FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

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NCBI To Launch PubChem

BRINGING THE SECRETS OF SMALL MOLECULES OUT TO THE PUBLIC

by James Swyers

Coming this fall, courtesy of the NIH Roadmap and NCBI is PubChem, a new database that seeks to do for small molecules what GenBank has done for nucleic acid sequences.

"PubChem will significantly improve researchers' abilities to explore and discover the biological properties of small molecules," says Stephen Bryant, a senior investigator in NCBI's Computational Biology Branch and PubChem team leader.

James Swyers

Stephen Bryant

PubChem is an initiative within the Molecular Libraries and Imaging component of the Roadmap.

At the bench end of the bench-to-bedside panorama of the Roadmap, the Molecular Libraries component provides funding and infrastructure for small-molecule screening and probe generation, an informatics platform for archiving and utilizing small-molecule data in the public sector, and technology development to expand the diversity and robustness of chemical libraries, assays, and detection technologies.

The cheminformatics aspect of the initiative calls for the creation of "a database of chemical structures, properties, and activities" to be established at NCBI, namely, PubChem.

Screening Centers

According to Bryant, much of the data that will be archived in PubChem

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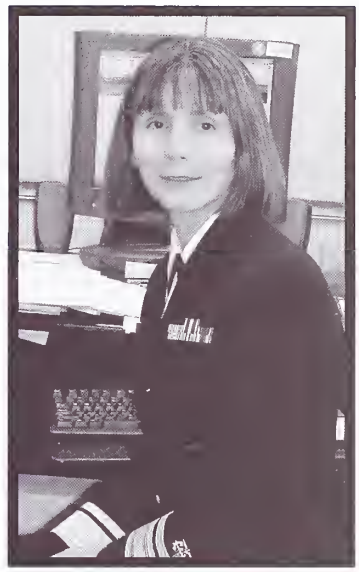
Interview with Dushanka Kleinman

AN AERIAL VIEW OF THE NIH ROADMAP AND THE INTRAMURAL-EXTRAMURAL CONVERGENCE

by Fran Pollner

It amounts to "a tiny drop in the large pool of NIH funds," Dushanka Kleinman notes, but, like a small stone in an insightfully aimed slingshot, the impact of the NIH Roadmap for Medical Research can be colossal.

"It's meant to transform how we do our science," says Kleinman, NIDCR deputy director, who's on Roadmap detail. Directing the early stages of the Roadmap's implementation, she says, is an "enticing" assignment.



Fran Pollner

Dushanka Kleinman

She sees the Roadmap as breaking down walls between NIH institutes to achieve common objectives that will in turn advance not only the research capacity and mission of each individual institute but all biomedical science and, especially, public health.

Charting the Roadmap

The product of intensive brainstorming sessions begun in the summer of 2002—"right after Elias [NIH Director Elias Zerhouni] came on board and with his leadership"—the Roadmap was hammered out by more than 300 extramural and intramural scientists who met in working groups chaired by IC leaders. Their charge was to define the roadblocks to progress in biomedical and behavioral research and envision the tools to shatter them.

Kleinman was involved in the project from the earliest days as a member of one of the working groups. The time was ripe, she recalls, for taking inventory.

"There was so much to look at, so much to get our arms around: the Human Genome Project and the complexity of human biology it revealed, the aging of the population and the shift from acute to chronic diseases, emerging and re-emerging infections, and the challenges of biodefense.

"How do you put your arms around all of this? That's what drove the Roadmap."

The groups devised three main themes for the Roadmap—New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise—that run the course from bench to bedside.

The Roadmap was officially unveiled at the start of fiscal year 2004, with funding projections out through fiscal year 2009 that total a little more than $2 billion.

Now woven into the research agenda

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When Last the Cicadas Peeked: 17 Years Ago at NIH—and Today

Michael Gottesman
c. 1987

Michael Gottesman
c. 2004

When Brood X of the 17-year locusts last emerged, NIH was celebrating its centennial year. The scientific directors were considering critical issues of the day (left, below, as uncovered by my staff from SD minutes). We were starting to figure out how to implement strong marching orders from Congress as demanded by the Federal Technology Transfer Act. Members of Congress told us in no uncertain terms that we needed to move swiftly and vigorously to establish ways to transfer NIH discoveries to the private sector, where they could be turned into treatments and products that would benefit the public health.

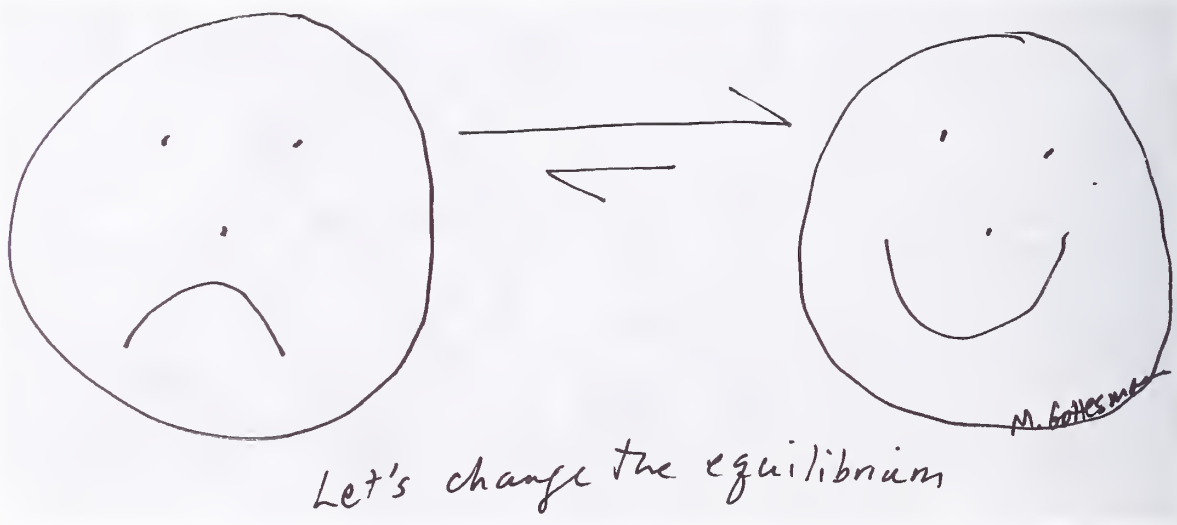
The HIV and hepatitis-C tests, drugs like AZT for the treatment of AIDS, and NICHD's *Haemophilus influenzae*–B vaccine are discoveries that have literally saved hundreds of thousands of lives in the decades since their transfer from the NIH bench to private industry to be developed into the tests, treatments, and vaccines used today.

The recent controversy about the extent to which NIH scientists can consult with industry reflects dramatic changes in the way science is conducted compared with 17 years ago.

And in another 17 years? Whatever may happen, I expect that the core values of cultivating first-rate research in the interest of public health will prevail at NIH. And I hope there's a shift to the right side of my cartoon-face equation below. I suspect there will be, and I welcome all your suggestions for catalysts to make that happen.

—Michael Gottesman

Then	Now
Psychological Well-being of Nonhuman Primates	Psychological Well-being of Rodents
Immigration Reform and Control Act of 1986	PATRIOT Act and Hosting Foreign Visitors
Clinical Center Ward for Study of AIDS Patients	Clinical Center Unit for Study of Obese Patients
Competitiveness and Fairness of the Summer Program	Competitiveness and Fairness of the Summer Program
Request for List of Important International Conferences	Attendance at Important International Conferences
Critical Issues of Laboratory Research Space on Campus	Critical Issues of Laboratory Research Space on Campus
Revitalization of the Commissioned Corps	Transformation of the Commissioned Corps
Scientific Faculty	SBRS and Title 42



NIH Scientists and Blue-Ribbon Panel Weigh In on Outside Activities

by Celia Hooper

Drawing on an outpouring of responses from intramural scientists, the NIH Blue-Ribbon Panel on Conflict of Interest Policies (a working group of the Advisory Committee to the NIH Director, or ACD) has completed a fast-paced, controversial study with "watershed" recommendations on NIH staff's outside activities. Delivering the panel's 80-page report and 18 recommendations on May 6, panel co-chair Norman Augustine told the ACD that the 66-day study treads a thin line.

Celia Hooper

Intermission: *Panel Co-chair Norman Augustine (left) and NIH Director Elias Zerhouni during a break in the action at the ACD meeting May 6 (in the foreground, back to camera, is Lana Skirboll, associate director for science policy)*

"The panel recognizes NIH as a national treasure, but we realized we could do harm to it. On the one hand, we had to avoid being too liberal and allowing NIH's credibility to be damaged. On the other hand, we had to avoid being too restrictive so that NIH would not be able to compete for world-class talent and [not] be able to translate research" discoveries into new medical advances, Augustine said.

"We tried to walk that narrow line between these concerns." Augustine is chairman of the Executive Committee of Lockheed Martin Corporation.

In a sparsely attended news conference after the panel's presentation, NIH Director Elias Zerhouni said the report was a watershed for NIH. "These are profound recommendations."

On May 12, Zerhouni, Augustine, and panel co-chair Bruce Alberts went on to present the report to the House Subcommittee on Oversight and Investigations, where it received a less-than-enthusiastic response. Zerhouni and other NIH leaders were due to make additional visits to Capitol Hill for further testimony on the report's issues before pursuing changes in policy, rules, and legislation governing what work scientists may pursue on their own time outside of official duties.

In presenting the recommendations on May 6, Alberts commended NIH for its energetic responses to conflict-of-interest concerns, which surfaced last fall and were reported in *The Los Angeles Times* in December. NIH responses included formation of the NIH Ethics Advisory Committee to review on a centralized basis requests from scientists to perform compensated outside activities.

Alberts also commended the testimony of 30 people appearing before the panel and more than 300 NIH staff members who contributed via a website for feedback on outside activities. Alberts, who is president of the National Academy of Sciences, said the panel was impressed by the thoughtful, long responses submitted by NIHers.

He also cited the work of NIH staff members who supported the panel by assembling statistics to put the issues in perspective. Of 17,526 full-time workers at NIH, 118 consult for biotechnology companies, a lower percentage than at most universities, he said.

Celia Hooper

Panel Co-chair Bruce Alberts

Augustine said a key overarching observation of the committee was that it would be a "grave error" to ban all outside activities. "We need more controls, but there are very good reasons for participation" by scientists in outside activities, including keeping current with what is happening in academic and industrial research labs—and helping them to keep in touch with progress at NIH. He noted that although the number of scientists who consult with outside groups is small, a ban would disproportionately alienate the most respected scientists and early career investigators who do not want to be denied opportunities in the future.

Another important general point, Augustine said, was to make COI policies sufficiently flexible to serve the public interest in the wide variety of situations that arise. "There are legitimate exceptions to every rule" that could be formulated, Augustine said. "The Director needs to be able to grant exceptions."

Summarizing the recommendations, Augustine said the panel wants to see tightening of restrictions on outside activities for top NIH officials and for staff involved in awarding grants. It also wants to see wider disclosure of outside income from staff members who are currently not required to disclose this information. Disclosure of possible conflicts of interest should accompany any delivery—written or spoken—of research findings.

On the other side of the narrow line it walked, the panel also urged liberalizing restrictions on some outside activities such as teaching, writing, and accepting awards—activities that are "part and parcel of being a member of the scientific community," Augustine said. "To deny this is a mistake."

An example of a restriction that should be lifted, Augustine said, is the one-year, postpublication embargo on scientists' discussing their NIH research. The panel said publication marked a point when scientists should be able to talk about their work with outside groups.

Members of Zerhouni's ACD blessed the Blue-Ribbon Panel's report with a thumbs-up as it set off on a hard journey through Congressional scrutiny and on to rule makers inside and outside NIH.

In the mean time, Zerhouni noted, there has been a sharp decline in new arrangements for outside activities at NIH. "Scientists do not want to deal with the hassle until this is settled," he speculated. The report itself noted, "many scientists sense that they are unfairly being forced to live under a cloud of suspicion."

The report called for the quickest possible action on its recommendations: "This is needed to assure the continued, deserved public confidence in the extraordinary work of NIH, to continue to enhance the quality of the scientific staff at NIH, and to rectify what the Panel perceives as a critical and growing morale problem among the agency's excellent staff." ■

The final report can be seen at the ACD website:

<http://www.nih.gov/about/ethics_COI_panelreport.htm>.

An Aerial View of the NIH Roadmap: Dushanka Kleinman

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of NIH, the Roadmap's three themes embrace nine implementation groups and the initiatives within them.

The NIH Catalyst interviewed Kleinman in mid-April, four months into her six-month detail as Roadmap coordinator and seven months after the earliest Roadmap RFAs had gone out.

Q: What do you find most exciting about this project?

KLEINMAN: Both the process and the promise.

The process of putting it together is like nothing I've experienced at NIH—and I've been here since 1980. It allows for representation across all institutes, a diversity of participants, with no one sector dominating. Everyone is stimulated to think creatively.

It's a collective process that melts down our organizational walls and even our disciplines and individual mission focus to focus on our common mission—acceleration of the conduct of science and the time it takes to transfer benefit to the public. That's the promise of the Roadmap.

Roadmap initiatives are not meant to replicate something already being done in the institutes, and they were selected by the IC directors because they were perceived as critical to their own missions.

The outcomes of the Roadmap initiatives will be of benefit to all the institutes and centers—and from my perspective as deputy director of a small institute, this is a highly positive thing.

Q: How will each institute benefit?

KLEINMAN: Have you been to the Roadmap website? You have to take a look at each of the component parts, lift the veil from each of the initiatives, and look at it all from the perspective of each of the institutes.

The initiatives within the "New Pathways to Discovery" theme, for instance, will develop new research resources—such as the Molecular Library—that will be accessible to any investigator in any institute and to the entire research community.

A focus of "Research Teams of the Future" is how to work across institutes to create interdisciplinary research teams and new fields. More effective partnerships across disciplines will benefit all of NIH.

And addressing infrastructure impediments to clinical research will facilitate clinical research across all the institutes, regardless of content area.

Q: Would you say that the Roadmap, then, runs parallel to ongoing NIH research?

KLEINMAN: One could say parallel, but it's actually integrated pretty well.

The Roadmap initiatives are not content-specific—not specific to, say, heart disease or cancer or diabetes. They are meant to be generic. They play a complementary role to the ongoing research of each institute. And several of the institutes are proceeding with their own initiatives to prepare their research community to benefit from the Roadmap itself.

Roadmap initiatives are intended to transform how we do our science and our funding to stimulate new fields. The ultimate outcome is to increase efficiency and instill creativity and novelty.

For example, one initiative under the "New Pathways to Discovery" is nanomedicine. In this fiscal year, the focus is on concept development—defining nanomedicine and how one would go about planning nanomedicine development centers.

Clinical research initiatives explore the feasibility of interoperability among clinical research networks and of expanding clinical research capacity through a national Clinical Research Associates Program, which would extend our research capacity to health providers in the field.

This not only would help in recruiting and retaining patients in clinical trials but would also accelerate the transfer of science into practice. Now it can take 20 years to actually bring something identified as a successful intervention into the hands of patients. If the provider is active in research, that time may be foreshortened.

Q: NIH already funds clinical research all over the country; is this initiative aimed at reaching more community clinics in addition to the major academic centers?

KLEINMAN: Reaching further into the community, expanding and diversifying the patient base, and more rapidly disseminating the findings is one aspect.

Fran Pollner

Dushanka Kleinman: "We're in a creation mode"

The other issue is that even though we currently have many ongoing clinical trials—and will continue to—the cost of setting up and then terminating a trial is tremendous. Basically, you're building a building and then wrecking it each time a trial is begun and concluded; time, money, and human resources are lost in the creation and demolition.

This initiative will look at whether currently established clinical research networks for trials in, say, arthritis or osteoporosis or cardiovascular disease can be used for other diseases as well.

Q: How much is the NIH intramural community involved in Roadmap research?

KLEINMAN: There's a role for intramural research in every part of the three themes. Here are a few examples.

Within "New Pathways to Discovery," six chemical genomic screening centers are being established. The first of these will be established in the IRP, and that center will serve to coordinate the network for all six centers (see "PubChem," page 1). The NIH center will be established this year, and then the others will be set up extramurally.

The Roadmap has already resulted in

the doubling of individuals (from 15 to 30) in the CC's Clinical Research Training Program, which is a critical part of the clinical research training aspect of the Roadmap.

The intramural program also plays a pivotal role in "Research Teams of the Future." One of the initiatives is to develop the IRP as a model for interdisciplinary research. The extramural community will get planning grants for this sort of activity.

Q: How will this IRP model be established?

KLEINMAN: We'll have to see how that develops. But clearly, we have the largest multidisciplinary enterprise in one physical setting and so we have the ability—and probably already the practice in our cross-institute collaborations—to define interdisciplinary research and demonstrate how it works. "Interdisciplinary" means the creation of a new field, a new discipline, a new science. It's not multidisciplinary research, where we work together to address a problem and then go back to our own labs and separate disciplines. It's a merger through which something new is created.

We're hoping that that will be the creative force for nanomedicine, for example.

Q: Where do you go from here?

KLEINMAN: Well, my six-month detail ends in June. But there's got to be

PubChem on the Way

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will be generated by these NIH-funded small-molecule screening centers. The screening centers—one to be based at NHGRI and several others at academic institutions around the country—will be analyzing thousands of chemically diverse small molecules via high-throughput screening processes to identify those compounds that are biologically active against a range of molecules, cells, or genes.

Chris Austin, a senior advisor on translational research at NHGRI and director of the NHGRI intramural screening center, which is also due to begin operations in the fall of 2004, says that PubChem will make available to the general research public small-molecule compounds and information that traditionally has been proprietary within the private sector.

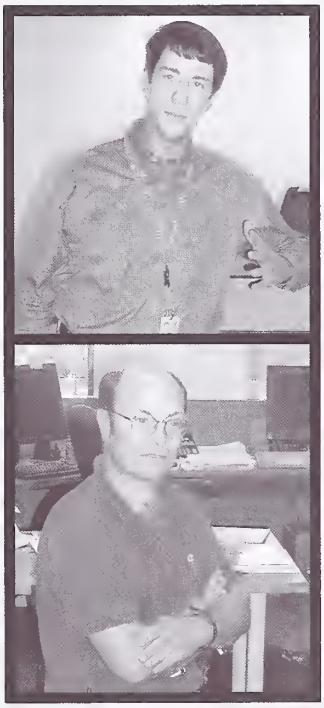
"People in the pharmaceutical industry have had access to this kind of information for some time. This is the first time that [such] comprehensive information on the chemical structures and biological activities of thousands of small molecules will be freely available to the public sector. . . . [It's] a tremendous step forward," Austin says.

Cross-linking

Bryant notes that PubChem will be cross-linked to NCBI's other databases, such as PubMed, in ways that can further enable research.

"Chemical structures in PubChem will automatically be neighbored, or compared to one another, and this will allow users to make new connections between articles in the literature, such as those concerning biological activity, toxicology, and animal or clinical studies. These articles might refer to the same or chemically similar compounds, but since compounds have many names, the connection can only be made by linking through chemical structure and structural similarity. We expect that these new cross-links will make PubChem an extremely powerful research tool," he says.

PubChem will initially contain "legacy" data, such as that from NCI's Developmental Therapeutics Program (DTP), a decades-old program that plans, conducts, and facilitates development of therapeutic agents for cancer and AIDS. DTP maintains a repository of synthetic compounds and fully characterized pure natural products that have been evaluated as potential anticancer and anti-HIV agents. It has an inventory of more than 140,000 nondiscrete compounds that have been submitted to DTP from a variety of sources worldwide.



James Swyers

Chris Austin (top) and Steve Bryant

Small Molecules as Chemical Probes

Another initiative of the Molecular Libraries component is the creation of a repository to collect and house the small molecules that will be analyzed by the NIH-funded screening centers.

This "Small Molecule Repository" will provide the centers with large sets of chemical compounds to be screened and will provide the biomedical research community with access to small-molecule probes generated by the screening centers.

Now being created, the repository has a mandate to acquire, maintain, and distribute a collection of approximately 1 million chemically diverse small molecules with known and unknown activities. Over time, this collection will be expanded and modified to include compounds that are capable of interacting with an increasing number and diversity of biomolecular target domains.

Bryant and Austin expect that the chemical probes generated will be used mainly as research tools for the study of genetic and cellular pathways in health and disease. But these tools should also give researchers developing new drugs a leg up, they note, and in selected cases may even be used directly as starting points for diagnostic tests or drug development, particularly for rare and orphan diseases.

"PubChem will be a huge cross-referencing resource for hundreds of thousands of small, biologically active molecules. It cannot help but speed up the drug development process," Bryant says. ■

An Aerial View of the NIH Roadmap:
Dushanka Kleinman

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central coordination for the Roadmap, whether it's myself or someone else. That has to be perpetuated.

Really, there's no set recipe for what needs to be done between now and July—and after that. We're in a creation mode.

It's my hope to be able to have the ability, the building blocks, the organizational structure that will allow the Roadmap to continue on over time. There is a tremendous amount of collaboration and contribution, not only within my immediate office but throughout the OD offices—a tremendous effort by so many people.

The initial plan takes the Roadmap out to 2009. Many initiatives are starting this year, but others will not start until 2005 and some not until 2006. Evaluation is a critical component.

Each initiative has a lifespan of its own, and, actually, we'll have to keep looking at each one, reviewing each one, to decide, as the time approaches, whether we will reissue existing initiatives and when another broad and open selection process will be held that would lead to new initiatives.

Q: How involved are intramural scientists in this process?

KLEINMAN: The working groups are the engines that drive the initiatives. And each working group is a virtual organization of NIH, with representation across all the institutes.

Each of these nine working groups has multiple project teams; within each project team are intramural scientists, grants managers, communications people—and all these people meet routinely. So, yes, many NIH scientists are greatly involved.

But whether NIH scientists are personally involved in today's activities is not the question. The question is whether the outcomes of the initiatives will affect NIH scientists personally in their research careers.

And, if these initiatives come to fruition—such initiatives as cheminformatics, PubChem, organic molecules, protein structures, computing centers, assay techniques, robotics, imaging probe database, and standards for proteomics and metabolomics—they will surely help scientists in their labs here. ■

The NIH Catalyst *plans to run a series of interviews with some of the NIH scientists who are serving as chairs of implementation groups and project team leaders.*

*The NIH Roadmap website—<**http://nihroadmap.nih.gov**>—contains a wealth of information on the objectives and scope of all Roadmap initiatives, together with the names of the dozens of NIH people involved in each of the nine implementation groups. The website is also an expanding universe of requests for applications, proposals, and information, as well as other related announcements.*

CML Treatment Pioneer New Fogarty Scholar

John Michael Goldman, the pioneer of stem-cell transplantation to treat chronic myelogenous leukemia (CML), is NIH's newest Fogarty Scholar and will be here for six to nine months between July 1, 2004, and June 30, 2005.

Goldman will be working closely with the NHLBI Hematology Branch, headed by Neal Young, and the Lymphoma and Leukemia Interest Group, chaired by NCI's Michael Bishop.

Goldman pioneered the use of bone marrow transplantation in the treatment of CML, was the first to use peripheral blood as a source of stem cells for autologous transplant in CML, and published a seminal report in 1986 on the use of allogeneic donors in CML transplant. He was a pioneer in introducing RT-PCR to monitor CML patients after treatment.

More recently, his team carried out some of the first trials of imatinib mesylate (Gleevac) in the treament of CML patients and described leukemias with other chromosomal translocations susceptible to control with this agent. His team is currently using gene profiling and other techniques to explore mechanisms underlying the heterogeneity of CML.

John Michael Goldman

Goldman is chairman of hematology at the Imperial College School of Medicine and the Hammersmith Hospital in London, the director of the Leukaemia Research Fund Centre for Adult Leukaemia, and the medical director of the Anthony Nolan Bone Marrow Trust.

According to Bishop, who nominated him, Goldman is "a world authority, perhaps the only world authority, on CML in all its aspects from basic biology to its clinical management." ■

Clinical Research Center 2004 Milestones

Substantial completion: August 25, 2004
Office/lab moves begin: September 2004
Ribbon cutting (proposed): September 22, 2004
Patient move date: December 4, 2004

Clinical Research Information System (CRIS) Status

Testing: April through July 2004
Training: June and July 2004
Go Live: July 31, 2004 (Medical Information System (MIS) is shut down just before midnight Friday July 30, 2004; CRIS is turned on after midnight the morning of Saturday, July 31, 2004; see *The NIH Catalyst,* November-December 2002, "From MIS to CRIS," page 2)
ProtoType/CRIS-AE: Beta testing spring and early summer 2004 (protocol writing and adverse-event reporting system; see *The NIH Catalyst,* November-December 2003, "ProtoType: According to Protocol," page 7)

Family Lodge Timeline

Design Begins: May 2000
Groundbreaking: October 2002
Construction starts: March 2003
Construction 70 percent complete: March 2004 (see below)
Family Lodge opens: November 2004

Quotations Selected for CRC Science Court

1. Research is "to see what everyone has seen, and think what no one has thought." —Albert Szent-Gyorgi

2. There are in fact two things, science and opinion; the former begets knowledge, the latter ignorance. —Hippocrates

3. ... we are too ignorant safely to pronounce anything impossible ... it has often proved true that the dream of yesterday is the hope of today, and the reality of tomorrow. —Robert Goddard

4. Concern for man himself and his fate must always form the chief interest of all technological endeavors ... in order that the creations of our mind shall be a blessing and not a curse to Mankind. Never forget this in the midst of your diagrams and equations. —Albert Einstein

5. You see things; and you say, "Why?" But I dream things that never were; and I say, "Why not?" —George Bernard Shaw

6. The first principle is that you must not fool yourself – and you are the easiest person to fool. —Richard Feynman

7. ... for in the sciences the authority of thousands of opinions is not worth as much as one tiny spark of reason in an individual man. —Galileo Galilei

8. Science and art belong to the whole world, and the barriers of nationality vanish before them. —Johann Wolfgang Von Goethe

9. To wrest from nature the secrets which have perplexed philosophers in all ages, to track to their sources the causes of disease, to correlate the vast stores of knowledge, that they may be quickly available for the prevention and cure of disease – these are our ambitions. —Sir William Osler

10. Liberty ... is the great parent of science and of virtue; and that a nation will be great in both, always in proportion as it is free. —Thomas Jefferson,

11. One never notices what has been done; one can only see what remains to be done. —Marie Curie

12. ... investigators ... should not trust ... authors who by employing only their imagination have wished to make themselves interpreters between nature and man, but only of those who have exercised their intellects ... with the results of their experiments. —Leonardo DaVinci

13. In science as in other human activities, the speed of progress is less important than its direction. —Rene Dubos

Additional Quotations for Consideration

14. Since new developments are the products of a creative mind, we must therefore stimulate and encourage that type of mind in every way possible. —George Washington Carver

15. Where there is no vision, there is no hope. —George Washington Carver

16. You will often reach patients and cure them by scientific use of your humanity. —Clara Marshall

17. We have a hunger of the mind which asks for knowledge of all around us, and the more we gain, the more is our desire; the more we see, the more we are capable of seeing. —Maria Mitchell

What's in a Picture? The Temptation of Image Manipulation

by Mike Rossner[1] and Kenneth Yamada[2]

It's all so easy with Photoshop®[3]. In the days before imaging software became so widely available, making adjustments to image data in the darkroom required considerable effort and/or expertise. It is now very simple, and thus tempting, to adjust or modify digital image files. Many such manipulations, however, constitute inappropriate changes to your original data, and making such changes can be classified as scientific misconduct. Skilled editorial staff can spot such manipulations using features in the imaging software, so manipulation is also a risky proposition.

Good science requires reliable data. Consequently, to protect the integrity of research, the scientific community takes strong action against perceived scientific misconduct. In the current definition provided by the U.S. government: "Research misconduct is defined as fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results." For example, showing a figure in which part of the image was either selectively altered or reconstructed to show something that did not exist originally (for example, adding or modifying a band in a polyacrylamide gel image) can represent falsification or fabrication.

Being accused of misconduct initiates a painful process that can disrupt one's research and career. To avoid such a situation, it is important to understand where the ethical lines are drawn between acceptable and unacceptable image adjustment.

Here we present some general guidelines for the proper handling of digital image data and provide some specific examples to illustrate pitfalls and inappropriate practices. There are different degrees of severity of a manipulation, depending on whether the alteration deliberately changes the interpretation of the data. That is, creating a result is worse than making weak data look better. Nevertheless, any manipulation that violates these guidelines is a misrepresentation of the original data and is a form of misconduct. All of the examples we will show here have been created by us using Photoshop®; although they may appear bizarre, it is remarkable that they are actually based on real cases of digital manipulation discovered by a careful examination of digital images in a sample of papers submitted (or even accepted) for publication in a journal.

Why Is It Wrong To "Touch Up" Images?

If you misrepresent your data, you are deceiving your colleagues, who expect and assume basic scientific honesty—that is, that each image you present is an accurate representation of what you actually observed. In addition, an image usually carries information beyond the specific point being made. The quality of an image has implications about the care with which it was obtained, and a frequent assumption (though not necessarily true) is that in order to obtain a presentation-quality image, you had to carefully repeat an experiment multiple times.

Manipulating images to make figures more simple and more convincing may also deprive you and your colleagues of seeing other information that is often hidden in a picture or other primary data. Well-known examples include evidence of low quantities of other molecules, variations in the pattern of localization, and interactions or cooperativity.

Journal Guidelines

It is surprising that many journals say little or nothing in their "Instructions to Authors" about which types of digital manipulations are acceptable and which are not. The following journals provide some guidelines, but they vary widely in comprehensiveness.

Molecular and Cellular Biology: "Since the contents of computer-generated images can be manipulated for better clarity, the Publications Board at its May 1992 meeting decreed that a description of the software/hardware used should be put in the figure legend(s)."

Journal of Cell Science: "Image enhancement with computer software is acceptable practice, but there is a danger that it can result in the presentation of quite unrepresentative data as well as in the loss of real and meaningful signals. During manipulation of images, a positive relationship between the original data and the resulting electronic image must be maintained. If a figure has been subjected to significant electronic manipulation, the specific nature of the enhancements must be noted in the legend or in the Materials and Methods."

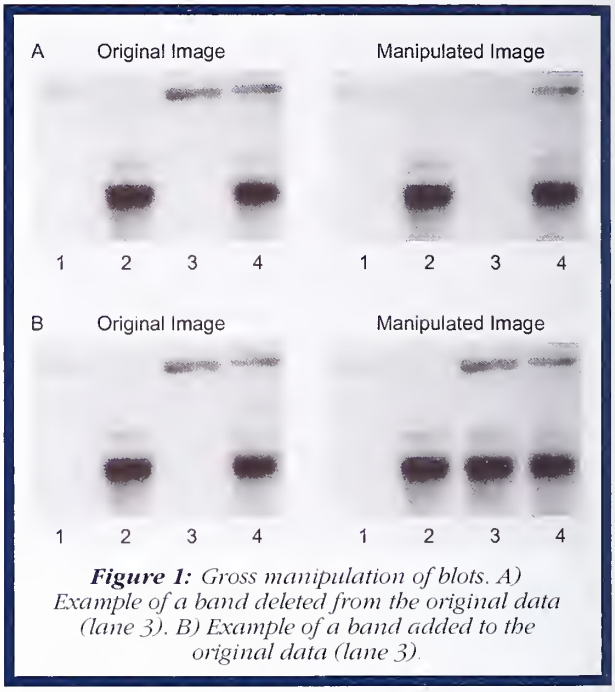

Figure 1: *Gross manipulation of blots. A) Example of a band deleted from the original data (lane 3). B) Example of a band added to the original data (lane 3).*

The Journal of Cell Biology: "No specific feature within an image may be enhanced, obscured, moved, removed, or introduced. The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., using dividing lines) and in the text of the figure legend. Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the whole image and as long as they do not obscure or eliminate any information present in the original. Non-linear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend."

Because the last set of guidelines is

1. *The Journal of Cell Biology*, Rockefeller University Press, 1114 First Avenue, 3rd Floor, New York, NY 10021

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3. The general principles presented here apply to the manipulation of images using any powerful image-processing software; however, because of the popularity of Photoshop®, we refer to several specific functions in this application. [Mention of this product does not constitute an endorsement.--ed.]

by far the most comprehensive we have found to date (full disclosure: we wrote them), we will continually refer back to them in the following discussions of the use and misuse of digital manipulations.

Blots and Gels

Gross Misrepresentation

The simplest examples of inappropriate manipulation are show in Figure 1. Deleting a band from a blot, even if you believe it to be an irrelevant background band, is a misrepresentation of your data (Figure 1A). Similarly, adding a band to a blot, even if you are only covering the fact that you loaded the wrong sample, and you know for sure that such a protein or DNA fragment or RNA is present in your sample, is a misrepresentation of your data. In the example shown in Figure 1B, the additional band in lane 3 has been generated by simply duplicating the band in lane 2.

Another example of using Photoshop® inappropriately to create data is illustrated in Figure 2, in which a whole single panel has been replicated (arrows) and presented as the loading controls for two separate experiments.

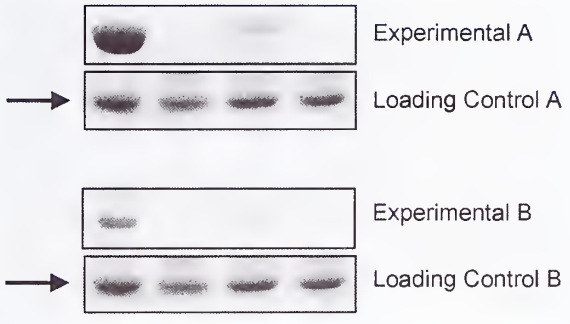

Figure 2: *Gross manipulation of blots. Example of a duplicated panel (arrows).*

More Subtle Manipulations

Brightness/Contrast Adjustments. Adjusting the intensity of a single band in a blot constitutes a violation of the widely accepted guideline that "No specific feature within an image may be enhanced, obscured, moved, removed, or introduced." In the manipulated image in Figure 3A, the arrow indicates a single band whose intensity was reduced to produce an impression of more regular fractionation. Although this manipulation may not alter the overall interpretation of the data, it still constitutes misconduct.

While it is acceptable practice to adjust the overall brightness and contrast of a whole image, such adjustments should "not obscure or eliminate any information present in the original" (Figure 3B). When you scan a blot, no matter how strong the bands, there will invariably be some gray background. While it is technically within the guidelines to adjust the brightness and contrast of a whole image, if you overadjust the contrast so that the background completely drops out (Figure 3B, part 2 vs. part 3), this should raise suspicions among reviewers and editors that other information (especially faint bands) may have dropped out as well.

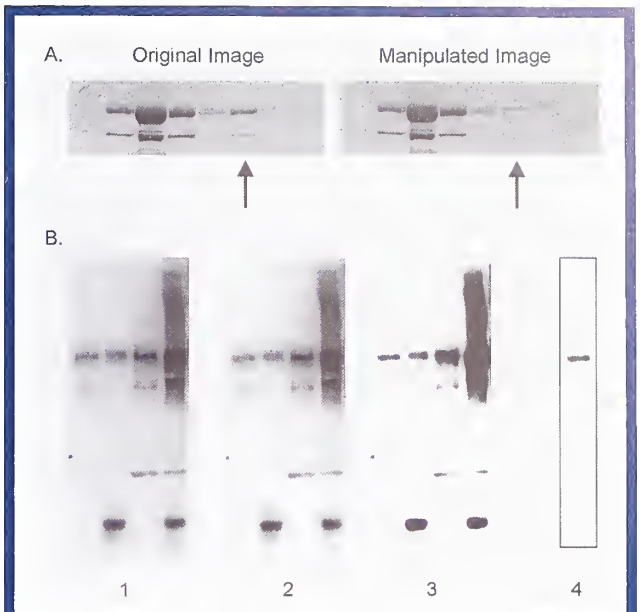

Figure 3: *Manipulation of blots: brightness and contrast adjustments. A) Adjusting the intensity of a single band (arrow). B) Adjustments of contrast. Images 1, 2, and 3 show sequentially more severe adjustments of contrast. Although the adjustment from 1 to 2 is acceptable because it does not obscure any of the bands, the adjustment from 2 to 3 is unacceptable because several bands are eliminated. Cutting out a strip of a blot with the contrast adjusted provides the false impression of a very clean result (image 4 was derived from a heavily adjusted version of the left lane of image 1). For a more detailed discussion of "gel slicing and dicing," see reference 2.*

It may be argued that this guideline is stricter than in the days before Photoshop®, when multiple exposures could be used to perfect the presentation of the data. Perhaps it is, but this is just one of the advantages of the digital age to the reviewer and editor, who can now spot these manipulations when in the past an author would have taken the time to do another exposure. Think about this when you are doing the experiment and perform multiple exposures to get the bands at the density you want, without having to overadjust digitally the brightness and contrast of the scanned image.

Cleaning Up Background. It is very tempting to use the tool variously known as "Rubber Stamp" or "Clone Stamp" in Photoshop® to clean up unwanted background in an image (Figure 4). Don't do it. This kind of manipulation can usually be detected by someone looking carefully at the image file because it leaves telltale signs. Moreover, what may seem to be a background band or contamination may ac-

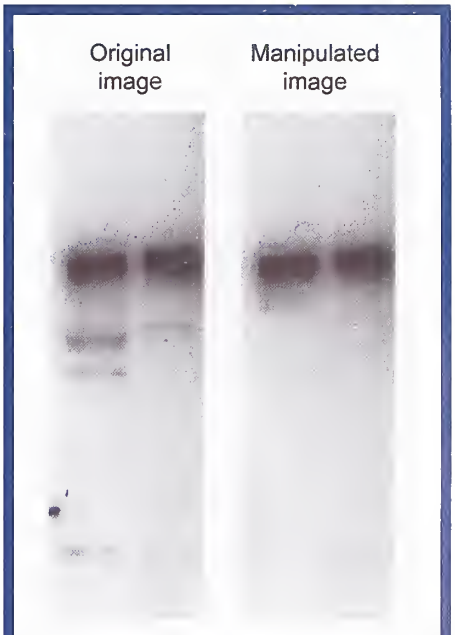

Figure 4: *Manipulation of blots: cleaning up background. The Photoshop® "Rubber Stamp" tool has been used in the manipulated image to clean up the background in the original data. Close inspection of the image reveals a repeating pattern in the left lane of the manipulated image, indicating that such a tool has been used.*

tually be real and biologically important and could be recognized as such by another scientist.

Splicing Lanes Together. It is clearly inappropriate manipulation to take a band from one part of a gel and move it to another part, even if you do not change its size. But it is within usual guidelines to remove a complete lane from a gel and splice the remaining lanes together. This alteration should be clearly indicated, however, by leaving a thin white or black line between the gel pieces that have been juxtaposed. Again, it could be argued that this guideline is stricter than in the days before Photoshop® when paper photographs of a gel were cut up and pieces were glued next to each other. This practice, however, usually left a black line indicating to the reader what had been done.

As it was with gel photographs, it is unacceptable to juxtapose pieces from different gels to compare the levels of proteins or nucleic acids. Rerun all of the samples on the same gel!

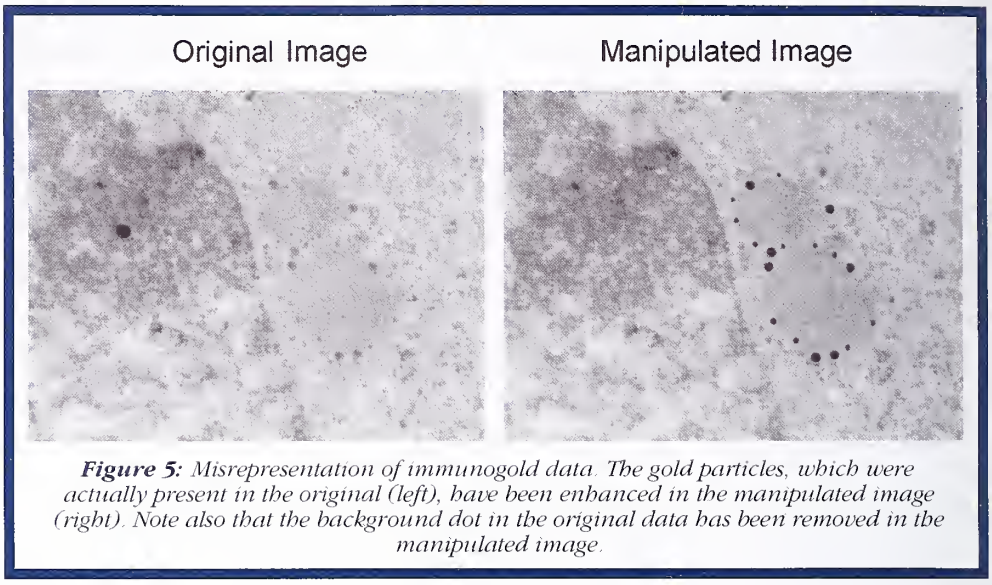

Figure 5: *Misrepresentation of immunogold data. The gold particles, which were actually present in the original (left), have been enhanced in the manipulated image (right). Note also that the background dot in the original data has been removed in the manipulated image.*

Micrographs

Enhancing a Specific Feature

An example of manipulation by enhancement is shown in Figure 5, in which the intensity of the gold particles has been enhanced by manually filling them in with black color using Photoshop®. This type of manipulation misrepresents your original data and is thus misconduct. There are acceptable ways to highlight a feature such as gold particles, which include arrows or pseudocoloring. If pseudocoloring is done with the "Colorize" function of Photoshop®, it does not alter the brightness of individual pixels, but pseudocoloring should always be disclosed in the figure legend.

Other examples of misconduct include adjusting the brightness of only a specific part of an image or erasing spots. Using the "Brightness" adjustment in Photoshop® is considered to be a linear alteration (see below), which must be made to the entire image.

Linear vs. Nonlinear Adjustments

Linear adjustments, such as those for "Brightness" or "Contrast" in Photoshop®, are those in which the same change is made to each pixel according to a linear function. It is acceptable (within limits noted above) to apply linear adjustments to a whole image. There are other adjustments in Photoshop® that can be applied to a whole image, but the same change is not made to each pixel. For example, adjustments of gamma output ("Color Settings" in Photoshop®) alter the intensity of each pixel according to a nonlinear function. Adjustments of "Curves" or "Levels" in Photoshop® alter the tonal range and color balance of an image by adjusting the brightness of only those pixels at particular intensities and colors. Such nonlinear changes are sometimes required to reveal important features of an image; however, the fact that they have been used should be disclosed in the figure legend.

Digitally altering brightness or contrast levels can be misleading with fluorescence micrographs. Some authors mistakenly change the contrast of an experimental compared with a control photo, or change individual panels in a time course, or use different contrast levels when making merged images compared with the original images. All of these changes in individual pictures used for comparisons can be misrepresentations. On the other hand, certain adjustments such as background subtraction or using a filter or digital mask may be needed to extract information accurately from complex images. Reporting the details and logic of such manipulations that are applied to images as a whole should resolve concerns about their use. Stan-

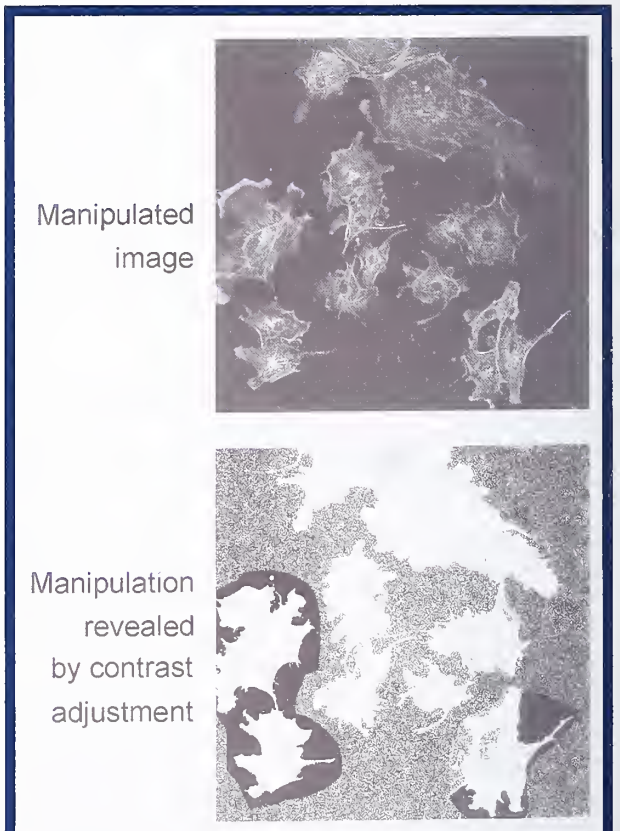

Figure 6: *Misrepresentation of image data. Cells from various fields have been juxtaposed in a single image, giving the impression that they were present in the same microscope field. A manipulated panel is shown at the top. The same panel, with the contrast adjusted by us to reveal the manipulation, is shown at the bottom.*

dards and guidelines in the field will continue to evolve, but full disclosure will always be the safest course.

Misrepresentation Of a Microscope Field

The reader assumes that a single micrograph presented in a figure represents a single microscope field. Combining images from separate microscope fields into a single micrograph constitutes a misrepresentation of your original data. In the manipulated image in Figure 6 (top panel), cells have been combined from several microscope fields into a single micrograph. This manipulation becomes visible when the contrast of the image is adjusted so that the inserted images become visible (bottom panel). You may want to combine images from several fields into a single micrograph to save space, but this assembly should be clearly indicated by thin lines between the different pieces.

Resolution

A pixel is a square (or dot) of uniform color in an image. The size of a pixel can vary, and the resolution of an image is the number of pixels per unit area. Although resolution is defined by area, it is often described using a linear measurement—dots per inch (dpi). Thus, 300 dpi indicates a resolution of 300 pixels per inch by 300 pixels per inch, which equals 90,000 pixels per square inch (see reference 1).

High-resolution digital cameras (in 2004) can acquire an image that is 6 megapixels in size. This can generate an image of approximately 2400 x 2400 pixels, or 8 inches x 8 inches at 300 dpi. Note that, with the right settings in Photoshop®, physical size and resolution can be traded off against each other without a gain or loss in the amount of information—that is, you can resize an image without altering the total number of pixels.

You should be aware of the resolution at which the image was acquired by the digital camera on your microscope. When that file is opened in Photoshop®, you have the option of setting the size and resolution of the image. You should not set the total number of pixels to be greater than that in the original image; otherwise, the computer must create data for you that were not present in the original, and the resulting image is a misrepresentation of the original data—that is, the dpi of an image can only be increased if the size of the image is reduced proportionately.

It is acceptable to reduce the number of pixels in an image, which may be necessary if you have a large image at high resolution and want to create a small figure out of it. Reducing the resolution of an image is done in Photoshop® by sampling the pixels in an area and creating a new pixel that is an average of the color and brightness of the sampled ones. Although this does alter your original data, you are not creating something that was not there in the first place; you are presenting an average.

Other Data-Management Issues

It is crucially important to keep your original digital or analog data exactly as they were acquired and to record your instrument settings. This primary rule of good scientific practice will allow you or others to return to your original data to see whether any information was lost by the adjustments made to the images. In fact, some journal reviewers or editors request access to such primary data to ensure accuracy.

There are other important issues concerning data handling that we have not addressed by focusing on manipulations of existing data. Examples include selective acquisition of data by adjusting the settings on your microscope or imager, selecting and reporting a very unusual result as being representative of the data, or hiding negative results that may contradict your conclusions. Any type of misrepresentation of experimental data undermines scientific research and should be avoided.

Conclusion

Data must be reported directly, not through a filter based on what you think they "should" illustrate to your audience. For every adjustment that you make to a digital image, it is important to ask yourself, "Is the image that results from this adjustment still an accurate representation of the original data?" If the answer to this question is "no," your actions may be construed as misconduct.

Some adjustments are currently considered to be acceptable (such as pseudocoloring or changes to gamma settings) but should be disclosed to your audience. You should, however, always be able to justify these adjustments as necessary to reveal a feature already present in the original data.

We hope that by listing guidelines and publicizing examples of transgressions, all of us can become more vigilant, particularly in guiding junior colleagues and students away from the tempting dangers of digital manipulation. Just because the tools exist to clean up sloppy work digitally, that is no excuse to do sloppy work.

If you would have redone an experiment to generate a presentation-quality image in the days before the digital age, you should probably redo it now. ■

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1. M. Rossner and R. O'Donnell, "The *JCB* will let your data shine in RGB." *J. Cell. Biol.* **164**:11 (2004)
<http://www.jcb.org/cgi/content/full/164/1/11>

2. "Gel slicing and dicing: a recipe for disaster." *Nat. Cell Biol.* **6**:275 (2004)
<http://www.nature.com/cgi-taf/DynaPage.taf?file=/ncb/journal/v6/n4/full/ncb0404-275.html>

The authors invite comments on the issues addressed in this article. Please send them to **<catalyst@nih.gov>**.

New Website Tracks Sequencing Projects Worldwide

The International Sequencing Consortium has launched a free online resource where scientists and the public can get the latest information on the status of sequencing projects for animal, plant, and other eukaryotic genomes.

The Large-scale Sequencing Project Database can be found at **<http://www.intlgenome.org>** and enables searching for sequencing project information by organism, by sequencing group, or by funding agency (such as NHGRI).

Information about each sequencing project includes timetables for completion and brief descriptions of the sequencing strategies used. The database also features links to websites of individual sequencing projects and their funding agencies, as well as links to other publicly run databases that contain actual DNA sequence data. ■

Postbac Posters Carry the Day in May

by Aarthi Ashok and Javier Lorenzo

More than 200 postbaccalaureate trainees opened their research projects to the scrutimy of the rest of the NIH community at the fifth annual Postbac Poster Day, May 5.

Postbacs are recent college graduates with an interest in biomedical research who were selected to spend a year or two in training at NIH to find out whether the life of a research scientist actually suits them. The great majority apply to (and are accepted into) a Ph.D. program and/or medical school during their stay at NIH. (More postbac posters will appear in the next Catalyst.*)*

Cindy Yang

Cindy Yang, Carnegie Mellon University, Pittsburgh, Pa.: *Iron Misregulation Leads to Neurodegeneration in Mice.*
Preceptor: Tracey Rouault, NICHD, Cell Biology and Metabolism Branch

Yang's research focused on iron imbalance as a cause of neurotoxicity. She worked with two mouse models of abnormal iron metabolism—one in which mice overexpress transferrin receptor, the receptor that escorts iron into cells, and the other in which there is loss of the IRP family of proteins, which are post-transcriptional regulators of ferritin and other iron-metabolism genes.

Mice that overexpress transferrin receptor performed poorly on behavioral and motor testing. These mice were prone to seizures and tremors and had a thinning coat; the males died within six months.

The second model had only one copy of IRP1 and lacked both copies of IRP2. These mice had a movement disorder and displayed large amounts of iron in the substantia nigra and the cerebellum, two regions of the brain involved in movement and coordination.

Yang hopes her research will add to the knowledge of such conditions as Parkinson's and Alzheimer's diseases. She'll continue her neuroscience studies in a doctoral program at the University of California at San Francisco beginning this fall.

—Javier Lorenzo

Wynne Morgan

Wynne Morgan, Colorado College, Colorado Springs: Drosophila melanogaster *as a Model for Hyperparathyroidism–Jaw Tumor Syndrome*
Preceptors: Jianhua Zhang and William Simonds, NIDDK, Metabolic Diseases Branch

Hyperparathyroidism–jaw tumor syndrome (HPT-JT) is an autosomal dominant disease characterized by benign and malignant parathyroid tumors, fibrous jaw tumors, and renal lesions. Previous work on HPT-JT uncovered mutations in *HRPT2*, a gene that codes for the protein parafibromin, whose function is not known. That the mutations in HPT-JT families inactivate *HRPT2*, however, suggests a tumor-suppressor function for parafibromin.

Morgan studied the *HRPT2* counterpart in the fly, which, she said, has a surprising 63 percent homology to the human gene. Through a series of removals and reinsertions of the gene, she found that eliminating both copies does not damage the fly in its earlier larval stages but is lethal prior to the adult stage. She next hopes to rescue the phenotype by restoring the gene. She hopes, too, that her research will help elucidate the tumor-suppressor function of *HRPT2*.

—Javier Lorenzo

Bridget Lynch, Gustavus Adolphus College, St. Peter, Minn.: *Protein Characterization of Stage-Specific Ovarian Cancer Biomarkers via Mass Spectrometry*
Preceptor: Mark Lowenthal, NCI-CCR, Laboratory of Pathology

Bridget Lynch explains her findings to postbac Matthew Crescenzo, whose own poster on "PET Imaging of Phosphodiesterase-4 with (C-11) Rolipram" (NIMH Molecular Imaging Branch, Robert Innis, Masahiro Fujita) was also on display

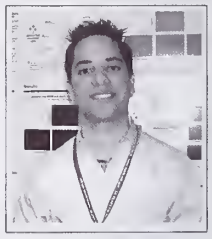
Diagnosis of ovarian cancer at stage 3, now possible with CA-125 peptide, is generally too late to intervene to improve outcome. Lowenthal's laboratory has been seeking biomarkers to detect ovarian cancer at stages early enough to intervene in a meaningful way. The lab has achieved a high degree of specificity in detecting early-stage ovarian cancer using a mathematical model based on the mass spectra profile of patient serum.

Concentrating on low-abundance proteins that bind to albumin, Lynch analyzed serum proteins by mass spectroscopy and uncovered 586 proteins not known to exist in serum. By sequencing samples from different patient populations, she also found that the concentrations of specific subsets of proteins vary between stage-specific cohorts.

This fall, Lynch will attend the University of New Mexico School of Medicine in Albuquerque and plans to become a family physician.

—Javier Lorenzo

Frank Diaz, Tufts University, Boston: *Initiation of Glial Differentiation from Human CNS Progenitor Cells Promotes JC Virus Susceptibility*
Preceptor: Eugene Major, NINDS, Molecular Virology and Neurogenetics Section



Frank Diaz

Diaz's work stems from the establishment of a unique cell-culture system that uses specific growth factors to induce selective differentiation of human CNS progenitor cells into populations of astrocytes or neurons.

Using this system, Diaz has started to ask questions about cellular determinants of susceptibility to infection by a neurotropic polyomavirus, JC virus (JCV), the etiological agent of progressive multifocal leukoencephalopathy.

JCV infects CNS progenitors at low levels and mature astrocytes robustly, whereas neurons are completely resistant. Infection of progenitor cells with JCV and subsequent differentiation showed that cells became susceptible to JCV infection within three days of differentiation towards an astrocytic lineage, as measured by viral DNA replication and viral late gene expression.

"It is too early to tell whether stem cells in the brain may be targets of JCV infection," Diaz said, although these results implicate molecular factors expressed early during glial cell differentiation in regulating JCV susceptibility.

According to Diaz, this work suggests that JCV susceptibility is likely governed by intracellular factors, rather than cell surface interactions. In previous work in the lab, differential expression of a transcription factor, NF1-D, was observed in mature glial cells and neurons. Future work, Diaz said, will determine whether NF1-D also confers susceptibility to JCV infection in astrocytic progenitors.

—Aarthi Ashok

Corinna Levine

Corinna Levine, Cornell University, Ithaca, N.Y.: *How Efficiently Do Cells Compartmentalize Proteins?*
Preceptor: Manu Hegde, NICHD, Cell Biology and Metabolism Branch

The aim of this study was to determine the efficiency of compartmentalization of signal sequence-bearing secretory proteins using a sensitive luciferase reporter assay in MDCK cells. The results were surprising, Levine said, in that the efficiency ranged widely between 60 and 95 percent, depending on the particular signal sequence.

Levine and her colleagues also found that the degree of confluency, or cell densoty, influenced the efficiency of protein compartmentalization. In the case of osteopontin, increased confluency was associated with increased localization in the cytoplasm and decreased localization in the endoplasmic reticulum.

This finding, Levine said, suggests that cells can modulate the levels of secretory proteins available in the cytoplasm in response to the environment—a significant finding in light of the fact that differential compartmentalization of several secretory and membrane proteins is being linked to disease states.

"There is a big impetus to understand the parameters that control the accumulation of proteins, such as prion protein, in the cytoplasm," she said. She hopes that future work leads to the design of novel therapeutic drugs that could change the balance of proteins in different cellular compartments in such conditions as prion diseases. *—Aarthi Ashok*

John Allen Houston

John Allen Houston, Stanford University, Stanford, Calif. *Intracellular trafficking of Apolipoprotein A1*
Preceptors: Silvia Santamarina-Fojo, H. Bryan Brewer and Edward Neufeld, NHLBI, Molecular Disease Branch

The ABCA1 transporter protein is essential for the removal of excess cholesterol (LDL) from peripheral cells such as macrophages, where accumulation of cholesterol can lead to arterial closing.

ABCA1 transfers intracellular cholesterol to the extracellular apolipoprotein A1 (apoA1) to form HDL particles that in turn transfer cholesterol to the liver, where it's removed from the body as bile. The critical interaction of ABCA1 and apoA1 has been thought to occur at the cell surface.

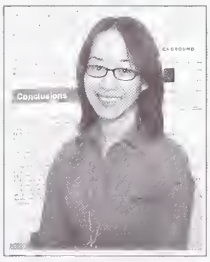
Work from the Molecular Disease Branch (J. Biol. Chem. 279:1557–15578, 2004) suggests, however, that this interaction may actually occur intracellularly. These researchers observed rapid endocytosis of labeled apoA1 in human fibroblasts transfected with ABCA1-GFP, and significant co-localization of these proteins occurred within late endosomes.

Their current model suggests that ABCA1 is present both in late endosomes and at the cell surface and that following endocytosis of apoA1, interaction of these proteins occurs in late endosomes with subsequent trafficking of lipidated apoA1 to the cell surface.

As a step towards defining a therapeutic strategy for Tangier's disease, a condition characterized by low HDL levels due to a nonfunctional ABCA1, Houston examined the effect of delivering ABCA1-GFP to Tangier's disease patients' fibroblasts in vitro.

Expression of ABCA1-GFP led to a marked decrease in the amount of intracellular cholesterol in Tangier patients' cells, as assessed by cytochemical staining of cholesterol with filipin. "This rescue of an in vitro phenotype may lead the way for future gene therapy," Houston said.

—Aarthi Ashok



Quynh Nguyen

Quynh Nguyen, *Philadelphia University, Philadelphia, PA: mTOR Cellular Localization*
Preceptors: Beverly Mock and Valery Bliskovsky, NCI-CCR, Laboratory of Genetics

Mouse plasmacytoma is a model for human lymphomas and myelomas. Plasmacytomas can be induced by peritoneal injections of prostane. A few mouse strains (including BALB/c mice) are genetically susceptible to this type of cancer; most others (including the DBA strain) are resistant. The goal of the project of which this study is a part is to understand how the genes responsible for susceptibility or resistance to cancer work.

In earlier work, it was found that tumorigenesis was controlled by several genes. FRAP/mTOR (mammalian target of rapamycin) was identified by positional cloning strategies as the most likely candidate for one of these genes. Comparison of nucleotide sequences revealed that the FRAP gene from susceptible and resistant mice differed by a single amino acid substitution. Of note, the amino acid present in resistant mice was conserved for about 1.3 billion years. Functional analysis revealed that the FRAP protein from the susceptible strain was less efficient as a kinase, consistent with the hypothesis that FRAP functions as a tumor suppressor.

Nguyen transfected cell cultures with fluorescently tagged versions of the two allelic variants of FRAP. She showed that while the allelic version of FRAP from resistant DBA mice localized specifically in the cytoplasm, the variant from susceptible BALB mice was distributed evenly throughout the cell. The cellular distribution of the mutated form was comparable to that of a nonfunctional truncated form of FRAP—providing a clue, Nguyen said, to the mechanism that impairs the tumor-suppressor activity of FRAP in the susceptible mice.

—Aarthi Ashok

Erica Sutton

Erica Sutton, McGill University, Montreal: *Truth-Telling and the Turner Syndrome*
Preceptor: Barbara Biesecker, NHGRI, Social and Behavioral Research Branch

Turner syndrome (TS) is a sex chromosome abnormality caused by partial or full deletion of one sex chromosome. It affects 1 in 2,500 women and confers short stature and, often, infertility.

Sutton worked on a project that ascertained the life concerns of women affected with TS and the effect of learning of their condition by adolescence vs. not knowing anything about it until they sought help because they could not become pregnant.

The researchers questioned a cohort of 97 girls and women about the disclosure of their condition by a parent or a healthcare provider. Those who had been told the truth by one or the other had more confidence in their ability to deal with the repercussions of the condition. They were better able to incorporate infertility into their self-identity at a younger age.

One-third of participants had learned of their infertility serendipitously from a stranger; they reported subsequent mistrust of family members and providers.

Sutton hopes the study will help to more fully elucidate the physician-patient relationship and its effect on health outcomes. Personally, she intends to pursue her interests in reproductive ethics and genetic counseling in graduate school.

—Javier Lorenzo

Recently Tenured

Jeffrey Baron *received his M.D. from Southwestern Medical School, University of Texas Health Science Center at Dallas, in 1983. He completed a residency in pediatrics at Yale–New Haven Hospital in New Haven, Conn., in 1986. In 1989, he completed a fellowship in pediatric endocrinology in the Developmental Endocrinology Branch, NICHD, where he currently heads the Unit on Growth and Development.*

The primary research interest of the Unit on Growth and Development is the cellular and molecular mechanisms governing skeletal growth and development. We have focused particularly on the process of longitudinal bone growth, which occurs at the growth plate—a thin layer of cartilage found near the ends of long bones and vertebrae.

Fran Pollner

Jeffrey Baron

The growth plate consists of three principal layers: the resting zone, the proliferative zone, and the hypertrophic zone. In the proliferative and hypertrophic zones, clones of chondrocytes are arranged in columns parallel to the long axis of the bone. Within these columns, the cells undergo clonal expansion followed by cellular hypertrophy. The hypertrophic cartilage is then remodeled into bone tissue. The net effect is that new bone is progressively created at the bottom of the growth plate, lengthening the bone.

The function of the resting zone is not well understood. We recently demonstrated that the resting zone can regenerate the proliferative and hypertrophic zones, suggesting that the resting zone contains chondrocytic stem-like cells that are capable of generating new clones of proliferative chondrocytes. We have also shown that ectopic resting-zone cartilage can induce a shift in the spatial orientation of nearby proliferative-zone chondrocytes, suggesting that the normal resting zone directs the spatial orientation of the proliferative clones, causing them to form columns parallel to the long axis of the bone.

The overall body proportions of vertebrates are determined by the size of the skeleton, which in turn is determined by the rate and duration of longitudinal bone growth. This rate falls progressively with age. In humans, fetal growth exceeds 100 cm/year. By birth, the growth rate has decreased to 50 cm/year, and by mid-childhood, 5 cm/year. A similar progressive decline in bone growth occurs in other mammals.

This decline in growth rate with increasing age is due primarily to a decrease in the rate of growth-plate chondrocyte proliferation.

In addition to functional changes, the growth plate also undergoes structural changes with age. We have termed these structural and functional changes "growth-plate senescence."

Our in vivo studies suggest that senescence occurs because the growth-plate stem-like cells have a finite proliferative capacity that is gradually exhausted. We are currently investigating the cellular and molecular mechanisms that limit proliferation of growth-plate chondrocytes.

Following a period of growth inhibition, the rate of longitudinal bone growth often does not just return to normal but actually exceeds normal. This phenomenon, known as catch-up growth, has been observed in humans and other mammals, following a wide variety of growth-inhibiting conditions.

It has long been speculated that catch-up growth is due to a central nervous system mechanism. However, we have shown that catch-up growth is due, at least in part, to a mechanism intrinsic to the growth plate, with evidence in particular that catch-up growth is caused by a delay in growth-plate senescence.

Growth-inhibiting conditions slow the proliferation of growth-plate chondrocytes, thus conserving the proliferative capacity of these cells and slowing growth plate senescence. If the growth-inhibiting condition resolves, the chondrocytes will have retained greater proliferative capacity than normal, will be less senescent than normal, and therefore will proliferate more rapidly and for a longer period of time than normal, resulting in catch-up growth.

Eventually, growth ceases and the growth plate is replaced by bone, a process known as epiphyseal fusion.

Our findings suggest that epiphyseal fusion is triggered when the proliferative capacity of the growth-plate chondrocytes is finally exhausted. We have found evidence that estrogen accelerates the proliferative exhaustion of these cells. As a result, estrogen leads to early termination of linear growth and early epiphyseal fusion. We recently found clinical evidence that estrogen accelerates growth plate senescence in girls exposed to estrogen because of precocious puberty.

One goal of this work is to improve medical treatment of growth disorders and childhood metabolic bone diseases. In addition, we seek to uncover general principles of developmental biology since the cellular processes underlying bone growth—such as cell proliferation, terminal differentiation, angiogenesis, and cell migration—are also essential for development in other tissues.

Thomas Bugge *received his Ph.D. from the European Molecular Biology Laboratory/University of Copenhagen in 1993. He performed his postdoctoral studies jointly at the University of Copenhagen and the University of Cincinnati from 1993 to 1995. He held faculty appointments as research leader at the University of Copenhagen and associate professor of pediatrics at the University of Cincinnati from 1995 to 1999 and was recruited to NIDCR in 1999. He is currently chief of the Proteases and Tissue Remodeling Unit, NIDCR.*

Extracellular proteolysis is essential to human development, homeostasis, tissue remodeling, tissue repair, learning, immunity, and fertility; excessive or impaired extracellular proteolysis is the cause of many human ailments. For example, abnormal extracellular proteolysis is the hallmark of cancer and enables tumor cell growth, survival, motility, invasion, and angiogenesis. Furthermore, inappropriate extracellular proteolysis by itself leads to genetic instability and can directly drive the malignant transformation of cells.

This "pruning" of the extracellular environment in health and during disease is performed by an array of proteases, several hundred in number, whose activities are tightly regulated by a series of inhibitors, receptors, associated proteins, and small molecules.

Research into how extracellular proteases modify their environment faces unique technical limitations, such as the lack of appropriate tissue-culture models and general methods to identify downstream protease targets. Moreover, a large number of extracellular proteases were discovered only very recently through the completion of the human, mouse, and rat genome sequences. Therefore, despite its imminent importance to human health and disease, the field of extracellular proteolysis is characterized by vast "frontiers of unknowns," which make the research particularly exciting and challenging.

Our laboratory has long been interested in understanding the functions of proteases that are bound directly to the surface of cells through membrane attachment or via the binding to specific cell-surface receptors. We use a collaborative, multidiscip-

linary approach that combines bioinformatics with targeted gene inactivation and overexpression in mice, advanced histology, proteomics, and degradomics to unravel the exceptionally diverse spectrum of functions of cell surface proteolysis in life.

Fran Pollner

Thomas Bugge

We previously performed an analysis of the overall biological function of the plasminogen activation system, a sophisticated cell-surface proteolytic cascade. We found that the system had a universal role in postnatal tissue homeostasis, remodeling, and repair, and that in most, but not all, cases the critical substrate was the provisional matrix protein fibrin.

More recently, we identified the molecular function of a new receptor for the urokinase plasminogen activator called uPARAP. Surprisingly, we found that the novel receptor has a dual role as a protease receptor and as a receptor for the connective tissue protein collagen that shuttles collagen from the outside of the cell to the phagolysosomal compartment of cells for proteolytic degradation. Moreover, we have shown that tumor cells trick normal stromal cells located around a tumor into expressing uPARAP, thereby aiding the invasion and destruction of healthy tissue by the tumor.

We have performed fundamental gene discovery and functional studies of a curious new family of transmembrane proteases that display an unusual orientation in the cell membrane, with the NH_2-terminus located inside the cell and the COOH-terminus containing the catalytic domain located outside the cell. This family of proteases, the type II transmembrane serine proteases, emerged from obscurity just a few years ago to represent one of the largest protease families known today.

By gene targeting in mice, we have shown that the absence of one member of this new protease family, matriptase/MT-SP1, impairs the maturation of epidermal surfaces and prevents the processing of the large epidermis-specific polyprotein profilaggrin, leading to perinatal death. Conversely, overexpression of matriptase/MT-SP1 in mice causes malignant transformation and the formation of invasive and metastatic carcinoma, which may explain the almost ubiquitous overexpression of this novel protease in human epithelial tumors.

We are also pursuing a quite different line of research in a close collaboration with anthrax researcher Stephen Leppla of NIAID. We exploit the fact that tumor cells vastly overexpress certain cell-surface proteases to engineer "made-to-order" bacterial toxins that are selectively activated on the surface of tumor cells.

We have found that the engineered toxins are much less toxic to to normal cells, but are endowed with potent tumor cell toxicity and can eradicate established tumors in animals. Our laboratories are working together toward the goal of introducing the modified bacterial cytotoxins into the growing arsenal of agents used for the treatment of cancer.

Fabio Candotti *earned his M.D. degree at the University of Brescia, Italy, in 1987. He did his postgraduate clinical training in pediatrics and pediatric allergy and immunology in Italy and postdoctoral research training at NCI and NHGRI. He was an assistant professor of pediatrics at the University of Brescia from 1996 to 1998, when he returned to NIH. He is now a senior investigator in NHGRI's Genetics and Molecular Biology Branch and is head of the Disorders of Immunity Section.*

Fran Pollner

Fabio Candotti

My laboratory studies the molecular basis of inherited disorders of the immune system and works to develop gene replacement strategies for this heterogeneous group of diseases.

Severe inherited immunodeficiencies can be cured with hematopoietic stem cell transplantation, which is most successful if performed using a perfectly matched sibling as a donor. However, most children with these disorders do not have matched siblings, which makes transplantation generally less successful and more risky.

As a pediatrician, I have long had an interest in finding alternatives to hematopoietic stem cell transplantation for the treatment of immunodeficient children.

Currently, we are focusing our research on adenosine deaminase (ADA) deficiency, Wiskott-Aldrich syndrome (WAS), and IL-12 receptor β1 deficiency.

Patients with ADA deficiency are unable to produce significant numbers of mature T or B lymphocytes and thus have a severe combined immune deficiency and no protection against viruses and bacteria. WAS is an X-linked recessive disorder characterized by a less profound form of immunodeficiency, along with eczema and thrombocytopenia. IL-12 receptor β1–deficient patients are extremely susceptible to salmonella and atypical mycobacterial infections.

For the past few years, we have been following a group of patients affected with ADA deficiency and WAS to learn about the natural history of these rare disorders and to evaluate whether genetic correction is a viable therapeutic option.

In addition, we are using in vitro and in vivo models to study the efficacy of corrective gene transfer into lymphocytes and hematopoietic stem cells, using viral vectors based on murine oncoretroviruses and lentiviruses.

At the bedside, we are evaluating novel retroviral vectors as gene-transfer tools for the genetic correction of ADA deficiency. A clinical gene-transfer trial is ongoing to test the hypothesis that these vectors will provide better reconstitution of the immune system than has been observed in previous trials.

In addition, this trial directly compares two viral promoters in the same patients to determine which confers higher expression in humans. The results of this clinical trial will provide important additional safety and biological information to the field of corrective gene transfer into human hematopoietic progenitors.

For WAS and IL-12 receptor β1 deficiency, we are performing preclinical gene-correction studies that have shown that retroviral-mediated gene transfer can correct the biological defects observed in lymphocytes and cell lines obtained from affected patients. We are evaluating similar strategies using in vivo models in knockout animals to test safety and efficacy of the gene transfer procedure in preparation for clinical applications.

Studies of primary immunodeficiency disease have been instrumental in defining multiple players and pathways critical for the correct development and function of the immune system. These unique experiments of nature have provided fertile ground for collaboration among clinical researchers and basic scientists not only in the field of immunology but also genetics and molecular and cellular biology.

Our ongoing explorations include

- The role of WASP, the protein mutated in WAS patients, in B lymphocyte development and autoimmunity onset
- The significance of the IL-12 receptor β-1 chain in the development and peripheral homeostasis of T cells

These subjects cut across the expertise of several research groups at NIH, which should afford the opportunity for idea exchange and collaboration. ■

Catalytic Reactions?

If you have a photo or other graphic that reflects an aspect of life at NIH (including laboratory life) or a quotation that scientists might appreciate that would be fit to print in the space to the right, why not **send it to us via e-mail: catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2W23.**

Also, we welcome "letters to the editor" for publication and your reactions to anything on the *Catalyst* pages.

In Future Issues...

- On the Road To Nanotechnology
- Chad Womack And the BSA
- Whither Obesity?

Kids' Catalyst

Save Your Breath: How To Blow Up a Balloon with Chemistry

It doesn't matter whether you're in 2nd grade or AP Chemistry—this experiment is always fun. Younger chemists can clearly see bubbles and a balloon expanding. Older chemists can write formulas, measure the balloons, compare the amount of gas produced with the amount of raw product, and guess what ratio will produce the best reaction.

I'm talking about what happens when you combine vinegar, baking powder, a balloon, and a curious mind. The specifics are:

■ **Safety glasses**. One day you'll need to wear a whole lot more than these to do an experiment, but for now protect yourself from spills, splashes, and exploding balloons. Swimming goggles will work, too, but protect those eyes! Always. ■ **Vinegar.** Any kind will work; I used plain old white vinegar. ■ **Baking powder.** ■ **Measuring spoons.** ■ **Measuring cups**, calibrated droppers, anything that you can measure liquid with (you can usually get 5-ml droppers from the pharmacy for free). ■ A piece of **paper** rolled like a funnel. This is for quick dispensing of the premeasured baking powder—and you'll need to be fast! ■ A small **glass**, a cup with a spout, or anything that will easily pour premeasured liquid. ■ **Chart** with five columns—*Experiment #, Vinegar, Baking Powder, Time, Size.* You can have as many rows as you wish, because since you're numbering your experiments you can go on for as long as you have materials! ■ **Plastic bottles** with mouths small enough for the balloon to fit over (and for you to pour liquid into without it spilling)—as many as you want, but five is a good number. ■ **Pre-expanded balloons**, at least one for each bottle. These things can and will pop, so test them first by blowing them about halfway up to make sure they don't have any holes. This also stretches them out. ■ **Masking tape** to wrap around the expanded balloons so you can accurately measure them.

Now start having some fun.

1. Write "1" in your experiment column. You'll make adjustments based on this first experiment, and it's important to document what you've done, no matter what. Write it down!

2. Put a tablespoon of baking powder in the paper funnel, laid on its side. Write down how much.

3. Take a tablespoon of vinegar and put that into the small cup. Write this down.

4. Test the balloon on the mouth of your plastic bottle by fitting it over the top. Better for it to break now than later! Write this down. Whoops . . . no space? Well, experiments are all about observing procedures, and how can you repeat a procedure if you don't know what it is? You can't. So make another test column—or a new chart with another test column. That's another thing experiments are all about—revision.

5. Pour the vinegar into the plastic bottle.

6. Get the balloon ready. . . .dump the baking powder in, and as fast as you can, put the balloon over the mouth of the bottle.

7. Watch the contents of the bottle bubble away, and watch the balloon expand. You might also want to watch the clock at this point to see how long it took for the balloon to start expanding. Give the bubbles a few minutes to calm down, and see what you have left. Is there extra baking powder at the bottom of the bottle? Maybe try some *more* vinegar this time? Sounds like Experiment # 2.

You could get pretty complicated with this, explaining reactions, drawing the formula for the process, measuring the balloon expansion with tape, predicting how large the balloon will be for a given experiment, and so on. . . . Or you could just watch a balloon expand without blowing a single breath!

—Jennifer White

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