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NIEHS Spotlight

Science Notebook

NIEHS Spotlight

New NIH Training and Education Director Visits NIEHS

By Eddy Ball

On July 30, NIEHS trainees and key administrators gathered in Rodbell Auditorium to meet Sharon Milgram, Ph.D., during her first visit to the Institute in her official capacity as the director of the newly reorganized NIH Office of Intramural Training and Education (OITE). The NIEHS Trainees Assembly (NTA) and NIEHS Office of Fellows Career Development hosted the workshop, which underscored the ongoing commitment by NTA, NIH and NIEHS administration to strive together for excellence in the Institute's training programs.

With her network of friends at UNC Chapel Hill, where she taught for 14 years before joining NIH, Milgram has personal and professional reasons for looking forward to visits to RTP. "I will be traveling here much more often than the previous acting director did," she promised. (Photo by Eddy Ball)

Nearly 70 of the approximately 260 trainees working at the Institute turned out to hear Milgram and other presenters offer their perspectives on the trainee-support network at NIH/NIEHS. An afternoon session on transitioning from training to careers featured a panel of five senior scientists and administrators. Milgram served as facilitator of the panel discussion and also shared her own career experiences during the question-and-answer session.

In their opening addresses to the audience, in-coming NTA Chair Anastasia Wise and steering committee representative Jennifer Adair, Ph.D., outlined the role of the NTA at the Institute and elaborated on the organization's many activities on behalf of trainees. In addition to organizing educational programs and workshops, holding the annual Biomedical Career Fair and putting together a semi-annual orientation program for new fellows, she explained, the NTA also serves as a social network for fellows, a liaison to the administration and an advocate for fellows.

Diane Klotz, acting director of the Office of Fellows Career Development (Photo courtesy of Steve McCaw)

As the featured speaker of the event, Milgram discussed the mission of OITE "to support the individual training offices in the individual institutes and centers and provide a broader view of the training experience.… We oversee the training of all trainees on all campuses."

"We are an office that responds to the needs of trainees all along the way," she continued. Along with supervising training at all levels and conducting training at the trans-NIH level, from pre-professional intern programs to advanced training for fellows, Milgram hopes that her office will soon be "the proud owners of an office of scientific career counseling."

Referring to the 2005 Sigma Xi Post-doc Survey, on which she was an advisor, Milgram closed her talk with a reminder to fellows about their responsibilities to take advantage of training and to be assertive about their professional needs. "The one thing that determines the satisfaction level of a post-doc and [his or her] productivity is whether that post-doc engages in meaningful career and professional development throughout the entire time as a post-doc.… Keep in mind how much power you hold in the process."

Milgram posed with NTA officers and steering committee representatives. Pictured, from left, Anastasia Wise, Scott Auerbach, Ph.D., Rose Ramos, Ph.D., Milgram, Karina Rodriguez, Ph.D., Jennifer Adair, Ph.D., and Friederike Jayes, D.V.M., Ph.D. (Photo by Eddy Ball)

Speaking on behalf of the NIEHS administration, Director of the Office of Translational Biomedicine Bill Martin, M.D., and Deputy Scientific Director Bill Schrader, Ph.D., articulated their roles in helping to mentor and nurture young scientists at the Institute. They also voiced their support for expanding the work of the Office of Fellows Career Development, which has been headed by a part-time administrator since its creation in 2003 by then NIEHS Director Ken Olden, Ph.D.

"We are certainly committed to having a full-time person run the office," Martin stressed. "We're here to serve you and help you through a transition to choose a career you want."

After the meeting, Anastasia Wise and Bill Martin chatted and shared a laugh. (Photo by Eddy Ball)

Acting Director of the Office of Fellows Career Development Diane Klotz, Ph.D., described herself as "very committed to ensuring the flow of communications among the various facets of the Institute that are making decisions about career development and training." Klotz completed her postdoctoral training at NIEHS and brings a trainee-centered perspective to her administration of the office. She characterized this time as offering all of the stakeholders "a really great opportunity" for benefiting trainees at NIEHS with "the best career development programs and the best scientific training opportunities."

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Advisory Panel Weighs in on Bisphenol A

By Eddy Ball

In its first step toward drawing conclusions about the human health risks of Bisphenol A (BPA), the National Toxicology Program (NTP) has released the findings of its Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel Evaluation held August 6 – 8, 2007 in Alexandria, Va. The panel was composed of 12 independent scientists who reviewed more than 500 scientific studies on BPA to assess the potential reproductive and developmental hazards of the estrogen-like chemical, which is used in the manufacture of polycarbonate plastics and epoxy resins. The panel's meeting was open to the public.

NTP emphasizes that the findings of its expert panels do not necessarily reflect the views of the NTP. The panel used a five-point scale, ranging from "negligible" to "serious," to rate the level of its concerns about the effects of exposure to the compound on human health:

- The panel's greatest level of concern was directed towards possible neural and behavioral effects caused by BPA exposure *in utero*. The panel expressed "some" concern for these types of effects, a level of concern that falls in the middle of the five-point scale.

- "Minimal" characterized the panel's level of concern that exposure to BPA *in utero* causes effects on the prostate or potentially causes accelerations in puberty. Likewise, the experts expressed minimal concern that children's exposure to BPA potentially causes accelerations in puberty.

- "Negligible" described the panel's degree of concern that exposure to BPA *in utero* produces birth defects and malformations in pregnant women and fetuses. The expert panel also had negligible concern that adverse reproductive effects may follow exposures in the general adult population to BPA.

A Statement on BPA by 38 Independent Scientists

A statement, signed by 38 scientists, is the outcome of another meeting sponsored by NIEHS Division of Extramural Research & Training, the National Institute of Dental and Craniofacial Research, the Environmental Protection Agency and *Commonweal* held in Chapel Hill, NC, November 28 -29, 2006. (See *eFactor* report "Expert Panel Debates Health Risks of BPA"). The meeting produced five review articles on specific aspects of BPA research with statements on the strength of the data, in addition to the consensus statement that integrated the data across research areas.

"Chapel Hill Bisphenol A Expert Panel Consensus Statement: Integration of Mechanisms, Effects in Animals and Potential to Impact Human Health at Current Levels of Exposure" is now in press for the online issue of *Reproductive Toxicology 24(2)*.

In the statement, BPA research scientists agreed that the range of exposures that most Americans experience are higher than those that cause a wide range of adverse health effects in animals. Although they recognized the short-comings of the current body of research and called for expanded investigations into the human health risks, they concluded, "The wide range of adverse effects of low doses of BPA in laboratory animals exposed both during development and in adulthood is a great cause for concern with regard to the potential for similar adverse effects in humans."

The group included several NIEHS grantees and two NIEHS representatives, DERT Administrator Jerry Heindel, Ph.D., and Laboratory of Molecular Toxicology Supervisory Biologist Retha Newbold. The group called for increasing research on human health risks based on the "extensive evidence from laboratory animal studies, particularly when common mechanisms that could plausibly mediate the responses are known to be very similar in the laboratory animal models, wildlife and humans."

The statement about the human health risks of BPA differed from conclusions in a European Food Safety Authority report released in January 2007, which found no risk to human health at current exposure levels.

(Note: The October 1 issue of the *eFactor* will feature a report on the Future Research on Endocrine Disruption Conference scheduled for August 27-29, 2007 in Durham. The conference is sponsored by the NIEHS Division of Extramural Research and Training and U. S. Environmental Protection Agency.)

The expert panel report will be finalized and available for public comment in fall 2007. The NTP will take the report into consideration as it reaches its own conclusions about BPA, using the same categories for level of concern as the panel used. The NTP will express its conclusions, which may differ from the panel's, in a document referred to as the NTP brief. During 2008 CERHR expects to release the NTP-CERHR monograph on BPA, which will consist of the NTP brief, the expert panel report and all public comments on that report. The center will solicit public comment on the brief and submit it for independent peer review prior to completing its report.

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Network Approaches Symposium Highlights Systems Biology Research

By Eddy Ball

NIEHS and the National Cancer Institute (NCI) co-sponsored a conference on August 16 in Rodbell Auditorium featuring talks by leading figures in the close-knit community of scientists working in basic, conceptual and translational research employing systems biology approaches. Titled "Network Approaches to Investigating Environmental Influences on Human Disease," the meeting was chaired by David Balshaw, Ph.D., program administrator in the Center for Risk and Integrated Sciences, a program in the Division of Extramural Research and Training (DERT).

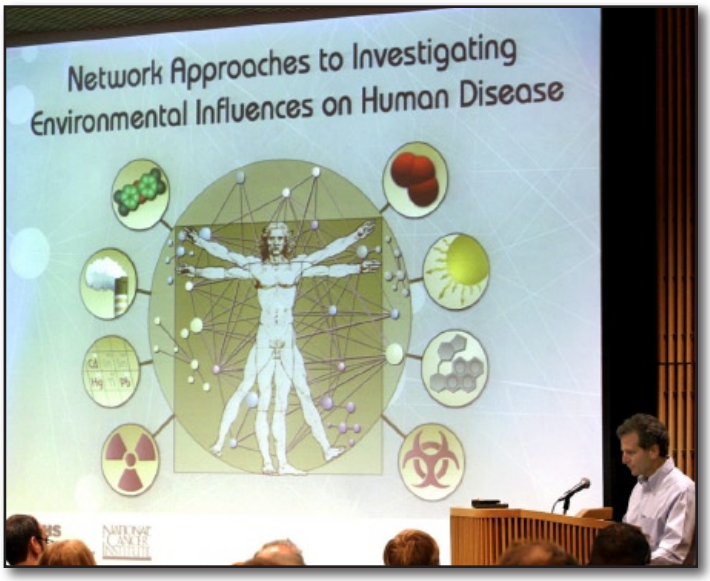
Before turning to the exciting new developments in systems biology, host David Balshaw joked about the poor showing of his division's ball team, the DERT Devils, at a recent match with Intramural Division players. (Photo courtesy of Steve McCaw)

In his welcoming address to symposium participants, several of whom were NIH grantees, David A. Schwartz, M.D., acknowledged the contributions by people at NIEHS, NCI, the National Heart, Lung and Blood Institute and the National Center for Research Resources. "This symposium will help us identify some approaches that we can take to understand how biological systems can be perturbed by environmental influences," he explained, "and how those perturbed biological systems relate to changes in human biology that lead to the development of disease."

Schwartz urged participants to keep that continuum in mind and help NIH identify "approaches that we can take in terms of programmatic development that will help us link environmental exposures to biological systems." He also stressed that the meeting would be an opportunity for the scientists to learn more about each other's work and identify future collaboration possibilities and new research directions.

Systems biology integrates many sciences, including biology, chemistry, physics, computation, engineering, mathematics and medicine, in an endeavor to understand the interaction and cross-regulation of biological pathways and characterize complete biological systems. The research involves extensive laboratory measurement, data mining and modeling in an effort to determine how to manipulate biological systems most effectively to treat and prevent disease.

Although the roots of system biology extend much farther back, the approach gained institutional and industrial support only in recent years. The Seattle-based Institute of Systems Biology was founded in 2000, and Harvard University established its first completely new department in over 20 years with the creation of its Department of Systems Biology in 2003. In the years since, system biology approaches have become a part of research by major biotech and drug development companies, and the approach is gaining a foothold in the toxicology testing and regulatory communities.



Schwartz described the way systems biology dovetails with the mission of NIEHS and issued participants several challenges that speakers referred to many times during the day's presentations. (Photo courtesy of Steve McCaw)

The Networks symposium was a day-long series of 11 presentations in four sessions:

- Biological Applications of Network Theory, chaired by Ben Van Houten, Ph.D., of DERT, and featuring lectures by University of California at San Diego molecular biotechnology specialist Trey Ideker, Ph.D., and University of Washington computational biochemist Herbert Sauro, Ph.D.

- Defining Connections in Biological Networks, chaired by Daniel Shaughnessy, Ph.D., of DERT, and featuring presentations by Harvard Medical School cancer geneticist Marc Vidal, Ph.D., NIEHS Tenure Track Investigator Karen Adelman, Ph.D., (see related story) and University of California at San Francisco computational biologist Tanja Kortemme, Ph.D.

- Dynamic Networks: Defining Location and Function, chaired by Sally Tinkle, Ph.D., of DERT, and featuring talks by Carnegie Mellon University machine learning developer Ziv Bar-Joseph, Ph.D., Indiana University School of Medicine bioinformatics specialist Keith Dunker, Ph.D., European Molecular Biology Labs protein complexes analyst Ann-Claude Gavin, Ph.D., and University of Albany cancer genomics investigator Tom Begley, Ph.D., an NIEHS Outstanding New Environmental Health Scientist (ONES) award winner.

Biological Applications keynote speaker Trey Ideker is emblematic of the youth, talent and energy the field of systems biology has attracted. Ideker moved into systems biology from an engineering background and quickly established himself as a leading voice in the field of computational biology. (Photo courtesy of Steve McCaw)

- Integration of Biological Networks and Phenotypic Endpoints, chaired by Richard Pelroy, Ph.D., of NCI, and featuring lectures by Massachusetts Institute of Technology biological engineering developer Mike Yaffe. M.D., Ph.D., and Stanford University pediatrician and bioinformatics specialist Atul Butte, M.D., Ph.D.

Systems biology has made significant advances in the past ten to fifteen years, but it faces challenges in achieving the ultimate goal of mapping what is called the "human interactome" — the nearly two million molecular interactions in the human organism. In his 2006 argument in *The Scientist* for creation of a Human Interactome Mapping Project, at a projected cost of some $100 million, symposium participant Marc Vidal observed that only 1% of the human interactome had been mapped and perhaps only 10% for the model organisms, yeast, worm and the fly.

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NIEHS scientist Karen Adelman focused on the systems aspect of her work with polymerase II stalling as she demonstrated some of the important applications of the approach to understanding stress response. (Photo courtesy of Steve McCaw)

NIEHS Outstanding New Environmental Health Scientist (ONES) award winner Tom Begley, center, returned to NIEHS to talk about "Network Abnormalities at the Transcription-Translation Interface." (Photo courtesy of Steve McCaw)

The full-day symposium was fast paced, and temperatures outside crept toward 100 — making it tempting for participants, such as Marc Vidal, foreground, to kick back and get more comfortable. (Photo courtesy of Steve McCaw)

Endocrinologist Lectures as Second Scientific Director Candidate

By Eddy Ball

NIEHS continued its search for a Scientific Director on July 24 in Rodbell Auditorium with a lecture and question-and-answer session featuring Evan Simpson, Ph.D. Simpson, whose talk was titled "Sex, Fat and Cancer," is a professor of Biochemistry at Monash University, lab director of the Victorian Breast Cancer Consortium and head, Sex Hormone Biology, Prince Henry's Institute.

The candidate's research interests include breast cancer, locally produced estrogen metabolism, osteoporosis and gene expression of aromatase, the enzyme that converts testosterone to the most potent estrogen, estradiol (E_2). His group is working to develop a breast tissue-specific aromatase inhibitor for use as a first-line alternative to current therapies and as an adjuvant and neoadjuvant therapy for estrogen-dependent tumors.

Simpson is also interested in the roles of phytoestrogens and potential endocrine disrupting compounds in the pathogenesis of breast cancer, the effects of sex steroids on cognition, personality and energy homeostasis, and the relationship between obesity and breast cancer risk.

According to Simpson, estrogen-dependent tumors occur in about 75% of postmenopausal breast cancer cases. "What we believe is happening [in these cancers] is that the adipose tissue [in breast] actually picks up the function of producing estrogen after menopause, when the ovaries cease to do so," he explained — with aromatase playing an essential role in localized production of the hormone.

In the 1980s, Simpson performed cDNA cloning of aromatase and, a decade afterward, developed the aromatase knock-out (ArKO) mouse. He and his lab characterized the ArKO phenotype in order to study the physical effects of inhibiting the enzyme. In addition to inhibiting aromatase, the scientists found that this phenotype resulted in spermatogenic arrest and failure of ovarian follicular development, as well as increased adiposity, reduced energy expenditure and sluggishness, obsessive compulsive behavior and insulin resistance.

Getting a Measure of the Candidate

When asked by David A. Schwartz , M.D., about his interest in becoming the new Scientific Director, Simpson cited personal and professionals reasons for wanting to join NIEHS and return to the United States. Although born and educated in Scotland, the candidate is an American citizen who has spent most of his professional life in this country and has family who live in North Carolina.

After eight years as director of Prince Henry's Institute, Simpson said, "I feel like I've done my bit… and I'm looking for something new to do, something challenging and satisfying." Simpson pointed to "so much exciting work going on here… much of it right up my alley."

Simpson emphasized the importance of obesity and related environmental health issues as he outlined the way he envisions the Institute's research mission developing in the future. "This is a global pandemic," he said, "and it may not be an issue simply of caloric intake." Like obesity, other environmental issues will also need to be addressed globally, and NIEHS is uniquely positioned to "spread the gospel of environmentalism" to emerging countries, such as China and India.

Asked to clarify his desire to leave the administrative demands of his current position only to face what appear to be similar demands in the role of Scientific Director, the candidate made a distinction between scientific administration, on the one hand, and the bureaucratic administration of dealing with state and federal regulators in Australia. "In terms of the administration of science, I really enjoy that," he said, "but [in my current position] I find myself doing more and more that isn't really related to science."

Toward the end of the question-and-answer session, Simpson underscored his commitment to the importance of basic research in the Institute's mission. "Integrated research and basic research should go hand in hand and feed back on each other," he explained.

The candidate described his management style as "horizontal" in response to a question from Senior Investigator Darryl Zeldin, M.D. "I believe in reaching decisions by consensus," Simpson said. "Decisions should be made by consultation, not by fiat."

Because of the side effects of this global inhibition of the enzyme, Simpson early on concluded that "it would clearly be of benefit to specifically inhibit the pathway of aromatase expression within the breast." His attention then turned to determining the best candidate among tissue-specific aromatase promoters to target.

Simpson ultimately identified breast tumor-derived factors such as prostaglandin E-2 (PGE2) as strong stimulants of aromatase expression via an alternative promoter, promoter II. He targeted the orphan nuclear receptor known as liver receptor homolog-1 (LRH-1) and its co-regulators as key players in modulating aromatase expression in breast adipose tissue. His group concluded that mutation of the nuclear receptor site could completely abrogate the action of LRH-1 in activating promoter II expression at the transcriptional level and inhibit the subsequent expression of aromatase in the breast.

As the question-and-answer segment progressed, Simpson shared several humorous anecdotes about life down under with his audience. (Photo courtesy of Steve McCaw)

In his search for LHR-1-specific inhibitors, Simpson and his colleagues are conducting computerized screening of the crystal structure of LHR-1 against potentially useful drug-like molecules to determine which ones show potential for selective binding at promoter sites. As promising as his discoveries are thus far, the candidate cautioned his audience that "this is very much a work in progress." Several promising compounds, he noted, have failed to demonstrate the degree of specificity required.

Research Fellow Yukio Yamamoto, Ph.D., of the Laboratory of Reproductive and Developmental Toxicology listened with interest to Simpson's discussion of aromatase synthesis in breast adipose tissue. (Photo courtesy of Steve McCaw)

Wilson moderated the question-and-answer session as scientists in the audience quizzed Simpson about his reasons for wanting this job. (Photo courtesy of Steve McCaw)

Simpson was the second of three candidates invited to lecture and interview with interest groups at NIEHS during the final phase of the selection process for this key position. Like the other two candidates, his talk was hosted by NIEHS Deputy Director Sam Wilson, M.D. As expected, Simpson attracted a variety of individuals from throughout the Institute interested both in his research and in whatever they could learn of the candidate's management style and philosophy.

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Like many in the audience, Office of the Scientific Director Biologist Joel Abramowitz, Ph.D., appreciated the candidate's self-effacing sense of humor. (Photo courtesy of Steve McCaw)

Cell Biologist Delivers Final Lecture in Scientific Director Series

By Eddy Ball

A lecture by Rudy Juliano, Ph.D., on August 6 brought the NIEHS Scientific Director Lecture Series to a close. Juliano spoke on "Integrin-Mediated Control of Cell Signaling Events" to a near capacity audience in Rodbell Auditorium. Following the lecture, he fielded questions from the audience about his management philosophy and his vision for the future of the Division of Intramural Research.

The candidate is the Cary C. Boshamer Distinguished Professor and former chair of the Department of Pharmacology at the University of North Carolina (UNC) at Chapel Hill. Juliano also serves as principal investigator at the National Cancer Institute-funded Carolina Center of Cancer Nanotechnology Excellence, where he oversees six research projects related to the use of nanoparticles in cancer therapy and imaging. His lecture was hosted by NIEHS Deputy Director Samuel Wilson, M.D.

Although he would set up a lab at NIEHS to pursue his research interests, Juliano speculated that he would have to give up one of his two lines of research if he is selected as Scientific Director. (Photo courtesy of Steve McCaw)

Although Juliano has worked in cell biology since his postdoctoral fellowship at Roswell Park Memorial Institute, he reminded the audience that he earned his doctorate in biophysics — helping explain the distinctive mix of his research interests. Juliano described his two-fold research program at UNC as

"sort of schizophrenic," embracing as it does his lab's basic research into cell adhesion molecules and signal transduction and the more translational orientation of his work in the development of macromolecular therapeutics for delivery of drugs.

Juliano's cell biology research has focused on the biology, biochemistry and molecular biology of the integrin family of membrane receptors. Integrins are a large family of cell surface proteins that regulate key signaling pathways, particularly those involving enzymes in the group of mitogen-activated protein kinases.

Integrin-mediated signals help to control gene expression and can influence cell differentiation, progress through the cell cycle and apoptosis. "There have been a number of knockouts of the various integrin subunits," Juliano explained, "and these have led to insights showing that integrins play a very important role both during development and in maintenance of normal cell interactions."

According to Juliano, integrins perform two important functions. In a structural context, they form a "physical bridge" across cellular membranes. In terms of inter-cellular communication, they modulate a variety of signal transduction processes. Aberrations in these cell interactions are hallmark characteristics of the invasive and metastatic behavior of malignant cells.

From his overview of the roles of integrins, Juliano moved on to a discussion of recent research in his lab involving a novel large integrin-binding protein called Nischarin and a cell-proliferation protein known as deleted in liver cancer 1 (DLC-1), both of which are associated with changes in normal cell interactions and tumor growth. In rodent studies, when Nischarin, which means "slow moving" in Sanskrit, is over-expressed and bound with the alpha five subunit of integrin, the protein strongly "down-modulates" cell motility and disrupts

Juliano on Vision and Leadership

Like the previous candidates, Juliano spent fifteen minutes before the audience answering questions about the kind of leader he might be and his vision for the future of the Division of Intramural Research (DIR).

Wilson opened the session with a question about how the candidate sees the management and evolution of the DIR over the next five years. "I think one of the key roles of the Scientific Director is to be highly involved in implementing the aspects of the Strategic Plan that relate to the Intramural Research program," Juliano answered. "I think you have a very good guideline for where you want the Institute to go. I guess the question is how to maximize Intramural resources… [and] to make sure that very high quality science is maintained."

David A. Schwartz, M.D., posed questions related to training, the development of minority leadership and the candidate's reasons for wanting to take on the responsibilities of the position.

In his responses, Juliano pointed to his years of experience with postdocs in his labs and his understanding of the difficulties they face in making the transition from trainee to successful independence as a junior investigator. He also referred to his experiences at UNC recruiting and promoting minority and female scientists. For both groups, he stressed the importance of mentoring and role models.

The candidate provided a thoughtful answer to one of the important questions on everyone's mind — why he would exchange "the pretty pleasant life" of a senior professor at a major research university for the challenges of administering DIR. "The interesting thing for me," he answered, "would be to have the chance to essentially 'write on a larger slate' in terms of developing new initiatives and new programs and also in terms of managing programs that already have a good dynamic."

Juliano also referred to the potential for influencing the future of environmental science. "I think there are lots of opportunities here to bring new perspectives and new technologies to bear on programs in the Institute."

In terms of conflict resolution and management, Juliano emphasized that "someone in a senior administrative position needs to serve as an honest broker." Like a university department chair, the Scientific Director has to deal with "hot button issues" such as space allocation. "You want to come out of [these kinds of interactions]," he elaborated, "with people feeling, if not good, at least that they've been treated fairly."

"When you go to a larger scale [as I will if I move from department chair to Scientific Director]," he said, "you have to be able to delegate responsibility.… [But] I think you also need to keep your fingers on the pulse of what's going on."

normal cytoskeleton organization by inhibiting the activity of a class of enzymes known as PAK-kinases. In carcinogenesis, low Nischarin levels coincide with more vigorous tumor development.

For its part, DLC-1 has emerged as a candidate tumor-suppressor gene. “What has gotten cancer biologists excited about DLC-1,” Juliano observed, “is that when this molecule is over-expressed in a variety of carcinomas, there is a significant inhibition of tumor growth and proliferation of tumor cells.” Named for its absence in liver cancer, DLC-1 has also been observed as missing or inactive in many breast cancer lines.

This initial work by Juliano’s lab to increase understanding of the mechanisms of integrins and the proteins Nischarin and DLC-1 has turned up intriguing possibilities. The lab has been able to reverse the effects of Nischarin over-expression and DLC-1 deletion, suggesting that one day these molecules may prove to be useful targets in therapeutic interventions.

Wilson hosted the lectures and moderated question-and-answer sessions for each of the top three candidates chosen by the search committee. His questions to Juliano helped to ensure that each candidate had the opportunity to respond to a set of core questions about vision and leadership. (Photo courtesy of Steve McCaw)

Joyce Goldstein, Ph.D., pondered Juliano’s answers. Wilson asked the candidate about conflict resolution, a topic Goldstein herself had addressed at a previous lecture in the series. (Photo courtesy of Steve McCaw)

Colleagues Mike Resnick, Ph.D., left, and Ken Korach, Ph.D., shared their impressions of the candidate. Both are group leaders who could be affected by how skillfully the next Scientific Director serves as a broker among stakeholders. (Photo courtesy of Steve McCaw)

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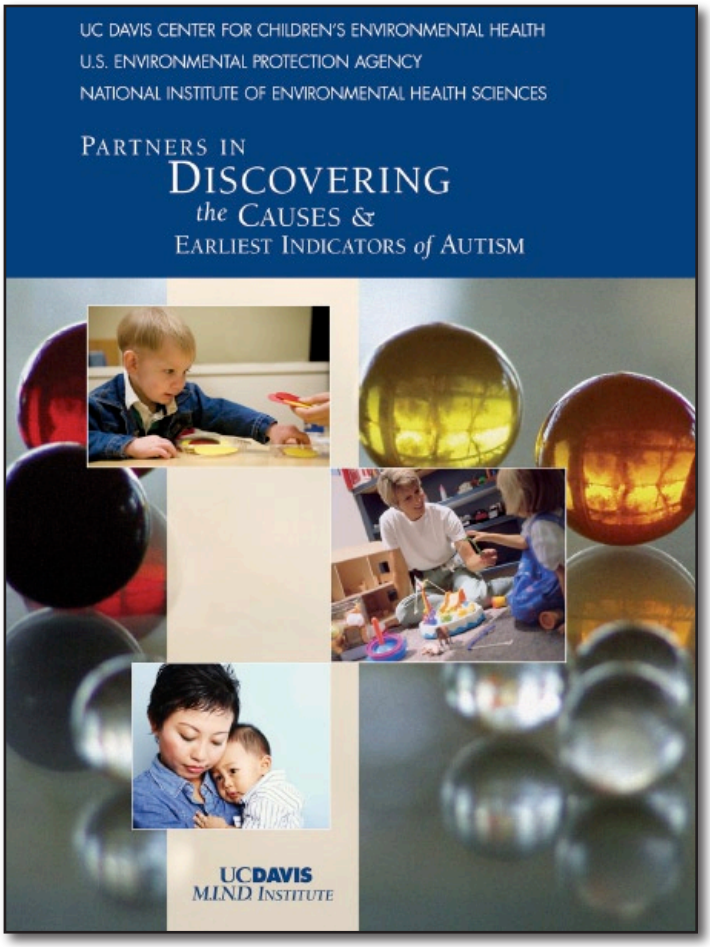
NIEHS and EPA Fund Innovative Autism Program

By Eddy Ball



A new five-year federal grant totaling $7.5 million will allow the University Of California – Davis Center For Children's Environmental Health to expand a pilot program that will become the first prospective study to begin the study of autism in the early gestation and infancy of at-risk children. The Environmental Protection Agency (EPA) and NIEHS, partners in the nation-wide Children's Environmental Health centers initiative, are jointly funding the program. The grant will be administered by Cindy Lawler, Ph.D., program administrator in the NIEHS Division of Extramural Research and Training.

The program, called Markers of Autism Risk in Babies—Learning Early Signs (MARBLES) is an extension of work underway as part of the Childhood Autism Risks from Genetics and the Environment (CHARGE). MARBLES will complement on-going CHARGE research into the influence of environmental factors and genetics and the interplay of the two in the development of autism.

MARBLES targets pregnant women who have at least one child with autism because they are at least ten times more likely to have another child with the disorder. The 20 participants currently enrolled in MARBLES undergo a battery of evaluations of their environmental exposures, genetics and immune systems during pregnancy, birth and nursing. Their children's development will be monitored closely until age 3.

"As comprehensive as CHARGE is," explained Principal Investigator Irva Hertz-Picciotto, Ph.D., "I realized the limitations of any study that begins looking for causes of autism after the diagnosis is made at age 2 or 3." According to Hertz-Picciotto, one outcome of the MARBLES study may be clarification of CHARGE data indicating that the immune systems of autistic children function differently than the immune systems of children developing normally.

Autism is a pervasive developmental disorder marked by poor verbal and communication skills, repetitive behaviors and an inability to form social connections. The condition has long been suspected of having its origins much earlier in a child's life — in early infancy, gestation and even prior to conception. Autistic children may seem normal during infancy and typically begin to show symptoms of the condition only after they reach age two to three.

"Autism is very complex. It is probably several disorders converging in a common diagnosis," said Isaac Pessah, Ph.D., who has led the CCEH since its establishment in 2001. "We actually don't anticipate finding just one factor that causes it, but will instead uncover patterns of susceptibilities and external influences that can lead to different forms of the disorder."

The program expects to increase participation and ultimately include 200 women who are pregnant or considering getting pregnant. MARBLES is currently recruiting women who live within a two-hour driving distance of Sacramento, meet the criteria of having at least one autistic child and are willing to make a four- to five-year commitment to the study.

"MARBLES is one component of a larger NIEHS and US EPA-supported Children's Center for Environmental Health and Disease Prevention located at the University of California at Davis," explained Program Administrator Cindy Lawler. "This Center supports an interdisciplinary team of scientists who are using many strategies to understand environmental contributors to autism, from studies in cells and animals, to epidemiologic and clinical investigations such as MARBLES."

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Committee Proposes Paradigm Shift in Toxicity Testing

By Eddy Ball

During a visit to RTP on July 31, Daniel Krewski, Ph.D., spoke to an audience of Environmental Protection Agency (EPA) and NTP/NIEHS scientists gathered at the EPA Conference Center. His topic was a report released on June 12 by the National Research Council (NRC) titled "Toxicity Testing in the Twenty-first Century." Krewski chaired the EPA-sponsored study committee that wrote the report.

Daniel Krewski, chairman of the Committee on Toxicity Testing and Assessment of Environmental Agents (Photo by Eddy Ball)

In his talk, Krewski described the report's recommendations as a "paradigm shift" in the way scientists, governments and the public should view toxicology — and a "far-reaching vision for the future of toxicity testing" worldwide that was three years in the making. "What we wanted to look at," he explained, "were totally different ways of approaching toxicity testing and pursuing efficiency gains not of five, ten or fifteen percent, but as much as 100, 500 or 5,000 percent."

Krewski has served as a consultant to EPA, NIEHS and several other scientific organizations, including more than 25 NRC committees. He is a professor of Epidemiology and Community Medicine and director of the R. Samuel McLaughlin Centre for Population Health Risk Assessment at the University of Ottawa. Krewski served as the facilitator for the consensus statement by the 21 North American and European Scientists who made up the Committee on Toxicity Testing and Assessment of Environmental Agents. The Executive Summary of their report is available as a PDF file and the full report can be read or purchased online from the National Academies Press.

As he concluded his presentation of the ambitious goals for toxicity testing over the next 20 years it will take to validate the tools needed to achieve the paradigm shift, Krewski underscored that "Effective communication of the vision is key to its success." The committee recommended several new directions for toxicology testing:

- **Significant increase in the nation's financial commitment** for developing alternative toxicity testing to address the backlog of over 80,000 chemicals now in use, a number that grows by approximately 2,000 new chemicals each year.

- **Increased use of *in vitro* studies using medium- and high-throughput assays** to replace in whole or part the use of animal testing, a radical change that some in the toxicology and regulatory communities, as well as in the private sector, may question.

- **Creation of a new institution** to foster the kind of cross-disciplinary research the vision demands — a move that could be perceived as threatening existing institutions and their spheres of influence and power.

During the question-and-answer session, the audience asked about some of the issues that might impact the success of the approach outlined in the report. These included concerns about the feasibility of using of cells derived from human tissue for *in vitro* testing, developing *in vitro* assays that would successfully replace complex animal response, such as occurs in reproductive and developmental toxicology testing, and the validation of links between perturbations in biological pathways and health outcomes. In a pointed comment on the phase out of animal testing called for by the report, one attendee joked, "You could end up trying to reconstruct an animal *in vitro* [when] it might just be easier to use an animal" in the first place.

On the day the report was released, Krewski met with top EPA officials and received what he characterized as "a very good reaction" from them. "They understood what we were proposing and understood that this is a long-term effort." He also made a presentation at the annual summer meeting of the Toxicology Forum in Aspen on July 11. Along with upcoming meetings in the United States during the fall, including ones with members of Congress, and publications about the report, Krewski is scheduled to make presentations for European officials in October and for scientists at the Tenth Annual Meeting of the Interagency Coordinating Committee on the Validation of Alternative Methods on February 5, 2008 in Washington, D.C.

Toxicity Testing Paradigms

The key difference in the existing toxicity testing paradigm and the new paradigm proposed by the Committee on Toxicity Testing and Assessment of Environmental Agents is in the choice of end-points — that is, what kinds of testing results may be used to justify classifying a chemical or compound as hazardous.

Existing Paradigm Endpoints: Currently, most toxicology studies rely on what are called apical endpoints determined by *in vivo* studies. The term "apical" is the adjective form of apex, referring to the end farthest away from the body; at or toward the tip. Apical endpoints are empirically verifiable outcomes of exposure, such as developmental anomalies, breeding behaviors, impaired reproduction, physical changes and alterations in the size and histopathology of organs, and, of course, death.

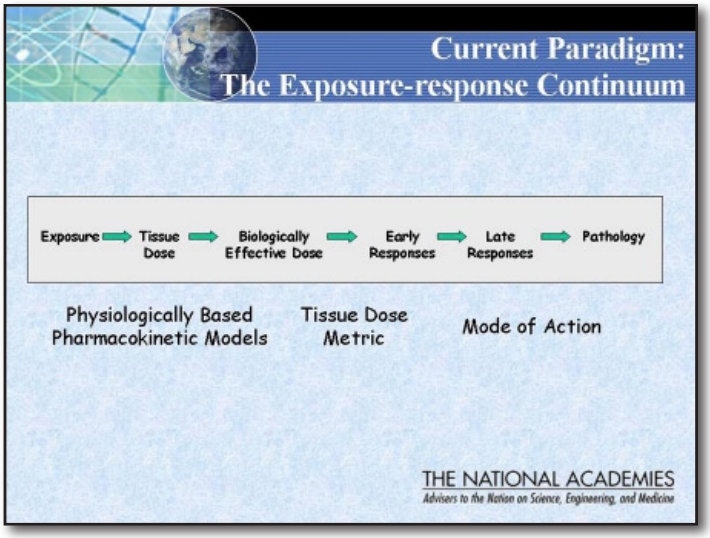
These gross changes observed *in vivo* can offer presumptive evidence of the toxicity of the chemical or compound under study.

Proposed Paradigm Endpoints: Medium- and high-throughput *in vitro* testing can detect what are described in the committee report as "biological perturbations" of a toxicology pathway. "The initial perturbations of cell-signaling motifs, genetic circuits, and cellular-response networks," the report explained, "are obligatory changes resulting from chemical exposure that might eventually result in disease."

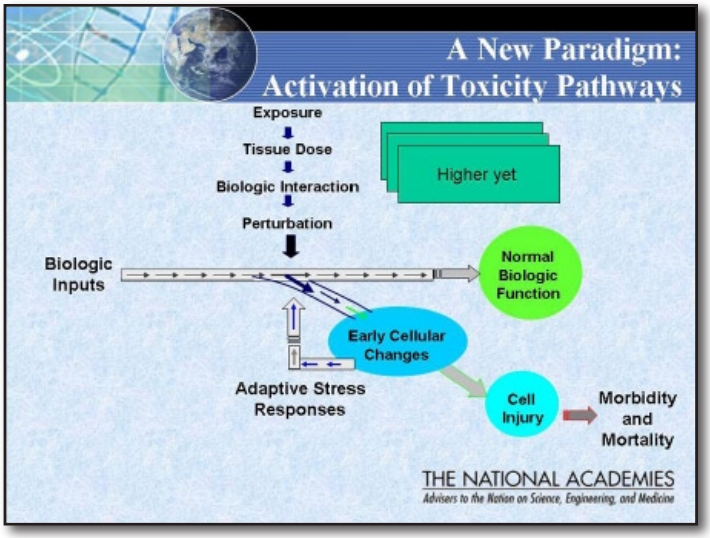
These subtle changes in metabolism occur much farther upstream than the observable and measurable physiological changes classified as apical endpoints. As such, their connection to adverse outcomes is not as immediately clear as the connections with apical endpoints.

If scientists can develop the paradigm proposed by the committee, their achievements will help overcome a limitation the toxicology community has itself recognized. As the *Toxicity Testing for Assessment of Environmental Agents: Interim Report* (2006) issued by the Board on Environmental Studies and Toxicology argued, "Apical tests provide little insight into the hundreds of molecular events, mechanisms, and targets responsible for toxicant action.… Future advances in testing will probably rely on our ability to discern the individual biologic underpinnings of toxicity, a complicated task in this setting."

Recognition of these limitations is the basis for the High-Through Put Screening initiative outlined in the NTP Vision and Roadmap and the rational for the EPA ToxCast™ Program.



The current protocol is considered by many as the "gold standard," of toxicology testing. According to the report, "the studies are expensive and time-consuming and can use large numbers of animals, so only a small proportion of chemicals have been evaluated with these methods." (Graphic courtesy of Daniel Krewski)



The report stated that "a critical feature of the committee's vision is the use of high-throughput methods that will allow economical screening of large numbers of chemicals in a short period." (Graphic courtesy of Daniel Krewski)

Options for Future Toxicity Testing Strategies

Option I In Vivo	Option II Tiered In Vivo	Option III In Vitro/In Vivo	Option IV In vitro
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

The committee envisioned phased implementation of its vision. Krewski conceded that toxicity testing may never completely achieve Option IV. (Graphic courtesy of Daniel Krewski)

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Science Notebook

Molecular Biologist Explores "Poised Polymerase" Phenomenon

By Eddy Ball

On August 7, a standing-room-only audience filled the NIEHS Executive Conference Room for the monthly meeting of the Receptor Mechanism Discussion Group. The featured speaker was Karen Adelman, Ph.D., who gave a talk titled "Poised Polymerases: Sitting in the Starting Gates and Ready to Respond." Adelman's talk was hosted by Ken Korach, Ph.D., senior research biologist in the NIEHS Laboratory of Reproductive and Developmental Toxicology.

Adelman spoke to an audience made up primarily of bench scientists during a lunch-time, brown-bag gathering of the NIEHS Receptor Mechanism Discussion Group. (Photo courtesy of Steve McCaw)

Adelman is a tenure-track investigator and head of the Transcriptional Responses to the Environment Group in the NIEHS Laboratory of Molecular Carcinogenesis (LMC). She is also the recipient of the 2006 NIEHS Early Career Award.

Adelman's research interests focus on the dynamic interplay between signals from the extracellular environment and transcription by RNA polymerase II [Pol II] in the minutes following an environmental stimulus. Pol II is the key enzyme responsible for transcription of protein-coding genes. Adelman's work has indicated that common mechanisms are involved in an organism's physiological response to many diverse stressors, such as heat shock, ultraviolet radiation, oxidative stress, toxins and carcinogens. By studying the way healthy cells deal with stress, Adelman hopes to discover what goes wrong with transcription profiles in disease states and after repeated environmental exposures.

Her investigations grew out of observations that Pol II in *Drosophila* behaved in manner counter to accepted theories about gene transcription. Rather than transcribing *Hsp70* heat shock genes immediately upon recruitment to the promoter, Pol II can begin transcription, but then become "stuck" near the promoter for up to ten minutes in a state called "proximal-promoter stalling." This stalled state precedes the transitions needed to allow the polymerase to function as a stable and productive elongation complex capable of penetrating chromatin barriers.

Conventional theory equated Pol II recruitment to a gene promoter, when it arrives at the "starting gates," with gene activation. "It had become dogma in the field," Adelman explained, "that if there was no polymerase brought to the promoter, the gene was off, and as soon as polymerase was brought to the promoter, the gene was on."

Using chromatin immuno-precipitation (ChIP) assays, Adelman's group set about trying to determine what other proteins were associated with Pol II at the stall site and might be responsible for proximal-promoter stalling. The most likely candidate, they found, was a protein complex known as negative elongation factor (NELF), which quickly disassociated from the Pol II when the transcriptionally stalled polymerase was released into the gene.

Performing array-based ChIP-chip (chromatin immuno-precipitation on a chip) studies, her genome-wide research turned up 1,000 other genes with stalled Pol II that are exceptions to that paradigm, suggesting that proximal stalling is far more widespread than previously suspected. In addition, Adelman "started to see a trend that the genes which are regulated by stalling were inducible by environmental or developmental stimuli," indicating that Pol II stalling may allow these genes to be "poised" for rapid regulation of gene expression

Adelman's lab is trying to understand those gene-environment interactions and the epigenetic changes that can generate altered patterns of gene expression. Among the handful of mammalian genes that have been shown to exhibit proximal-promoter stalling are the proto-oncogenes *c-Myc* and *c-FOS*, several genes regulated by estrogen receptor-alpha (*ER-α*) and the human immunodeficiency virus (*HIV*). "By studying this stalling process in *Drosophila* genes, we hope to get insight into some very important genes," she said.

According to Adelman, there is no shortage of rapid response genes in humans, and humans have a conserved form of the NELF complex, which includes the protein known as cofactor of BRCA1 (COBRA1). The group's goal is to continue investigating the role of Pol II stalling in the regulation of inducible genes, and to characterize the mechanisms of activity of a number of factors that function in both cellular survival pathways and the development of cancer.

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Duke Physician Discusses Gene-Environment Matrix in Stress Response

By Eddy Ball

NIEHS welcomed Duke University Professor Redford Williams, M.D., to its Frontiers of Environmental Sciences Lecture Series on August 10 in Rodbell Auditorium. Redford, who is the director of the Behavioral Medicine Research Center at Duke, spoke on "Stressful Social Environments and Genes: Effects on Mental and Physical Well-Being." His talk was hosted by Liam O'Fallon, a program analyst in the Division of Extramural Research and Training.

Williams concluded his lecture with an impassioned call for action. "We've got to do this research," he told the audience. (Photo courtesy of Steve McCaw)

A well-known authority on the interplay of genes, the social and physical environment, behavior and pathology who has written over 100 peer-reviewed studies, Williams served as a participant in an NIEHS-led trans-NIH program on health disparities. He has also received several other grants from NIH to pursue his research interests.

Williams opened his talk with an overview of the current disease-focused medical practice model and the changes in philosophy that need to be made in order to improve health care in the United States, which currently costs payers an estimated $1.5 trillion each year. Most of that amount is spent on what Williams called "the usual suspects" in a belated attempt to "find and fix" chronic diseases long after they have developed.

Among the psychological and social risk factors Williams has studied are hostility and anger, depression, childhood adversity, social isolation and low socioeconomic status. “These factors tend to cluster in the same individuals and groups, especially in people of low socioeconomic status,” he explained, “and when the psycho-social factors cluster, they have an enhanced effect.”

According to Williams, these factors can interact with particular genes to affect emotional, behavioral and physical health. In combination, genetic and environmental factors have a disproportionately greater effect on outcomes than they do individually. These interactions, some of them gender- or race-specific, can mean that individuals with different polymorphisms may react much differently to the same environmental stresses.

David A. Schwartz, M.D., asked the speaker to respond to social, ethical and legal implications of choosing the people best suited to be caregivers based on genotype. (Photo courtesy of Steve McCaw)

The combination of genotype and environmental stress can determine whether subjects develop emotional problems that in turn may accompany or trigger disease risk and pathology. Williams presented study results based on outcomes such as high blood pressure, elevated glucose, high C-reactive protein levels, major depression, anti-social behavior and recurrent cardiovascular events following myocardial infarction as a function of genotype and life experience.

Using a study on depression as an example, Williams explained, “If you don’t have the wrong genotype, stressful life events don’t affect your risk of depression. If you don’t have stressful life events, genotype doesn’t affect your risk of depression.”

Williams then turned to the translational aspects of his research, reviewing some of the promising research into the benefits of incorporating behavioral therapy with other treatment. He referred to studies of post-myocardial infarction patients who showed significant improvements in recurrent cardiovascular event rates and hospitalization rates after receiving behavioral and cognitive therapy interventions to reduce type A behavior, manage stress or control hostility along with usual care.

Director of the Office of Risk Assessment Chris Portier, Ph.D., wanted more details about the studies of polymorphisms in the gene encoding serotonin transport. (Photo courtesy of Steve McCaw)

Results of a study of incorporating structured coping-skills training into treatment of patients with chronic disease in Hawaii showed a 20% decline in the costs of primary care, a decrease in systolic blood pressure (SBP) reactivity and increases in emotional health indicators. Primary care costs for patients who did not

receive the training increased by 20%, and a group receiving individualized psychotherapy fared even worse. These control groups also showed worse outcomes in terms of SBP and emotional health indicators.

In contrast to the disease-oriented practice of medicine, Williams concluded, "we need to have a prospective evaluation that involves a health profile summary, health risk analysis, including genetic and lifestyle factors, and a five-year plan [of preventive interventions]. We can, instead of spending most of our healthcare dollars after disease has developed, spend them on prevention."

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The audience included a number of employees in the Division of Extramural Research and Training. Shown here are David Balshaw, Ph.D., left, and Beth Anderson. (Photo courtesy of Steve McCaw)

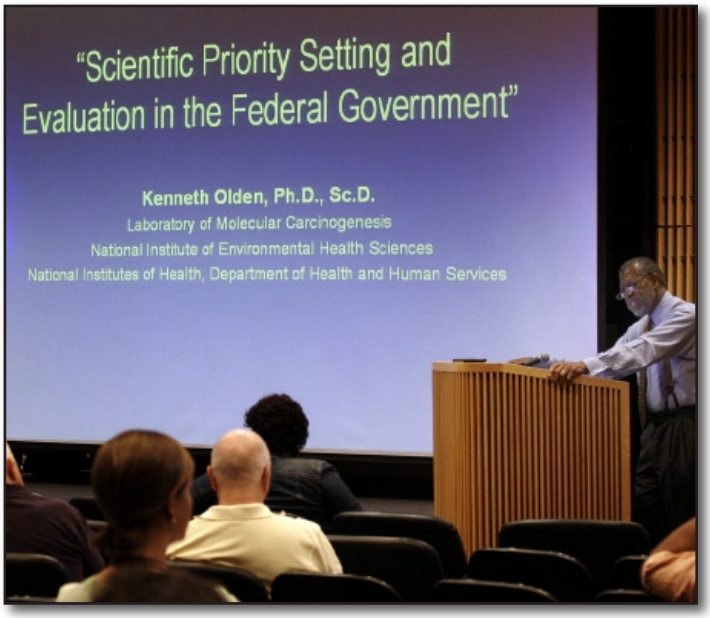
Olden Calls for Public Involvement in Science Policy

By Eddy Ball

The Frontiers in Environmental Sciences Lecture Series featured a talk by NIEHS Director Emeritus Ken Olden, Ph.D., on August 17 in Rodbell Auditorium. Olden argued for increased public involvement in a lecture titled "Science Policy Setting and Evaluation in the Federal Government." The host of the lecture was David A. Schwartz, M.D.

As Schwartz noted in his introduction, Olden was responsible for spearheading transformative changes at NIEHS during his tenure as director. "After coming to the NIEHS," Schwartz said, "he quickly reversed the status of NIEHS from being one of the lowest institutes in terms of percent increases [in budget] to one of the highest.… He also developed very substantial programs during his tenure as Institute director."

As head of the Institute during 14 years of unprecedented growth and program expansion, Olden spoke from first-hand knowledge about the lack of effective decision-making in science policy at NIH. "I'm going to propose that the President and



At the beginning of the lecture, Olden joked about his dark shirt and tie. As a principal investigator, the director emeritus is more often seen in casual attire. (Photo courtesy of Steven McCaw)

Congress should create a commission to develop a rational science policy for the nation." Without this central, integrated approach, he continued, "there cannot be interagency coordination."

Olden also pointed out that the nation currently does not have a consensus about what percentage of the gross domestic product should be allocated to scientific research, resulting in up-and-down cycles that make long-term planning almost impossible. "Stability and continuity are critical for intelligent planning," he said.

Looking back to 1945, Olden referred to the last example of truly integrated, long-term planning, a report to President Truman by Vannever Bush titled "The Endless Frontier." This document set scientific priorities emphasizing basic science that have not been fundamentally revisited for over sixty years. "Clearly, we need a new science policy" that addresses the country's current needs and offers the public a way to be involved in policy setting and program evaluation, he said.

In the 1945 report, Bush advocated letting scientists themselves set the priorities of science. However, according to Olden, in practice scientific leadership does not always rise to the occasion. He offered AIDS and environmental health as just two examples of the many scientific investments that came about because of public pressure to bring them to the forefront of scientific research — not because of advocacy by scientists.

Olden quoted a 1998 Institute of Medicine report on "Improving Priority Setting at the NIH," which emphasized the need for better collaboration with the American public. What we have today, Olden said, is a "closed system" in which scientists, operating as a classic "interest group," tend to support "the science that scientists want to do" and not necessarily "the science that the nation needs done."

"As a community of scientists, we need to turn the search light inward," Olden observed, "If we are willing to view our profession dispassionately, such an exercise can be liberating."

Schwartz praised Olden for implementing innovative environmental justice and community involvement programs, as well as for spearheading unprecedented growth in the Institute's budget. (Photo courtesy of Steve McCaw)

Lockhead-Martin Contractor Nick Staffa, Ph.D., seemed to ponder what a new kind of policy protocol could mean for NIEHS — and contractors who face cuts during lean budget years. Faced with a flat budget, many in the audience could empathize with Olden's call for "stability and continuity." (Photo courtesy of Steve McCaw)

In the end, the public must be involved in the search for an effective means for involving all the stakeholders to help ensure that "the power of ideas will set the terms of the debate." He concluded that "the objective of the NIH really centers around public health impact.… That's what we're here for." All of the other things we do, he argued, should further progress toward that objective and address the wider social and economic needs of the nation.

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NTP Scientist Bill Jameson, Ph.D., background, Pat Mastin, Ph.D., center, and Jerry Heindel, Ph.D., both of DERT, listened as Olden described his vision of a policy and evaluation mechanism with more public involvement. (Photo courtesy of Steve McCaw)

Mouse Haplotype Map Published

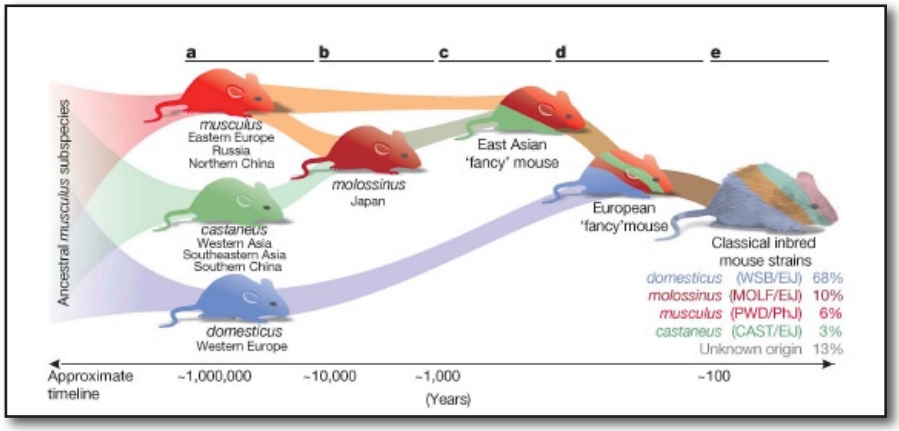
By Robin Mackar

Research on the DNA of 15 mouse strains commonly used in biomedical studies is expected to help scientists determine the genes related to susceptibility to environmental disease. The body of data is now publicly available in a catalog of genetic variants, which displays the data as a mouse haplotype map, a tool that separates chromosomes into many small segments, helping researchers find genes and genetic variations in mice that may affect health and disease. The haplotype map appearing online in the July 29th issue of *Nature* is the first published full descriptive analysis of the "Mouse Genome Resequencing and SNP Discovery Project" conducted by NIEHS.

The paper describes in detail the laborious and technology-driven approaches that were used to identify 8.27 million high quality SNPs distributed among the genomes of 15 mouse strains. Single Nucleotide Polymorphisms, or SNPs (known as snips), are single genetic changes, or variations, that can occur in a DNA sequence.

Much of the project was conducted through a contract between the National Toxicology Program at NIEHS and Perlegen Sciences, Inc. of Mountain View Calif.

"The database of mouse genetic variation should facilitate a wide range of important biological studies and help demonstrate the utility of this array technology approach," said David R. Cox, M.D., Ph.D., chief scientific officer at Perlegen Sciences, Inc.



Modern mouse strains evolved from a single ancestor approximately one million years ago. They are ancestors of mice inter-bred for coat color in Japan and Victorian England as "fancy" mice to serve as pets. In the 20th century, breeding programs in the United States produced the classical inbred mouse strains.

The Perlegen scientists used C57BL/6J, the first mouse strain to undergo DNA sequencing, as their standard reference to conduct the re-sequencing on the four wild-derived and eleven classical mouse strains. The technology used, the oligonucleotide array, was also used to discover common DNA variation in the human genome.

The arrays looked at about 1.49 billion bases (58 percent) of the 2.57 billion base pair of their standard reference strain. The data were then used to develop the haplotype map, which contains 40,898 segments.

"The NTP is looking forward to exploring the responses of these strains of mice to various environmental agents," said John Bucher, Ph.D., the new associate director of the NTP. The National Toxicology Program (NTP) is an interagency program, headquartered at NIEHS, with the mission to coordinate, conduct and communicate toxicological research across the U.S. government.

Frank M. Johnson, Ph. D., an NTP research geneticist and one of the authors of the *Nature* paper, adds that systematically characterizing even more mouse strains for susceptibility to toxins will not only help with genetic analysis, but better position researchers to do intervention studies.

The data are publicly available on the National Center for Biotechnology Information Web site at http://www.ncbi.nlm.nih.gov/SNP/ and at a Web site developed by Perlegen at http://mouse.perlegen.com which allows researchers to download SNPs, genotypes and LR-PCR primer pairs, which are currently mapped to NCBI Build 36.

In addition to the NTP and Perlegen Sciences researchers, other key collaborators on the project included scientists from the University of California - Los Angeles; University of California - San Diego; The Jackson Laboratory, Broad Institute of Harvard and MIT; and the Center for Human Genetic Research, Massachusetts General Hospital.

Citation: Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, Beilharz EJ, Gupta RV, Montgomery J, Morenzoni MM, Nilsen GB, Pethiyagoda CL, Stuve LL, Johnson FM, Daly MJ, Wade CM, Cox DR. 2007. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. Nature. (doi:10.1038/nature06067)

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Cancer Drug Researcher Is Upcoming Distinguished Lecturer

By Eddy Ball

On September 11, NIEHS will welcome Susan Cole, Ph.D., as the first speaker in the 2007-2008 Distinguished Lecture Series. Cole, who is the Bracken Chair in Genetics and Molecular Medicine at Ontario's Queen's University, will speak on "The Complex Role of GSH in the Function of the MRP1 Drug and Organic Anion Transporter." Her lecture will be hosted by David Miller, Ph.D., senior investigator in the Laboratory of Pharmacology and Chemistry.

Cole is a professor in the departments of Pathology and Molecular Medicine and Pharmacology and Toxicology and conducts research in her lab at the Cancer Research Institute at Queen's. She received her doctorate at the university and served a postdoctoral fellowship with NIH.

The central focus of Cole's lab is the investigation of mechanisms of resistance to natural product drugs that may be relevant to lung cancer and other human tumors. By using *in vitro* selected drug resistant lung cancer cell lines as model systems, she and her colleagues have identified two novel forms of multi-drug resistance to drug therapy among cancer patients.

Cole studies a protein known as Multidrug Resistant Protein (MRP1). MRP1 confers resistance by pumping drugs out of cancer cells. The protein is also found in normal cells where it can act as a barrier to protect tissues from drugs or environmental toxins.

Cole's lab has identified several types of drugs and environmental agents, including herbicides and tobacco related chemicals, that are transported by MRP1. She is interested in the molecular structure of MRP1 and the specific features of the protein that determine whether or not it will pump a particular drug or toxin. Cole is also investigating the way the protein and several of its most closely related homologs perform as efficient energy-dependent cellular efflux pumps of glutathione (GSH) and glucuronide conjugated xenobiotics (the so-called Phase III elimination step of drug metabolism).

The 2007-2008 NIEHS Distinguished Lecture Series continues in October with speakers scheduled each month from September 2007 to August 2008.

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Distinguished Lecturer Susan Cole. (Photo courtesy of Queen's University)

Extramural Update

Have you been asked to include your research protocol in ClinicalTrials.gov? Was your first response, "Not me, I'm not doing a clinical trial!" Because of new directions in environmental sciences research, when it comes to ClinicalTrials.gov, the answer may not be what you think.

What is ClinicalTrials.gov?

Established in 2000 by the National Library of Medicine under the Food and Drug Administration Modernization Act, ClinicalTrials.gov was designed originally to serve as a means for critically ill patients to find a trial in which they might participate. The ClinicalTrials.gov registry now includes information on federally and privately supported observational studies (examples: mechanisms of human disease, development of new technologies, epidemiologic and behavioral studies, or outcomes and health services research) and interventional clinical research in human volunteers. A clinical trial is a subset of clinical research. The only conditions required for inclusion are that the study must be approved by an institutional review board or an equivalent and that it must conform to the regulations of the appropriate health authority when applicable.

NIH Definitions

Clinical Research: (1) Patient-oriented research conducted with human subjects (or on material of human origin such as tissues, specimens and cognitive phenomena) for which an investigator (or colleague) directly interacts with human subjects. (2) Epidemiologic and behavioral studies. (3) Outcomes research and health services research.

Clinical Trial: A prospective biomedical or behavioral research study of human subjects that is designed to answer specific questions about biomedical or behavioral interventions (drugs, treatments, devices, or new ways of using known drugs, treatments, or devices).

NIH Mandate

Registries serve the ethical function of ensuring that the public has information about new, ongoing and previously conducted clinical research. They also serve as a scientific resource on all clinical research and published results. NIH has mandated that all research using human subjects be included in ClinicalTrials.gov.

Why me?

If you are interacting with human subjects or you have identifiable information on human tissue, your study qualifies as clinical research and should be included in ClinicalTrials.gov. Some journals require that a clinical trial be included in a registry before enrolling the first patient, in order for papers from that trial to be included in the journal. The International Committee of Medical Journal Editors issued a joint statement in September 2004 promoting registration of all clinical trials [N Eng J Med. 2004, 351:1240-1251]. ClinicalTrials.gov is an approved registry.

How do I include my NIEHS-supported study in ClinicalTrials.gov?

To include your NIEHS-supported study in ClinicalTrials.gov, contact Martha Barnes.

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Extramural Papers of the Month

By Jerry Phelps

Parkinson-like Degenerative Changes Linked to Reduced Dopamine Storage

Mice with a decreased ability to package and store dopamine undergo a degenerative process that mimics Parkinson's disease, report NIEHS-supported neuroscientists at Emory University and The Parkinson's Institute. Mice genetically altered to produce only five percent of the normal levels of a protein called vesicular monoamine transporter 2 (VMAT2) were used in the experiment. VMAT2 is responsible for packaging dopamine for future release by neurons.

Lack of the neurotransmitter dopamine is responsible for many of the symptoms related to Parkinson's disease, a progressive neurodegenerative disease that strikes Americans at the rate of about 20/100,000 per year. Estimates of the current number of Parkinson's cases in the U.S. vary between 300,000 and 750,000. Current treatments include administration of the dopamine precursor, L-dopa.

The mice, known as VMAT2 LO, were carefully bred so that they were only deficient in the VMAT2 gene. Previous research found that this mouse strain included a chromosomal deletion spanning the α-synuclein gene locus. The mice used in the current study were screened to verify the presence of α-synuclein. This study represents the first data on VMAT2 LO mice with normal α-synuclein expression. The investigators will continue this line of research using these mice to test compounds that can possibly slow the course of the Parkinson symptoms.

Citation: Caudle WM, Richardson JR, Wang MZ, Taylor TN, Gullet TS, McCormack AL, Colebrook RE, Di Monte DA, Meson PC, Miller GW. 2007. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J Neurosci 27(30):8138-8148.

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Sunlight in Youth — Protective for MS?

Members of the Southern California Environmental Health Sciences Center funded by NIEHS report in the July edition of the journal *Neurology* evidence from a study of 79 pairs of identical twins that exposure to sunlight through various activities at an early age may be protective against developing multiple sclerosis (MS).

The study assessed sun exposure of 79 pairs of identical twins in the US and Canada. One twin in each pair had been diagnosed with MS. Study participants were questioned about a variety of exposures including the amount of time spent tanning, going to the beach and playing outdoor sports during childhood. The researchers found that the twin with MS usually had been exposed to less sun overall as a child than the twin without the disease. However, the protective effect was only found in female twins. According to the researchers, the lack of a protective effect in males may be due to a relatively small number of male twins in their study.

This study adds to the growing body of evidence that sunlight acting directly through an unknown mechanism or indirectly through stimulation of vitamin D production plays a role in preventing the development of multiple sclerosis as an adult.

Citation: Islam T, Gauderman WJ, Cozen W, Mack TM. 2007. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. Neurology 69(4):381-388.

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IL-6 and Gender Differences in Liver Cancer Rates

A study in mice carried out by NIEHS grantees at the University of California San Diego may shed light on why the most common form of liver cancer strikes men with three to five times the frequency as women. Hepatocellular carcinoma arises from a variety of causes including viral hepatitis infection, chronic alcoholism and exposure to aflatoxin or a combination of these factors.

The research team treated mice with the potent liver carcinogen diethyl nitrosamine. All of the male mice — but only 10-20 percent of the female mice — developed liver tumors. Further investigation showed that the male mice produced much more of the inflammatory protein interleukin-6 (IL6) than the females. When IL-6 was eliminated in the male mice, the liver cancer rate dropped by about 90 percent bringing it in line with the rate in the female mice. Treating the male mice with estrogen also lowered IL-6 production and reduced liver disease to the same level as the female mice.

The researchers postulate that similar mechanisms may be responsible for the different rates of liver cancer in men and women and suggest potential interventions for humans as well. This discovery may have implications for bladder cancer, which also occurs more frequently in men.

Citation: Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. 2007. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317(5834):121-124.

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Poor Diet Affects Respiratory Health of Teenagers

New epidemiologic research on teenagers in North America shows that a diet poor in essential vitamins, minerals and other antioxidant compounds is linked to increased risk for developing respiratory conditions — especially among smokers. The results suggest that higher dietary intake of antioxidant and anti-inflammatory micronutrients, such as vitamins A, C, and E and omega-3 fatty acids, such as those found in fish and algae, is linked to lower reports of cough and respiratory infections and less-severe asthma symptoms.

Lung growth and development parallels growth in physical stature; therefore the study subjects in late adolescence were near their peak of lung function. Analysis of questionnaires showed that 33 percent of the study subjects' diets were below USDA recommendations. One-third of the teenagers were overweight, another contributing factor for asthma, 72 percent did not take multivitamins, and 25 percent smoked.

This study adds to the body of knowledge that a healthy diet high in antioxidants is important for proper lung growth and development to reduce the risk of asthma as well as improve the general health of teens. The researchers conclude that snacks of fresh fruit and a simple nutritious family meal would be easy ways to help teens consume the proper amounts of essential nutrient.

Citation: Burns JS, Dockery DW, Neas LM, Schwartz J, Coull BA, Raizenne M, Speizer FE. 2007. Low dietary nutrient intakes and respiratory health in adolescents. Chest 132(1):238-245.

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Intramural Papers of the Month

By Eddy Ball

Acquired Tolerance to Inorganic Arsenic

In an NIEHS-funded study published in *Toxicological Sciences*, scientists from NIEHS and Dartmouth College reported novel insights into the mechanisms that convey acquired tolerance to inorganic arsenic in killifish — a model species with renal xenobiotic transport mechanisms similar to those found in higher vertebrates including man.

Killifish were collected from a creek near Bar Harbor, Maine, and kept in aquaria at the Mount Desert Island Biological Laboratory where they were exposed to sub-lethal levels of arsenic. Controls were kept in seawater alone. The researchers measured the effects of arsenic exposure on renal tubular expression (mRNA and protein) and transport function of multidrug resistance-associated protein 2 (Mrp2). They also measured tissue arsenic levels and evaluated mitochondrial function in tubules as an indicator of toxicity.

Although arsenic stimulation of MRP2 expression in liver and renal cell lines has been described previously, this study was the first to investigate the effects on MRP2 expression and function *in vivo* in intact renal proximal tubes at environmentally relevant levels of exposure. The researchers found an increase in MRP2 abundance and transport activity likely due to posttranslational processes. They proposed that up-regulation of MRP2 expression and activity was partly responsible for the tolerance to acute arsenic toxicity the fish developed. "These data have important implications for effects of arsenic on xenobiotic excretion and bioavailability," they concluded.

Citation: Miller DS, Shaw JR, Stanton CR, Barnaby R, Karlson KH, Hamilton JW, Stanton BA. 2007. MRP2 and acquired tolerance to inorganic arsenic in the kidney of killifish (Fundulus heteroclitus). Toxicol Sci 97(1):103-110.

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Chronic Bronchitis and Non-Smoking Farm Women

Using data from the Agricultural Health Study, a large, prospective study of Iowa and North Carolina pesticide applicators and their spouses, researchers found an association between agricultural risk factors and chronic bronchitis among non-smoking farm women. The study, funded jointly by NIEHS and the National Cancer Institute, was published in the *Journal of Occupational and Environmental Medicine*.

Researchers examined enrollment data provided by 21,541 non-smoking female spouses. The researchers analyzed current farm activities, lifetime non-farm job history and lifetime pesticide history. Not only was chronic bronchitis associated with common farm exposures (manure, driving combines, and organic dusts), but more importantly, the study was the first to demonstrate an association with pesticides. The team found at least a 50% increase in chronic bronchitis associated with the pesticides dicholorvos, DDT, cyanazine, paraquat and methyl bromide.

The cross-sectional analysis was the first to investigate pesticide use and chronic bronchitis in farm women. It was one of the only studies with sufficient power to study non-smokers alone and the largest such study to date. The research team concluded, "Our results suggest that farm women have similar risk factors as men and that some specific pesticides may also be associated with chronic bronchitis."

Citation: Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP, Hoppin JA. 2007. Chronic bronchitis among nonsmoking farm women in the agricultural health study. J Occup Environ Med 49(5):574-583.

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Novel Genes and Pathways in Cadmium Toxicity

In an NIEHS/NTP-funded study of gene expression following exposure of *C. elegans* to cadmium, a team of scientists reported the discovery of novel genes and functional protein interacting networks associated with cadmium exposure. The results were published in *Genome Biology* by the research team of scientists from NIEHS, the National Heart, Lung and Blood Institute and Duke University.

The researchers used whole genome *C. elegans* DNA microarrays to monitor global changes in the nematode transcription following cadmium exposure at 4 and 24 hours. They mapped a total of 290 genes that were up-regulated and down-regulated after exposure and calculated fold changes in expression of each. They performed functional analysis of cadmium-responsive genes using RNA interference to investigate the biological consequences of changes in gene expression.

This study is important for its identification of gene families that have not been well-characterized in regard to cadmium exposure using alternative toxicological testing methods and protein-interaction analysis.

The researchers also identified six novel pathways, one of which they studied in detail. "Because more than 60% of *C. elegans* genes and many signaling pathways are evolutionarily conserved," they concluded, "these results contribute to understanding of functional roles of various genes in cadmium related diseases in humans."

Citation: Cui Y, McBride SJ, Boyd WA, Alper S, Freedman JH. 2007. Toxicogenomic analysis of Caenorhabditis elegans reveals novel genes and pathways involved in the resistance to cadmium toxicity. Genome Biol 8(6):R122 [Epub ahead of print (DOI:10.1186/gb-2007/8/6/R122)].

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DNA Polymerase Epsilon and Leading-Strand DNA Replication

Researchers from the NIEHS Laboratory of Molecular Genetics and Laboratory of Structural Biology, in collaboration with scientists from Sweden's Umeå University, have reported findings that add significantly to understanding the roles of polymerase proteins in the process of DNA replication. In a careful examination of replication error patterns, published in the journal *Science*, the researchers presented a series of observations to support the inference that yeast polymerase epsilon (pol ε) participates preferentially in leading-strand DNA replication.

The researchers reached that conclusion after conducting experiments to create mistakes in replication in the yeast *Saccharomyces cerevisiae* using a strain containing a mutation in *POL2*, the gene that transcribes pol ε. The resulting mutant DNA polymerase, known as M644G pol ε, offered insight into the process due to its ability to retain replication activity and grow at rate similar to wild-type, while generating a distinct mutational signature *in vivo*. They observed the mutants's behavior in replicative mutagenesis triggered at different base pair locations and orientations.

This research is especially important because it provides evidence about the identity of the polymerase(s) that replicates the leading strand during the chain elongation phase of replication, something which had remained unclear for over fifty years.

Citation: Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. 2007. Yeast DNA polymerase epsilon participates in leading-strand DNA replication. Science 317(5834):127-130.

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