining how these posttranslational protein modifications alter gp120's stimulatory effects on the immune system.

Recently, in collaboration with Hannelore Ehrenreich, now at the University of Göttingen in Germany, we demonstrated that monocytes exposed to gp120 also produce endothelin-1 (ET-1), a peptide with potent vasoconstricting activity, which could mediate alterations in the cerebral perfusion pattern associated with AIDS dementia complex. This possibility is supported by the observation that cerebral macrophages in HIV encephalopathy and circulating monocytes from HIV-infected individuals strongly express ET-1, whereas expression is not detectable in healthy, noninfected individuals — suggesting that HIV infection may cause chronic activation of this gene. Our laboratory is currently investigating the regulation of ET-1 expression, as well as the expression of other cytokine genes during acute HIV infection of human monocytes in vitro.

We have extended our studies to include potential inhibitors of TNF-mediated upregulation of HIV. In particular, in collaboration with William Farrar at NCI, we have examined two forms of soluble, recombinant p80 TNF receptors as inhibitors of TNF-induced HIV activation in vitro. We found that a soluble, dimeric p80 TNF receptor, formed by fusing the extracellular, ligand-binding portion of the receptor to a truncated human IgG heavy chain, can block TNF-induced HIV expression in both monocytoid and lymphoid cells, when the receptor is used at a molar ratio at least 5:1 over TNF concentrations. In contrast, comparable ratios of a monomeric form of this receptor actually enhance TNF-mediated HIV-1 expression. We are continuing to investigate how these receptors enhance and suppress the biological effects of TNF. This may illuminate the interactions of TNF with its natural soluble receptors in vivo, and, like all of our research, could have significant implications for our understanding of AIDS pathogenesis. ■

Ann Dean *joined the Laboratory of Cellular and Developmental Biology at NIDDK in 1984. She is currently a Senior Investigator. She received her PhD. in 1981 from George Washington University.*

The human ß-globin genes represent a paradigm for the study of regulated gene expression during development. The

continued on page 12.

RECENTLY TENURED
continued from page 11.

expression of each of the five globin genes is spatially and temporally restricted. For example, the embryonic ε-globin gene is transcribed during the first six weeks of development

in erythroid cells of the yolk sac. Later in development, the site of erythropoiesis shifts, and other globin chains predominate. Because of globin's physiological role in the transport of oxygen, defects in the structure or regulation of the adult gene can result in serious human diseases (thalassemia and hemoglobinopathies). If we could induce a recapitulation of an early developmental program, it could lead to an amelioration of these diseases.

Expression of the ß-globin family of genes is controlled by distant DNA sequences, termed the locus control region (LCR). One hypothesis envisions that stable complexes, assembled by chromatin-binding proteins, exist between the LCR and each of the globin-gene promoters as each promoter is activated during development. My laboratory has investigated regulation of the ε-globin gene. By using in vitro binding techniques and DNAse footprinting, we mapped the sites where nuclear proteins interact with the promoter of the gene and with one region of the ß-globin LCR. We found that two erythroid-specific nuclear proteins, GATA-1 and NF-E2, bind to these regulatory regions of DNA (in addition to several ubiquitous transcription factors). Further, in vivo expression studies in our lab showed that binding of GATA-1 and NF-E2 to the ε-globin promoter and the LCR enhancer, respectively, is required to mediate the transcriptional effect of the enhancer.

The experiments strongly support the idea that GATA-1 and NF-E2 cooperate in vivo in the regulation of the human ε-globin gene. These proteins are good candidates for mediating a complex between enhancer and promoter that could form by the looping out of the intervening DNA sequences. The juxtaposition of DNA regulatory elements through the formation of loops of intervening DNA has been demonstrated in prokaryotic and viral systems. Current studies in my laboratory are focused on whether a direct interaction occurs between the ε-globin regulatory regions in chromatin. ■

David Kaslow *came to the NIH Inter-institute Genetics Program and the Laboratory of Parasitic Diseases in 1986. Kaslow, who received his M.D. from the University of California at San Francisco in 1983, now heads the Molecular Vaccine Section of NIAID's Laboratory of Malaria Research.*

Malaria accounts for an estimated 2 million childhood deaths each year and continues to exact an increasingly heavy toll on much of the human population living in sub-Saharan Africa and the subtropics. Research efforts in our section of the Laboratory of Malaria Research are focused on developing a transmission-blocking vaccine for malaria that may stem or even reverse the spread of the disease. Our program has three components: 1) broad-based basic research to elucidate mechanisms involved in normal development of the parasite stages responsible for transmission, 2) applied vaccine development to identify and characterize potential target antigens and then to clone and recombinantly express those antigens to determine their utility in transmission-blocking vaccines, and 3) advanced development of fermentation and post-fermentation processes for the production of vaccines, followed by testing of the most promising transmission-blocking vaccines in human trials.

When I first joined the Laboratory of Malaria Research, I started working toward cloning Pfs25, the gene encoding the major surface protein of the malaria parasite zygote. Monoclonal antibodies to Pfs25, when mixed with infectious malaria parasites and fed to mosquitoes, completely prevent the insects from becoming infected. In collaboration with the Biological Resources Branch, we microsequenced several immunoaffinity-purified, trypsin-digested peptides encoded by *Pfs25*, which I have now cloned. The deduced amino acid sequence predicted a cysteine-rich protein. Early on, it became clear to me that recreating disulfide bonds in recombinant Pfs25 was going to be essential in using this protein in vaccine development. Stuart Isaacs and Bernie Moss of the Laboratory of Viral Diseases produced a recombinant vaccinia virus expressing properly folded Pfs25, and we showed that mice immunized with this virus developed antibodies that completely blocked transmission. More recently, I found that a Pfs25 vaccine produced in yeast elicits similar transmission-blocking antibodies with an alum adjuvant. I determined appropriate fermentation conditions, developed a postfermentation purification scheme, and am now in negotiations with a company interested in manufacturing recombinant Pfs25 for use in a human vaccine. I hope to begin a phase I human trial of this vaccine in the Clinical Center next spring. ■

Roberta Shahin *is a staff scientist in the Food and Drug Administration's Laboratory of Pertussis, Division of Bacterial Products, CBER. She received her Ph.D. from Johns Hopkins University in 1985 and came to CBER in 1987.*

My research has focused on protective immunity to *Bordetella pertussis* infection, with a special emphasis on the mucosal immune response to pertussis antigens. Whooping cough remains a significant disease, with its greatest morbidity and mortality effects in infants. Intense efforts in recent years have been aimed at identifying purified antigens of *B. pertussis* that would be highly effective vaccine components with fewer adverse effects than the current whole-cell pertussis vaccine.

My laboratory group demonstrated that a novel 69-kDa outer-membrane protein, called pertactin, purified from *Bordetella pertussis* protected mice against experimental infection. Antibody to pertactin was suffi-

cient to mediate protection. We demonstrated that pertactin was immunogenic in children and that this previously unrecognized protein is present in significant concentrations in one of the first-generation acellular pertussis vaccines licensed for use in the United States. Pertactin is now included as a purified component of several second-generation acellular pertussis vaccines currintly in clinical-efficacy trials.

Using a mouse model of respiratory *B. pertussis* infection, we have defined other protective *B. pertussis* components and discovered three previously unknown virulence factors of the bacterium. One of these virulence factors is environmentally up-regulated when all other known toxins and adhesins necessary for virulence are down-regulated.

B. pertussis preferentially associates with the cilia of the respiratory epithelium, and during the course of disease, it does not disseminate from the lungs. Natural *B. pertussis* infection confers long-lasting protection from reinfection, whereas intramuscular vaccination induces protection that wanes in young adulthood. These observations suggest that induction of a potent local respiratory immune response to antigens of *B. pertussis* would be an effective vaccine strategy.

My current work focuses on mucosal delivery systems for pertussis antigens, especially biodegradable, biocompatible microspheres and liposomes bearing the antigens. We have shown that intranasal administration of either microspheres or liposomes containing low doses of pertussis antigens results in production of high concentrations of antigen-specific immunoglobulin G (IgG) and IgA antibody in the serum and respiratory tract, and decreases experimental infection of the lungs and trachea of mice. Our goal is to define the immune mechanisms triggered by antigens presented as particles in the respiratory tract. We hope that this knowledge will lead to the rational design and improvement of vaccines against a variety of mucosal pathogens. ■

Elizabeth Snyderwine *joined the Laboratory of Experimental Carcinogenesis, Division of Cancer Etiology, in 1986 as a Guest Researcher and began a Senior Staff Fellowship in the laboratory in 1990. She is currently Head of the Chemical Carcinogenesis Section. Snyderwine received her Ph.D. in 1984 from East Carolina State University in Greenville, N.C.*

My research has focused on the metabolic processing, DNA adduction, and mutagenicity of the heterocyclic amines, a group of carcinogens found in cooked proteinaceous foods. In the late 1970s, scientists first recognized that potent mutagens, subsequently indentified as heterocyclic amines, form during the broiling of beef and fish. These amines may play a role in diet-related human cancers, including colon and, possibly, breast cancer. In my initial studies, I synthesized and characterized the reactive metabolites of the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and showed that the major DNA adduct of IQ is at the C8-guanine position. I carried out studies that showed that cytochrome P450IA2 and phase II esterification enzymes are involved in the metabolic activation of IQ and other heterocyclic amines, including 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (8-MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-ß]pyridine (PhIP). Using the ^{32}P-postlabeling method for measuring DNA adducts, I examined the binding of IQ, 8-MeIQx, and PhIP to DNA in animal models. The results of these studies reveal that in vivo N-hydroxylation of heterocyclic amines is a critical step in DNA adduction and carcinogenicity. Recently, I examined the in vivo metabolic processing of IQ, 8-MeIQx, and PhIP, and characterized urinary, biliary, and fecal metabolites indicative of pathways of metabolic activation and detoxification of these chemicals.

Researchers in my laboratory are currently studying the molecular and cellular consequences of metabolic activation and DNA adduction of heterocyclic amines. We are examining the sequence-specificity and mutagenic spectra of heterocyclic-amine–DNA adducts in genes of mammalian cells and in a shuttle vector system. Identification of specific patterns of cellular damage from heterocyclic amines could be useful in understanding the carcinogenic potential of these chemicals in the diet. We are also researching the effects of these agents on isolated cardiomyocytes, and our results have implicated N-hydroxylamine metabolites in heterocyclic amine-induced cardiotoxicity. Most recently, we have begun investigating the mammary carcinogenicity of the heterocyclic amines in model systems in order to assess the possible etiological role of these agents in human breast cancer. ■

Richard Spencer *earned his Ph.D. from the Massachusetts Institute of Technology in 1987 and his M.D. from Harvard in 1988. He came to NIA's Laboratory for Cellular and Molecular Biology at the Gerontology Research Center in Baltimore in 1991 as Chief of the Nuclear Magnetic Resonance Unit.*

In my laboratory, we have been using nuclear magnetic resonance (NMR) spectroscopy in a variety of in vivo studies, including clinically oriented work and studies of experimental animal models. Our overall goal is to gain insight into organ- and tissue-level processes associated with aging, and the interactions of aging with other processes and interventions. To this end, we are also engaged in methodological studies. Our work has focused on the bioenergetics of peripheral muscle metabolism, and we use, primarily, a 1.9-Tesla, 31-cm horizontal-bore spectrometer.

One of our goals is to distinguish among basic hypotheses proposed to account for decline of muscular performance with age. Drawing subjects from the Baltimore Longitudinal Study on Aging, we have found that age-associated muscle weakness is not necessarily correlated with decline in inherent bioenergetic efficiency. Further studies, now under way, may uncover more subtle changes and enable us to define a dose-response relationship between isometric exercise and metabolism. This research will provide a foundation for investigations into nonnormative aging and the metabolic underpinnings of frailty.

We are also using NMR spectroscopy to evaluate anti-aging regimens. We are currently

continued on page 18.

COMMENTARY

EPIGENETIC INHERITANCE: THE CHROMATIN CONNECTION

Alan P. Wolffe, Ph.D., Chief, Laboratory of Molecular Embryology, NICHD

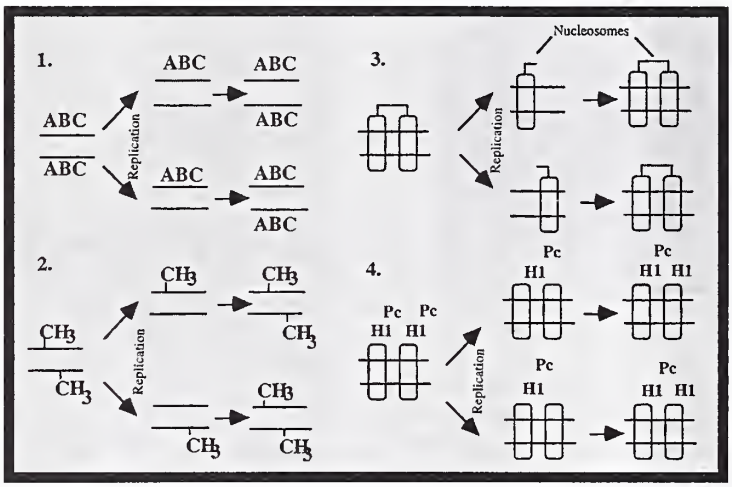
Most — but not all — homologous genes behave identically within the same type of cell. In the rare instances when copies of a gene behave differently within one cell type, epigenetic mechanisms are probably at work. Recent experiments have highlighted the role of epigenetics in developmentally important events in mammals, especially events involving imprinting. Moreover, human genetic disorders such as Prader-Willi and Angelman syndromes and cancers such as chronic myelogenous leukemia also have an epigenetic component (1). Evidence is accumulating that supports the idea that the way in which a gene is packaged into chromatin and the chromosome plays a key role in the molecular mechanisms responsible for epigenetic effects. Our understanding of how the packaging of a gene influences its expression is still rudimentary, and researchers in our lab and others are now focusing attention on this research area.

Imprinting and X-Inactivation

Imprinting occurs when the expression of a gene depends on whether it is inherited from the father or the mother. Examples in the mouse include the gene that encodes the receptor for insulin-like growth factor-2 (IGF-2), which is expressed only when inherited from the mother. In contrast, the gene encoding the receptor's ligand, is expressed only if derived from the male parent. This type of imprinting ensures that both the maternal and paternal chromosomes are present and being expressed; successful embryogenesis does not occur without the expression of both genes. In Prader-Willi syndrome, failure to inherit the chromosomal region 15q11-13 from the father leads to retarded motor development and severe hypotonia, whereas in Angelman syndrome, failure to inherit the same chromosomal region from the mother leads to contrasting symptoms, including ataxia, seizures, and hyperactivity. Thus, some specific paternal and maternal imprint must be made on a particular gene or group of genes in the 15q11-13 region. This imprint must be perpetuated through the differentiative events of oogenesis and spermatogenesis, and retained through embryogenesis until it is time for the gene to be expressed.

The related and well-studied epigenetic phenomenon of X-chromosome inactivation requires genes on one of the two X-chromosomes in female cells to be coordinately repressed early in embryonic development. In contrast to imprinting, which is a local effect involving single genes, X-inactivation involves almost all of the genes on a particular chromosome. X-inactivation results in the equivalent expression of X-linked genes within chromosomally XX females and XY males. Once established, the inactive state of a particular X-chromosome will be stably propagated in all progeny cells. Thus, like the imprinting phenomenon, a fixed pattern of gene expression is established that will be maintained through many cell generations, probably by a self-templating process (2).

Although imprinting and X-inactivation represent dramatic examples of epigenetic phenomena, all developmental processes rely on differential regulation of gene activity. The response of a particular gene to a new signal depends on the developmental history of the chromosome and cell in which it functions. Eventually every chromosome in a differentiated cell will consist of a patchwork of active and repressed regions that must be faithfully reproduced in identical differentiated cells following chromosomal replication and cell division. Replication of DNA duplicates only one component of the chromosomes; the distribution of active or repressed genes must also be duplicated. This requires reassembly of the preexisting nucleoprotein complexes that activate or repress genes. Resolving how specific aspects of chromosomal structure affect the activity of individual genes and how such structures are maintained (or altered) during chromosomal replication is a central concern of contemporary biology (3). This work could have important ramifications for genetic engineering and clinical research on human genetic diseases, cancer, and development.



Hypothetical mechanisms for imprinting

1. Maintenance of specific trans-acting transcription-factor (ABC) interactions through replication, followed by cooperative sequestration of additional transcription factors (second arrows).

2. Maintenance of methylation. Methylated cytosine in a cytosine-guanine sequence segregates to daughter DNA duplexes after replication. Methyltransferase preferentially methylates C-G at a hemimethylated site (second arrows).

3. Maintenance of nucleosomal structure. After replication, positioned nucleosomes segregate to daughter DNA duplexes but direct the phasing of adjacent nucleosomes (second arrows).

4. Maintenance of long-range chromatin structure. After replication, repressive proteins such as histone H1 or Polycomb (Pc) are reduced in abundance. Direct or indirect cooperation between these proteins or structure-dependent features might direct the sequestration of additional H1 or Pc (second arrows).

Nucleoprotein complexes that regulate gene expression

Researchers have made considerable progress in determining how sequence-specific transcription factors associate with DNA to assemble transcription complexes recognized by RNA polymerase. The genes and their regulatory elements involved in this process do not exist as naked DNA within the chromosome, but are instead packaged by core histone proteins. Histones determine the access of transcription factors and RNA polymerase to DNA and, thus, both have important roles in gene activation.

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GENETIC CONTRIBUTIONS TO SEROTONIN METABOLISM AND SUICIDAL BEHAVIOR

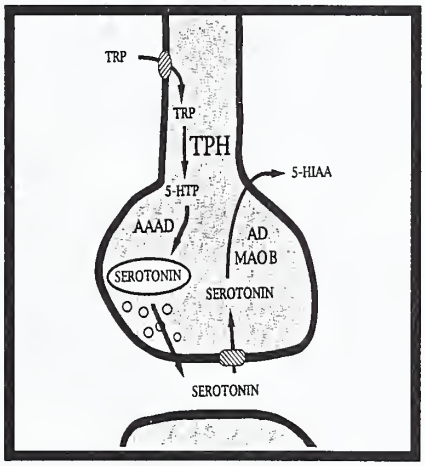
David A. Nielsen, Ph.D., Markku Linnoila, M.D., Ph.D., and David Goldman, M.D., Section of Molecular Genetics, Laboratory of Neurogenetics, Division of Intramural Clinical and Biological Research, NIAAA

Twin and family studies on the inheritance of behavior implicate genetic factors as influences in certain kinds of antisocial (1) and suicidal behaviors (2,3), alcoholism (4,5), and several aspects of personality (6). The causes of most or all behavioral differences are probably complex and heterogeneous. The behaviors that are genetically influenced show clinical heterogeneity and interrelationships because they often coexist in patients and their blood relatives. The studies we are conducting are aimed at identifying and explaining the interrelationships of the genetic components of behaviors that are postulated to be regulated by serotonin.

The principal metabolite of the neurotransmitter serotonin is 5-hydroxyindoleacetic acid (5-HIAA). The concentration of 5-HIAA in cerebrospinal fluid (CSF) obtained by lumbar puncture is positively correlated with the concentration of 5-HIAA in frontal cortex (8), an area thought to be important in attention and impulse control. Low CSF 5-HIAA is associated with suicides and suicide attempts (2,3,9-11), impulsive violence (12,13), depression (14,15), and alcoholism (16). The underlying characteristic of these behaviors is an anxiety-related intolerance to delay and a deficiency of impulse control (17).

Although the specific genes regulating serotonin biosynthesis and catabolism are largely unknown, CSF 5-HIAA concentration in rhesus monkeys is heritable (18). Among the likely genetic determinants of serotonin turnover are genes involved in serotonin metabolism, receptor function, and signal transduction. Researchers have now cloned most of these genes, including tryptophan hydroxylase (TPH) (19), aromatic l-amino acid decarboxylase, serotonin transporter, and monoamine oxidases A and B (MAO-A and -B). Numerous serotonin-receptor genes have also been cloned. Variants of many of these genes could modulate serotonin-dependent behaviors at various points in the metabolism of serotonin. For example, a recent study has implicated a one-codon change in the gene encoding MAO-A as a determinant in aggressive behaviors in men (20). The MAO-A alteration was also associated with low concentrations of 5-HIAA in urine and borderline mental retardation indicative of developmental abnormalities.

Our initial focus has been on the role of the TPH gene in modifying behavior, because hydroxylation of tryptophan is rate-limiting in serotonin biosynthesis (21). TPH catalyzes the oxidation of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to form serotonin. Serotonin is subsequently deaminated by MAO-A and -B (MAO-B is the predominant variant in serotonergic neurons) to 5-hydroxyindoleacetaldehyde, whose oxidation product is 5-HIAA. We hypothesized that a genetic variant of TPH is found in some individuals with low CSF 5-HIAA concentrations.



Serotonin synthesis and catabolism in the brain

After entry into the serotonergic neuron, tryptophan (TRP) is hydroxylated to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH). 5-HTP is decarboxylated by aromatic l-amino acid decarboxylase (AAAD) to yield serotonin, which is sequestered in vesicles by uptake through the vesicular monoamine transporter. Stimulation of the neuron releases serotonin into the synapse. Serotonin exerts its action by binding to various serotonin receptors on pre- and postsynaptic membranes. Serotonin action is terminated by re-uptake into the presynaptic terminal through the serotonin transporter. Serotonin is then deaminated by monoamine oxidase B (MAO-B) to 5-hydroxyindoleacetaldehyde, then oxidized by aldehyde dehydrogenase (AD) to 5-hydroxyindoleacetic acid (5-HIAA) and released into the cerebrospinal fluid.

We identified such a polymorphism within the TPH gene by using the single-strand conformational polymorphism (SSCP) method (22,23). In this method, regions of the TPH gene are amplified by the polymerase chain reaction, cut with a restriction enzyme, and denatured with sodium hydroxide and formamide. When the denatured DNA is separated in a nondenaturing polyacrylamide gel, it folds up into a single-strand conformation that allows electrophoretic separation of DNA strands with as few as one nucleotide difference. SSCP revealed a polymorphic site in the human TPH gene at a site corresponding to intron seven of the mouse TPH gene. To confirm the location of this polymorphism in the TPH gene, we mapped the polymorphism by linkage analysis to the short arm of chromosome 11, in close proximity to β-globin and tyrosine hydroxylase (22). In Caucasians, two TPH alleles, U and L, were identified. Both alleles are abundant, with the L allele present at a frequency of 0.60.

To test the hypothesis that the TPH genotype may associate with behaviors postulated to be controlled by serotonin, we assessed TPH genotype (22), CSF 5-HIAA concentration (7), and history of suicide attempts in a Finnish population that was behaviorally extreme and genetically homogeneous. The population consisted of Finnish control subjects and incarcerated, alcoholic, violent offenders and arsonists. We classified the criminals as impulsive or nonimpulsive, based on whether their crimes were premeditated or not. All subjects were subsequently interviewed and diagnosed by a psychiatrist using DSM-III criteria (24) and evaluated for a history of suicide attempts. To quantify serotonin metabolism, we measured 5-HIAA concentration in CSF.

The data revealed that for the impulsive violent offenders — but not for nonimpulsive offenders or controls — serotonin metabolism, as indicated by CSF 5-HIAA concentration, is associated with TPH genotype (25). The impulsive violent-offender group with the LL genotype had low CSF 5-HIAA (45 ± 4 nmol/L) whereas the UU genotype group had the highest CSF 5-HIAA concentration (76 ± 9 nmol/L). Impulsive offenders in the UL genotype group had low CSF 5-HIAA concentrations (46 ± 5 nmol/L). The data suggest that impulsive offenders may have an unknown abnormality that allows TPH to function as the primary regulator of serotonin metabolism.

How could a TPH variant linked to the L allele reduce serotonin turnover? There are several possible explanations. The association could indicate a genetically linked mutation elsewhere in the TPH gene that alters enzyme activity or stability. The processing of TPH mRNA could also be altered, because the TPH polymorphism is located within an intron. Other possible mechanisms include alterations in gene transcription or mRNA stability. Considering that TPH is active as a tetramer (26), one allele with low activity could act in a

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VARMUS Q & A
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there are ways to improve — to develop trans-institute programs along disciplinary lines, to start outstanding lecture series. The Structural Biology Interest Group, the recently formed Cell Biology Group, and the incipient Genetics Group are examples of ways we can expand and diversify the intellectual environment. I expect many areas to benefit from programs along these lines.

I have also heard a lot about the problems of working as a scientist in the government — and it is something I have experienced myself in setting up my lab — the difficulties that we have as a result of the excessive rules and regulations and cumbersome procurement procedures.... I am working hard to get Vice President [Albert] Gore to come out here. He has offered to provide some assistance on how to apply the rules for reinventing government at NIH. There are some ways in which we can improve NIH on our own — by changing the way we evaluate, recruit, and retain people for positions, for example. But there are other aspects of the way science works here — for example, the types of regulations governing permission to travel or purchasing from small vendors — that will require some new authority to change. I have talked to [HHS] Secretary [Donna Shalala] about these problems, and she is very receptive to the idea that the place needs to work better. She has assured me that I will have the authority to make appointments of scientists—up to just below the level of Institute Directors — without having to send everything down to the Department for validation. And the SBRS [Senior Biomedical Research Service] privileges that the Congress accorded us in 1990 and that have still not been approved by the Office of Management and Budget — I think — will come to us in the very near future.

Q: What has pleased or surprised you most in your talks with people on campus?

A: I have greatly enjoyed the people I have met. I wouldn't call this a surprise because I knew in advance that there were a lot of people here who are serious about high-quality science — I have a lot of friends here — but I feel that there is a sense of re-commitment, that there is going to be a change here as a result of my arrival. People have been extremely eager to help me out. I have seen some of the interinstitute activities that I encourage coming into bloom — the Cell Biology, Structural Biology, Neuroscience, and the Genetics Groups for example [see story, page 6]. The emergence of these groups is a very good sign. I am very pleased to have Francis Collins and his colleagues in Building 49, where my own lab is going to be housed.

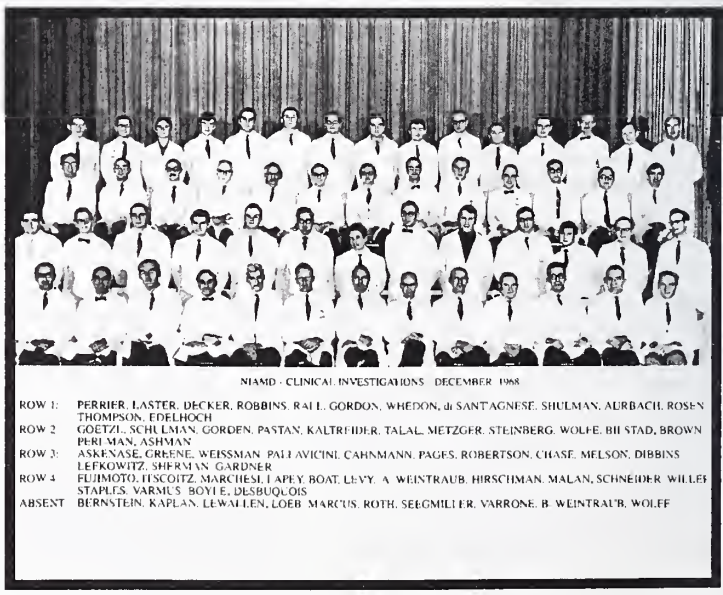
I am optimistic about a positive reception of my own lab group. My own work will be in the area of cancer genes and mouse genetics, developmental biology, and hematology. I have been talking to people in all these areas and find a very high level of enthusiasm for doing the highly interactive science that I am used to. ...I feel the potential for having the kind of intense communal experience in doing science that I came to love at UCSF. My laboratory group came to visit in September, and they went away with smiles on their faces. There were a couple of low moments when they felt that some of the bureaucratic aspects of life here might makes things difficult, but they found the [NICHD Cell Biology and Metabolism Branch] lab retreat energizing.

Q: What has changed the most since you were here before [as a Clinical Associate in the National Institute of Arthritis and Metabolic Diseases from 1974 to 1979]?

A: It is hard to answer that question because my perspective has changed. When I was here before, I really did not know what was going on at NIH as a whole; I knew mainly what was going on in my lab and in adjacent labs. If you look at a picture of my cohort of Associates, you can see one difference — all but one of the hundred or so people were white males. This indicates a healthy change — more women and minorities on campus. But it was an extremely talented group. We were all dying to come to NIH to do postdoctoral work. In part, we were coming to NIH to perform alternative service [rather than going to war in Vietnam] but we were a bunch of academically inclined M.D.s who thought this would be a great place to learn science. In that generation, we were getting the best minds just out of medical school competing in a very active way for the positions that were available here. I don't believe that exists anymore — that sense of urgency.... I would like that to be recreated — without another war, of course.

Q: How do women, minority, and disabled scientists fit into your priorities for NIH?

A: Underrepresentation is a trans-NIH problem. Many of the minority issues and problems have nothing to do with science — they are traditional management problems.... But there are some special problems for women and minorities as scientists in achieving high levels of stature in the community... Michael Gottesman and I certainly will be paying attention to the data being collected for the Women's Advisory Committee to the Deputy Director for Intramural Research (DDIR), and the perceived inequities have been noted. I don't see any reason why women scientists should be paid less than men scientists.... I don't enjoy having to issue politically correct statements, but I am as strong a supporter [of the advancement of women and minority scientists] as anybody. I believe in action more than words. I have a record of supporting my own women and minority students and post-

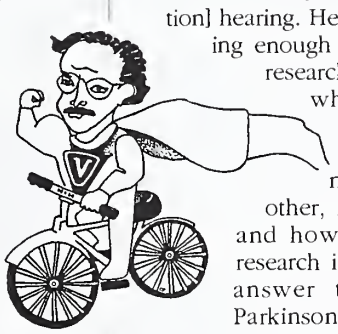


docs, and feel strongly about having a diverse workplace and taking advantage of the contributions of all scientists.

The problem is not just having prominent women senior scientists right now, but also having a diverse work force for the next century—a work force that is racially more diverse and scientifically more advanced. NIH needs to be fair to all people and representative of all society. These issues go way beyond inequities in the salaries of women.

Q: How will you improve the excellence of NIH and its science, given current fiscal constraints?

A: The crux of excellence is in personnel. The IRP has the extraordinary opportunity to provide the best working environment in the country for people who want to do outstanding science. We need to make sure that positions that become available here are open to competition; every position that is open should be filled with a national search. Among my own postdoctoral fellows [back at the University of California at San Francisco], NIH is not thought of as a place that they could easily go. There is a perception that this is a closed shop. Scientists need to know they can come here and work in a way that is free of the vexations of the grant-application process.... There are differences that sometimes make it harder to work here — the IRP has advantages and disadvantages, but most outsiders would welcome access to so much intellectual strength, the Clinical Center, equipment and facilities — with no grant application. For each position that opens up, these advantages should be used to attract the best possible talent.



I am not planning to eliminate positions, but there are going to be continued, government-wide FTE restrictions. There may be some differences in the ways we evaluate how people are performing. I see the new tenure-track program as an improvement over the previous processes, and I will support Dr. Gottesman's efforts to be sure that new tenure-track positions are widely advertised and that appointments are closely reviewed at a central level. We are considering other mechanisms, but tenure inevitably is what is going to attract the very best scientists here. We will be advertising nationally for positions that become available at all levels — including Scientific Directors. The quality of science varies from Institute to Institute, and a lot of that depends on who the SD is. I also think that the review process [of the Boards of Scientific Counselors (BSC's)] needs to be looked at carefully to be sure that programs are responding to judgments made by the BSC. I plan on putting some teeth into the process to be sure their recommendations are followed and that resources are appropriately reallocated. We will also be looking at the way the BSC members are selected. That is being examined by the External Advisory Committee.

Q: How will you balance political influences and pressure coming from Congress and HHS with scientific priorities?

A: I will stand up for my principles. You have to be compromising in some ways — you especially have to listen to those who feed you — but then you stick up for your principles. Take the example of [Sen.] Paul Wellstone [D-Minn.] at the [confirmation] hearing. He said NIH is not spending enough on Parkinson's disease research. First you have to say what it means to study Parkinson's: we have to understand how nerve cells talk to each other, how they differentiate, and how they die. The basic research investment we make to answer these questions for Parkinson's disease may not be all that different from the investment we make to understand multiple sclerosis or Alzheimer's disease, etc. Research on these diseases is not just conducting clinical trials. I can make that case, and we all need to make that case.... My experience with members of Congress is that they are educable, but in the past, scientists have not done a good job of educating them on the role of basic research

In making this case, it is useful to move backwards from research that is clearly in the clinical domain — for example, the development of gene therapy for cystic fibrosis or the discovery of the colon cancer gene — to the roots of this work in basic science. We can show how, 20 years ago, the essential research that made these discoveries possible was not at all connected to the diseases.... It is extremely likely that the things that we are doing now in basic research will pay off in the long run — just as work on obscure DNA-repair mechanisms ultimately paid off in identifying the colon cancer gene — but right now, we do not know exactly where this work will lead us.

Q: In the past, you have said that NIH should be an independent agency like the National Science Foundation. Will you pursue a separation from the Department of Health and Human Services?

A: Right now, no, but the question is in the air, and from time to time, we will be revisiting it. At the moment, I am more comfortable with NIH being within HHS.

Q: What changes would you like to see in NIH's role in science education?

A: I think that we should be playing a very large role in science education. This is actually one of the big missions of the next 10 to 15 years — to help make the improvements that have to occur, especially in K - 12 education. Every place I went in NIH, I found that there were programs — almost every institute has some involvement in the education of either the general public or in the schools — but often the programs seemed unconnected with each other and fairly short-term in nature. I would like to see us develop a more coordinated program with a more long-term effort to target schools or other audiences, to educate the public about what NIH does and what biomedical research is. One idea would be to have a road show — to stage an NIH fair to teach about the role of science in improving health, to help in the transfer of technology, and to have lectures in various fields. Or perhaps we could rent a convention center for a weekend and have the public see demonstrations, hear people talk, and engage in panel discussions that raise issues from basic science to bioethics.

I have had some experience with this in San Francisco. We staged a program called "Winding Your Way Through DNA" that explained to the general public how biotechnology—DNA science—developed and how it will affect society. I think we should do more of that type of direct, hands-on educational experience, which can be more effective than TV. It may reach a smaller audience, but the ripple effect of such public symposia produces results that are much more long lasting.

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In the realm of K - 12 education, I think that NIH staff — not just bench scientists but also program managers and science administrators — have the ability to advise in hands-on science education.... All NIH scientists should be encouraged to be involved in teaching or providing in-service training to teachers. For example, the National Academy of Science has a huge education program under way now that my good friend Bruce Alberts, who heads the Academy, has set as his primary mission. I have been talking to him about how to make use of the talents of NIH personnel to help with a demonstration project that is going on in Washington, D.C. In addition, [NICHD's] Rick Klausner is in charge of an Academy program to set education standards in biology. There are many ways in which our energies could be directed to ongoing projects in science education. I would like to see an ethic arise in which people feel that they have a public responsibility to spend a certain amount of time transmitting science — not only to their competitors or close colleagues, but to general audiences and to the schools.

Q: What is your favorite book?

A: I don't have a single favorite book. Right now, I am reading a book by one of my favorite novelists: *The Holder of the World,* by Bharti Mukerjee. I like to read novels, but I haven't read that many since coming to NIH. I have been swamped with position papers and have been trying valiantly to keep up with the scientific literature. ■

RECENTLY TENURED
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assessing the improvement in muscle bioenergetics that results from administering growth hormone - releasing hormone to healthy older men. In addition, we are investigating the metabolic consequences of long-term dietary restriction in primates — another intervention that holds promise for retarding the aging process.

Our methodological work has centered on improving measurements of NMR relaxation times and determining reaction rate constants for muscle-enzyme systems. For example, we recently developed a technique for measuring reaction rates that does not require selective radiofrequency excitation of a spectral line, thus making the measurement simpler and more accurate. This is important because determining reaction rates through enzyme systems may provide a more sensitive gauge of bioenergetics than can be achieved through measurements of metabolite concentrations alone.

We are also conducting basic research on energy transfer and double-quantum excitation in dipolar-coupled spin systems in rotating solids. With this work, we hope to extend the rotational resonance technique for analyzing molecular structure of non-crystalline solids. In work published in 1991, we used rotational resonance to elucidate the structure of an important nine - amino acid fragment of β-amyloid, a brain protein associated with Alzheimer's disease. Other researchers have successfully applied the technique in studies of other proteins and enzyme systems.

Our laboratory recently procured two NMR spectrometers: a high-field vertical-bore system and a full upgrade of the existing low-field horizontal-bore system. These instruments will greatly enhance our imaging capability and physiological sensitivity and the range of the experiments we can perform. ■

Scott Stibitz *received his Ph.D. from the University of Wisconsin at Madison in 1983. He came to CBER in 1987 and now works in the newly created Laboratory of Enteric and Sexually Transmitted Diseases.*

The primary focus of work in my laboratory has been the regulation of virulence in the human bacterial pathogen *Bordetella pertussis*. Until recently, these studies have centered on a genetic locus called *vir*, which regulates the expression of all known protein virulence factors of *B. pertussis*, in response to environmental conditions. Examples of such virulence factors include adhesins — such as filamentous hemagglutinin, pertactin, and fimbriae — which allow the bacteria to adhere specifically to host tissues, and toxins—such as pertussis toxin, invasive adenylate cyclase, and dermonecrotic toxins — which are responsible for host pathology and evasion of the immune response. The *vir* locus encodes proteins that belong to the family of two-component regulatory systems that govern a variety of environmentally regulated phenotypes in a variety of bacterial species. In addition to sequence similarity in conserved domains, these systems share a common mode of signal transduction and intracellular signaling that combines a transmembrane protein kinase and a phosphate-accepting DNA-binding protein.

Although *vir* ultimately regulates all the *B. pertussis* virulence proteins, in many cases, the regulation appears indirect. Using a system for genetic mapping in *B. pertussis* that was developed in our lab, we recently began a search for potential intermediate regulators. The system uses a conjugative plasmid vector that can mobilize chromosomal sequences by inserting at various locations in the *B. pertussis* chromosome and that is used in conjunction with a set of kanamycin-resistance gene markers at defined positions in the chromosome. The exact physical locations of these markers are known from a physical map of the *B. pertussis* genome that was recently completed in our lab. Genetic linkage and appropriate map positions of interesting mutant phenotypes can thus be obtained and used to direct subsequent cloning and characterization of newly identified genes.

In addition to these studies, we have developed a new mapping method that permits relatively rapid physical localization of a set of genes of interest for a particular isolate of *B. pertussis* or another *Bordetella* species. This has allowed us to compare the genomic organization of several strains and led to the observation that the genomes of the *Bordetellae* appear remarkably fluid. We are currently applying the same technique to strains of *Vibrio cholerae* in the hope of shedding light on the relationship of recent serotype O1 strains to newly emerged strains of a novel serotype that are responsible for what is believed to be the beginning of the eighth pandemic of cholera. ■

FIAU STUDY
continued from page 1.

When the first wave of the crisis finally passed in September, the ordeal took another turn: the review of how an institution protects the safety of research subjects. This process is time-consuming and involves gathering reams of records, undergoing extensive reviews, and responding to congressional requests for information.

The whole incident has cast a pall over clinical researchers, who are now concerned that inside and outside review groups may suggest new regulatory checkpoints in the already complicated path of conducting clinical trials. But, says Straus, Chief of the Laboratory of Clinical Investigation, NIAID, "if that is what it takes to ensure public trust and increase safety for human subjects, clinical researchers must be willing to go that further step." Alan Sandler, Director of the Office of Human Subjects Research (OHSR), agrees. "The protection of human subjects is the first and foremost priority of clinical research, and if a review of the FIAU incident shows that there is something we can do to further protect the safety of patients, then that is what we should do," says Sandler.

Stephen Straus

Alan Sandler

Various review committees both inside and outside NIH are now gathering the information from which will emerge the lessons to be learned from the FIAU study. NIH has appointed an independent group of experts to review records of NIH's experience with FIAU, and the Secretary of HHS has asked the Institute of Medicine to examine all aspects of the FIAU research. FDA held a public hearing, spent several days at NIH gathering details, and has issued one of its expected reports. The Subcommittee on Human Resources and Intergovernmental Relations of the House Committee on Governmental Operations is also reviewing the case and may hold public hearings this spring.

The NIH Catalyst will be following the events in the FIAU review process as they unfold in the coming months and will provide updates in future issues. ■

An FIAU Retrospective

Although a tragic turn of events has only recently thrust FIAU into the public eye, the drug has been under investigation by researchers for about 20 years. The history behind FIAU's discovery, development, and use in humans dates back to the 1970s, when a family of antiviral drugs known as fluorinated pyrimidine analogs was synthesized at Memorial Sloan Kettering Cancer Center in New York and demonstrated greater activity against herpes viruses than any other drug to date. During the 1980s, the first member of this family of drugs, FIAC, was studied as a potential treatment for herpesvirus infections, including herpes simplex, varicella (chicken pox), zoster (shingles) and cytomegalovirus (CMV). But by 1990, after a decade of studies in humans and experimental animals, researchers concluded that the drug, at the doses required to treat herpesvirus infections, was too toxic for that use in humans, and FIAC was abandoned.

But in the meantime, work at Memorial Sloan Kettering Cancer Center, Bristol-Myers, and Oclassen Pharmaceuticals, which picked up the rights to FIAC from Bristol-Myers, showed that FIAC is converted to FIAU once it is absorbed in the body, and that FIAU and FIAC have identical activity. In 1990, investigators began exploring the possibility that FIAU might have the same beneficial effect against herpesvirus infections, with less toxicity than FIAC. FIAU was first tested in HIV patients with CMV infection and later in HIV-infected patients with other herpesvirus infections. But the protocol was further modified to include HIV-infected patients with hepatitis B virus (HBV), because researchers learned that FIAC and FIAU have strong activity against HBV. The chronology below follows FIAU's journey from its preliminary investigations in HIV-positive patients with HBV to the phase II clinical trial that was halted in June. ■

10/90– 6/92
Researchers at NIH, the University of Washington, and the University of California, San Diego, enroll 43 patients — 13 with HIV and 30 with HIV and HBV — to receive FIAU in a 14-day trial.

3/92
FDA grants permission to Straus and Hoofnagle to explore FIAU's use in hepatitis patients who do not have HIV infection.

4/92–10/92
FIAU is tested in 24 patients with hepatitis, about half of whom had already failed interferon treatment Patients receive the drug for 28 days.

9/1/92
Eli Lilly buys the rights to share development of FIAU from Oclassen Pharmaceuticals and plans multi-center, large-scale phase III studies in the United States

10/92
The study of FIAU in hepatitis patients is completed and data are sent to the FDA for review. Hoofnagle and Straus begin to design a third, phase II, six-month study to explore the safety, tolerance, and anti-HBV activity of FIAU in longer-term treatment for chronic HBV infection.

3/93–6/93
15 patients are enrolled in the study, 11 of whom had previously received FIAU in the 4-week study in 1992.

6/17/93
One patient from the study is hospitalized for liver flare and develops pancreatitis after FIAU treatment is stopped.

6/25–6/28/93
A second patient comes in with a life-threatening condition. FIAU seems to be the culprit, and investigators stop the study immediately. Investigators contact all patients who received FIAU, and inform the FDA, the drug companies, and the two other centers beginning to study the drug

6/28–8/31/93
All patients come in for tests and observation.The two patients that came in on June 17 and June 25 receive liver transplants, but subsequently die. Of three other patients receiving transplants, two survive. The third dies of severe lactic acidosis, as do two other patients who did not receive transplants. The remaining surviving patients continue under medical observation for delayed toxicity, and thus far show no signs of progressive illness other than hepatitis. ■

EPIGENETIC INHERITANCE
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Core histones are highly conserved in eukaryotes and consist of two domains: a positively charged amino (N-) terminal tail and a globular carboxyl (C-) terminus. Together with DNA, the four histone proteins (H2A, H2B, H3, and H4) form the core of the nucleosome. Organization of DNA and protein components into nucleosomes is considered necessary for transcriptional regulation. Within the nucleosome structure, two turns of DNA (166 bp) are wrapped around the globular domains of the histones, with the N-terminal tails protruding toward the outside of the nucleosome. These N-terminal tails permit signal-transduction pathways to affect chromosomal structure, and are thus the sites of many posttranslational modifications. For example, acetylation of these domains can modulate the access of transcription factors to specific genes (4). A fifth specialized histone, H1, interacts with the linking DNA between nucleosomes and mediates the folding of nucleosomal arrays into higher-order structures such as the chromosome fiber. Nucleosomes do not form randomly on DNA. Intrinsic structural features, such as the curvature and rigidity of the double helix, together with modifications such as methylation, influence the exact position of histone-DNA interactions (5,6). Nucleosomes are positioned with very specific spatial relationships to particular regulatory DNA sequences. These positioned nucleosomes modulate the action of specific transcription factors, either by facilitating or preventing the association of a transcription factor with DNA, thus potentiating or repressing the gene activity (7).

THE CAST OF PLAYERS IN EPIGENETIC INHERITANCE HAS BEEN DEFINED, AND THE FUTURE OFFERS MUCH EXCITING RESEARCH AS WE BEGIN TO APPRECIATE THE PLAYERS' ROLES IN EMBRYOGENESIS.

Nucleoprotein complexes determine epigenetic phenomena

The view that the histone proteins, nucleosomes, and chromatin have a role in gene regulation is substantiated by genetic experiments on epigenetic phenomena. In *Drosophila*, the expression of many genes is influenced by their chromosomal position; homologous genes are expressed differently when located at different positions on the chromosome. This position-effect variegation depends on the presence of normal concentrations of histone proteins and on acetylation of the N-terminal tails of the core histones (8). It also depends on proteins that recognize chromatin, but not naked DNA — such as the chromatin modification organizer (chromodomain) proteins HP1 and Polycomb (9). HP1 is associated only with inactive chromosomal regions, but Polycomb interacts with at least 50 different chromosomal sites in *Drosophila*, including developmentally important loci encoding homeodomain proteins. Polycomb would normally repress expression of genes encoding these homeodomain proteins; however, mutations in a second gene, *brahma*, allow the genes to remain active (10). *Brahma* is homologous to a family of genes in yeast that interact with the histone proteins. Thus, the genes for Polycomb, brahma, and the histones appear to regulate gene expression in a way that depends on chromosomal position. All of these genes have mammalian homologs that may influence comparable epigenetic events in animals.

Hypotheses To Explain Imprinting

How might chromatin structure contribute to imprinting at the gene or chromosomal level? At least four hypothetical mechanisms could be operative. Sequence-specific nucleosome positioning demonstrates that position effects occur at the local level of individual promoter elements. Functional actions of transcription factors with DNA will depend on the exact context of histone-DNA interactions. Stable, propagated covalent modifications of DNA, such as cytosine methylation, may also influence nucleosome positioning. If a positioned nucleosome includes a DNA regulatory element to which a particular transcription factor cannot bind, the gene or genes governed by the regulatory element will be repressed. Theoretically, such repression would continue in daughter cells if the nucleosome (or the component histone proteins) remain stably associated with the regulatory element through DNA replication and reassociate with the daughter chromatids (11,12).

This means of maintaining a stably repressed state could be extended to the association of proteins such as histone H1, HP1, and Polycomb that might alter the stable repression of larger chromatin domains during replication. Alternatively, chromatin domains containing stably associated enzymes capable of modifying histones (e.g., by acetylation) could maintain a local chromatin structure through a comparable mechanism.

Another possible mechanism for the propagation of stable, repressed chromatin states may be replication timing. Because active genes normally replicate early in S-phase, and repressed genes replicate late in S-phase, if the type, abundance, or modification of chromatin proteins is changed during S-phase such that a higher concentration of repressive proteins (e.g., H1, HP1, or Polycomb) is present late in S, a global repression of gene expression could be achieved. These mechanisms, singly or in combination, may contribute to the establishment and maintenance of stable repressed states (see figure).

Experimental evidence supports the existence of these mechanisms. In the inactivation of the X chromosome, stable propagation of DNA methylation, positioned nucleosomes, removal of N-terminal histone acetylation, and late replication all occur (2). However, the causal relationships among these four phenomena have not been established. For imprinting of other paternal and maternal chromosomes, even the nature of the transcription factors or of the chromatin modifications influencing gene expression remains undescribed, in part because of the difficulty in determining the regulatory elements of imprinted genes. Nevertheless, an encouraging sign for future experimentation is that differences in transcriptional activity exist between maternal and paternal pronuclei after microinjection of various genes in the fertilized mouse egg (13).

Future Directions

Developmentally regulated changes in the gene expression that are dependent on chromatin structure can serve as useful tools in defining the molecular mechanisms responsible for the establishment and propagation of genetic imprints. For example, regulated expression of the *Xenopus 5S* rRNA genes depends on both specific transcription factor-DNA interac-

tions and selective histone - DNA interactions (14,15). We have examined the events that lead to the assembly of an array of positioned nucleosomes containing histone H1 on a family of 5S rRNA genes that are repressed in somatic cells (16).

The repressive influence of chromatin structure on the 5S rRNA gene is dominant, occurring even in the presence of excess transcription factors in vivo. Moreover, this repressive state is maintained through chromosomal duplication (P. Bouvet, S. Dimitrov, and A. P. Wolffe, unpublished observations). In contrast, genes that remain active must be "re-programmed" by association with transcription factors after every replication event because their transcription interactions with DNA that establish gene activity are "erased," or removed, by passage of the replication complex through the gene (17). Subsequently, the mechanism by which chromatin is assembled facilitates transcription-factor access to these active genes (18). Timing also plays a role in this system: The repressed 5S rRNA genes are late-replicating during S-phase, whereas the active genes replicate early (19). Thus, many features of the X-inactivation phenomenon are reproduced in this accessible and easily manipulated experimental system.

Molecular details gleaned from these studies will eventually allow us to determine the influence of individual aspects of activating or repressing chromatin structure on the establishment and maintenance of differential gene expression. Evaluation of the relative impact of histone modifications, histone composition, and replication timing on the expression of these simple genes will set the stage for examining similar mechanisms in mammalian and human systems. The cast of players in epigenetic inheritance has been defined, and the future offers much exciting research as we begin to appreciate the players' roles in embryogenesis. ■

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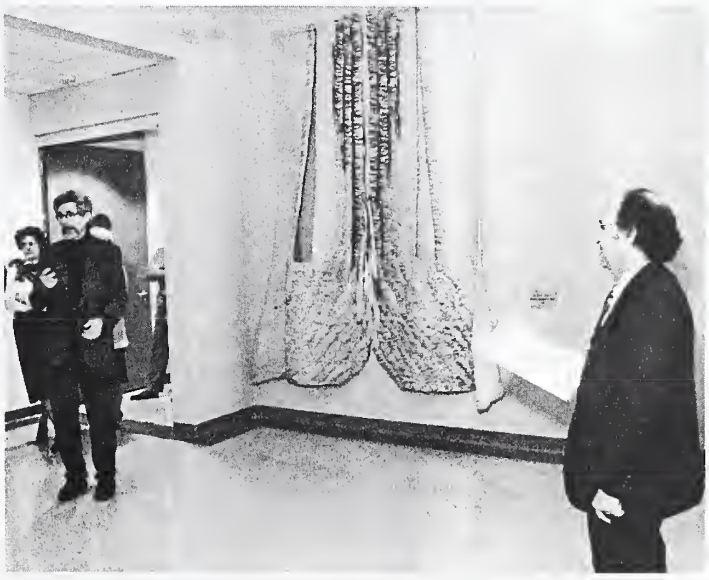

Art Appreciation: *NICHD Scientific Director Art Levine dedicated Building 49's new wall hanging by Sirpa Yarmolinsky on Nov.30.*

SEROTONIN METABOLISM
continued from page 15.

dominant fashion to reduce the activity of the enzyme more than 50% in heterozygotes. We cannot discount the possibility that there may be linkage disequilibrium of the polymorphism with a nearby gene involved in serotonin turnover.

More than half of the violent offenders had attempted suicide. Because suicidal behavior is associated with low serotonin turnover (9 - 11), it was not surprising that we observed a significant ($p<0.016$) association of TPH genotype with a history of suicide attempts (25). Most of the subjects who attempted suicide (34 of 36) had either the LL or UL genotype, independent of CSF 5-HIAA concentration. Furthermore, only subjects with the UL or LL genotypes had attempted suicide more than once, and subjects with the LL genotype had made more suicide attempts than UL heterozygotes.

The ethnically matched patients and controls in the Finnish population in this study were an ideal group in which to explore genetic associations of serotonin-related behaviors. A relatively homogeneous population compared with U.S. Caucasians, they have a high suicide rate (1 in 4,000 per year) (27). In addition, many of the violent offenders in this study had very low CSF 5-HIAA concentrations. Their low serotonin turnover may have increased the probability of finding a linked mutation in the TPH gene. It may also be possible that alcohol use or abuse is a necessary component, because these associations were obtained in a population with these problems.

An important caveat in interpreting these recent results is that genetic associations may also arise due to chance. To eliminate this possibility, we are now locating and identifying possible linked mutations and splicing variants in the TPH gene by sequencing TPH genes and cDNA clones from subjects with low and high CSF 5-HIAA concentrations. We are also attempting to replicate the results from the Finnish violent offenders in studies of other population samples and families.

A link between TPH genotype and serotonin turnover might have implications for other psychiatric diseases in which abnormal serotonin metabolism has been implicated. Both autism and Tourette syndrome have a genetic component (28) and are associated with abnormal serotonin metabolism. The discovery of functional variants of neurotransmitter genes will lead to improved diagnostic tests for neuropsychiatric diseases and insights into their pathophysiology. ■

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HUMOR ON CAMPUS
ontinued from page 9.

Or consider the "dangerous" mocking of scientific, professional issues by *Cool*, a five-page parody of the front pages of *Cell*. Volume 1, Number 1, of *Cool*, the only issue produced, was dreamed up in July, 1990 by two anonymous graduate students at a prestigious East Coast university. The bogus journal was never published per se, but hundreds of copies made their way across the country by fax as researchers told their colleagues about the parody and duplicated nearly illegible copies for one another. The predominant theme running through *Cool* is the clubbiness of contemporary cellular-molecular biology — the exclusive pack of elite publications in which every scientist wants to be published, the clique of first-name-basis scientists that serve on editorial boards, the bandwagon research topics....

"I was unnerved by the extent to which people responded to it," recalls "Jonathan," a graduate student studying bacterial physiology and one of the authors of *Cool*. "Everyone was talking about it. The reaction said, 'You hit home.' We were sort of surprised because we were making fun of *Cell* and aspects of scientific culture that people felt pretty bitter about."

Jonathan says that, with the exception of Ben Lewin, the editor of *Cell* — who was definitely not amused — most people took the parody in the light-hearted vein in which it was intended. "The saving grace of *Cool* is that it was sophomoric," says Jonathan. "People knew it was not a hate letter" or a personal attack intended to hurt anyone in particular. He says that he and co-author "David" were worried that Lewin might seek some revenge after *Science* ran a brief paragraph about the spoof. "That was a bit unnerving. There is a cat-and-mouse game between *Cell*, *Science*, and *Nature*. When *Science* ran their article [about *Cool*] it was a way of sniping at *Cell*. I'm not sure it was such a good thing for us to get involved" in the crossfire, Jonathan says. Since producing *Cool*, Jonathan and David have been sticking to their studies, but Jonathan doesn't rule out the possibility that another issue of *Cool* will emanate again someday from the fax machine in his department's graduate student lounge.

Dent and Maile also emphasize that their humor is not intended to offend. Maile says there have been some complaints about a few boards. "There was one cartoon that generated some heat.... And a few boards have been completely erased." Maile says that, in the spirit of the movie "Indecent Proposal," one offending board posed the question, "What would you do for $1 millon?" Some of the answers included, "Run around Building 10 in the buff," "Go in a date with Roseanne Arnold," and "Take Madonna home and introduce her to your mother as your fiancee." Maile isn't sure which answer prompted the anonymous erasure. "We don't intend to make people mad or to be offensive," Maile says. He does admit, however, "We like to get a rise out of people."

Dent says that for him, personally, the board has a good and a bad side. On the bad side, having raised the concentration of receptors in the funny bones of his appreciative audience, coming up with fresh ideas day after day can actually cause, rather than relieve, his own stress. But on the good side, the process seems to stimulate creativity. "I like to think that creativity spills over into other areas," Dent says. And if things don't work out with the B cell genes . . . maybe Letterman could use some help. ■

FAX-BACK

In this issue we are asking for your feedback in four areas: The conduct of clinical trials in the wake of the FIAU tragedy; new techniques we should cover in the "hot methods" clinic; your consumer complaints or raves about scientific products (reagents, kits, equipment, instruments, etc.); and suggestions for improving the intellectual environment at NIH. Please fax your responses to 402-4303, or mail it to us at Building 1, Room 134.

In Future Issues. . .

- NIDR's New Scientific Director
- Intramural-extramural cooperative research
- New predoctoral IRTA's

1) What are your concerns about clinical research in the wake of the FIAU episode?

2) What hot techniques would you like to see discussed in our new feature, the "Hot Methods Clinic?" What hints can you offer for our next "Hot Methods Clinic" on the yeast two-hybrid system, a new way to identify and clone genes for proteins that interact with a protein of interest?

3) We are considering starting a new feature in which we discuss the merits and demerits of scientific products. As a "consumer" of scientific gear, have you had particular problems with a particular reagent, kit, or piece of equipment? Has a particular product worked especially well for you? What products would you most like to see reviewed?

4) Harold Varmus, the new Director of NIH, wants to improve the intellectual atmosphere on campus. What suggestions do you have that would help accomplish this?

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