National Institute of Environmental Health Sciences

Environmental Factor

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NIEHS Spotlight

Birnbaum presents plenary at meeting of Canadian toxicology group

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EPA releases chemical screening data and launches challenges

Researchers gained access to more chemical screening data with the U.S. Environmental Protection Agency release of new information Dec. 17 on 1,800 chemicals.

NTP shares new toxicology approaches with international colleagues Video

Associate Director John Bucher, Ph.D., delivered the keynote lecture at the Karolinska Institutet Nov. 20, on new approaches in toxicology research and testing.

Validating civic perspective, grassroots resources for environmental science Audio

Research by members of the public can meet the rigors of science and help advance environmental health efforts, according to Sara Wylie, Ph.D., of Northeastern University.

Science Notebook

2013 papers of the year

From the more than 2,800 NIEHS-funded studies published in 2013, leaders of the Institute's three research divisions selected 30 for special recognition as Papers of the Year.

Lewis Cantley discusses cancer metabolism in Rodbell Lecture

The Dr. Martin Rodbell Lecture Series Seminar Dec. 10 featured Lewis Cantley, Ph.D., discussing the role of phosphoinositide 3-kinase signaling in cancer progression.

Seminar showcases Toxicogenetics Challenge winners

A panel of experts peer-reviewed the two most recent draft NTP Report on Carcinogens documents, known as monographs, at a public meeting Dec. 12-13 at NIEHS.

NTP panel peer reviews substances for potential listing as carcinogens

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Preterm birth linked with maternal phthalate exposure during pregnancy

A new study by Superfund researchers found the women with the highest levels of phthalate exposure during pregnancy had up to five times the odds of preterm birth.

NIEHS Spotlight

Newly dedicated facility to house NIEHS-funded labs Video

Officials at the University of North Carolina, Wilmington cut the ribbon Nov. 18 for a new facility that will serve NIEHS-funded labs on the N.C. coast.

Autism studies build on past investments and guide future research

Cindy Lawler, Ph.D., discusses three new reports on autism illustrating the diverse nature of current research and its influence on future directions.

New evidence of gene–environment interaction in autism Video

Children possessing a specific genetic risk factor appear more likely to develop autism spectrum disorder when prenatal exposure to air pollution occurs.

Parents are right: autistic children experience more GI symptoms Video

Young children with autism spectrum disorder and developmental delays are at least three times more likely to experience frequent gastrointestinal problems.

Autism in Minneapolis higher among Somalis and whites than other groups

Report finds autism more prevalent among Somali and white children in Minneapolis.

Science Notebook

LSB speaker discusses the role of mobile DNA elements in nature

Understanding the significance of mobile DNA elements is important because half of human genome is derived from them, according to Phoebe Rice, Ph.D.

Researchers' work translated into positive results for public health

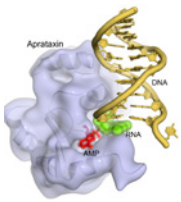
The NIEHS-funded study of 6- to 8-year-old girls showed that the duration of being breast fed is associated with higher levels of certain polyfluoroalkyl chemicals.

New study finds toenails help trace arsenic exposure from foods Audio

Diet alone can be a significant source of arsenic exposure regardless of arsenic concentrations in drinking and cooking water, according to a new study.

Researchers find elevated flame retardant exposure among U.S. gymnasts

Gymnasts may be ingesting or inhaling dust created by foam blocks that contain hormone-disrupting flame retardant chemicals, according to a new study.



NIEHS study identifies protein involved in DNA damage removal

The discovery may help uncover some of the genetic mishaps that contribute to neurodegenerative diseases, as well as point to new therapies for cancer patients.

NIEHS Spotlight

Latoni tapped to head Scientific Review Branch

NIEHS welcomed its new Scientific Review Branch chief Nov. 18, as Alfonso Latoni, Ph.D., joined the leadership of the Division of Extramural Research and Training.

Students in NIEHS program WOW! supporters with interactive presentations

NIEHS scientists reaped the rewards of teaching and service as they participated in the Citizen Schools WOW! Event Dec. 12 at Lowe's Grove Middle School in Durham, N.C.

Duke brings Superfund research to the Elizabeth River Learning Barge

The end of 2013 marks the first full semester of Duke University Superfund Research Program field work and outreach on the Elizabeth River Project Learning Barge.

Industry-academic collaboration streamlines decades of data

Scientists from Pfizer and North Carolina State University culled more than 88,000 studies for information about toxicity for some 1,200 drugs.

Science Notebook

NIEHS research helps unlock one of the secrets of stem cells

A new study by NIEHS researchers helps to explain the molecular basis of self-renewal and differentiation in embryonic stem cells.

NTP seminar explores functional ecology

An ongoing lecture series sponsored by NTP made an unexpected turn into animal ecology Dec. 10 with a talk at NIEHS by zoologist Vladimir Vershinin, D.Sc.

This month in EHP

The January issue of Environmental Health Perspectives highlights the role of environmental factors in myopia and the unique qualities of wind turbine noise.

Extramural Research

Extramural papers of the month

- Epigenetic changes associated with pancreatic cancer might lead to early detection
- Phthalate exposure linked to preterm birth
- A 3-D map of chromatin interactions
- Girls are reaching puberty earlier

Intramural Research

Intramural papers of the month

- tssRNAs associated with paused Poll II serve as scaffold for transcription factors
- Asthmatic reaction is dependent on dose and timing of endotoxins
- Modest changes in dNTP levels affect cell's ability to repair mutations
- Distinct features of RNA binding protein make it unique among its family

Inside the Institute

Employees make the 2013 Giving Tree a success

This year's Giving Tree allowed 160 area children to have a brighter holiday.

NIEHS scientists join other volunteers to teach children about nutrition

Thanks to volunteers from NIEHS and public service groups, six children in a Durham, N.C. program learned important lessons about healthy lifestyle and diet.

Calendar of Upcoming Events

- **Jan. 7**, in the Executive Conference Room, 12:00-1:00 p.m. — Receptor Mechanisms Discussion Group seminar on "The Role of Estrogen Receptor Signaling in Endometriosis," by Katherine Burns, Ph.D.
- **Jan. 10**, in Rodbell B, 2:30-3:30 p.m. — Exposome Faculty Meeting
- **Jan. 13**, in Rodbell A, 9:00-10:00 a.m. — Keystone Science Lecture Seminar Series presentation on "Uncovering Early Life Exposure to Chemical Mixtures," by Manish Arora, Ph.D.
- **Jan. 17**, in Rodbell A, 11:00 a.m.-12:00 p.m. — Laboratory of Reproductive and Developmental Toxicology Seminar Series, featuring David McClay, Ph.D., exploring "A Systems and Cellular Analysis of Primordial Germ Cell Homing to the Gonad"
- **Jan. 24**, in Rodbell Auditorium, 10:30 a.m.-12:00 p.m. — Distinguished Lecture Series with Joseph DeSimone, Ph.D., "Exploiting the Manufacturing Tools of the Microelectronics Industry to Make Precise Organic Particles for Therpeutics and Vaccines"
- **Jan. 30**, in Rodbell A, 11:00 a.m.-12:00 p.m. — Seminar featuring Sadis Matalon, Ph.D., exploring "Mechanisms and Modification of Halogen-induced Bronchial Hyperreactivity"
- View More Events: NIEHS Public Calendar

NIEHS Spotlight

Birnbaum presents plenary at meeting of Canadian toxicology group

By Eddy Ball

NIEHS and NTP Director Linda Birnbaum, Ph.D., added an interesting twist on the famous quote from Paracelsus during her plenary talk Dec. 4 in Ottawa, "Does dose make the poison? A current assessment of nonmonotonicity."

Addressing the Society of Toxicology of Canada (STC) 45th Annual Symposium on "Mechanistic Paradigms for Toxicological Regulation," Birnbaum, who was joined by an NTP toxicologist and two NIEHS grantees at the meeting, discussed an emerging paradigm of dose response.

The quote from Paracelsus (1493-1541), who is acknowledged as the father of toxicology, points to the traditional notion of dose response — that increasing dose increases toxicity in a monotonic pattern. "All things are poison," Paracelsus is quoted as saying, "and nothing is without poison; only the dose permits something not to be poisonous."

Increasingly, toxicologists in the field of environmental health science, such as Birnbaum, are recognizing that for some compounds, especially hormones and hormone-like chemicals, the reverse may hold true. In these cases, a much smaller dose may have a disproportionate impact on toxicity, while greater doses may actually blunt effects through several antagonistic mechanisms, including the saturation of receptors.

"The question is no longer whether nonmonotonic dose responses are 'real' and occur frequently enough to be a concern," Birnbaum told her audience. "Clearly these are common phenomena with well-understood mechanisms. Instead, the question is which dose–response shapes should be expected for specific environmental chemicals and under what specific circumstances."

Does dose make the poison?

Birnbaum built the case for her central premise, with examples of essential nutrients, which exercise their beneficial effects at low doses, and hormones, specifically prolactin, which exerts both stimulatory and inhibitory effects upon testicular steroidogenesis. She also outlined

Supporting the goals of our colleagues to the north

Joining Birnbaum at the meeting were NTP toxicologist Scott Auerbach, Ph.D., and NIEHS grantees Dean Jones, Ph.D., of Emory University, and Tom Gasiewicz, Ph.D., of the University of Rochester, who is also a member of the National Advisory Environmental Health Sciences Council. Their talks were prominent, among the 11 presentations during the symposium's three themed sessions:

- Auerbach explored "Characterization and application of toxicogenomic perturbation space," and Jones argued for new directions in toxicology with his talk, "Sequencing the human exposome: a call to action." Their talks were part of session two, "Genome, Epigenome, and Exposome: Future Roles in Carcinogenesis and Toxicity."

- Gasiewicz discussed "The Ah receptor in hematopoietic stem cells (HSCs): regulation of signaling pathways associated with HSC function," as part of session three, "Molecular Toxicology: Mapping the Pathways."

One member of the STC board of directors, incoming president David Josephy, Ph.D., had a special reason to appreciate the contributions of NIEHS scientists and grantees at the symposium. An award-winning molecular toxicologist at the University of Guelph since 1983, Josephy completed a postdoctoral fellowship at NIEHS in 1982.

eleven mechanisms for nonmonotonic dose response, including tissue-specific shut-off response, biochemical modification of receptors, and endocrine feedback loops.

This complicated and somewhat counter-intuitive concept, Birnbaum noted, has gained increasing acceptance among scientists and regulators. She pointed to a growing body of literature and statements in 2012 and 2013 by The Endocrine Society, U.S. Environmental Protection Agency (EPA), and European Commission Office of the Chief Scientific Advisor. She also referred to an NTP review in 2001 of literature on endocrine disrupting compounds (EDCs).

Birnbaum, right, enjoyed an opportunity to talk with STC President Louise Winn, Ph.D., of Queen's University, about environmental health concerns common to people in the U.S. and Canada. Birnbaum's elegant argument for expanding perspective on dose response set the stage for the first session of the meeting, which examined future directions in the areas of carcinogenesis and toxicity. (Photo courtesy of David Josephy)

A central issue in environmental health science, Birnbaum continued, is the low-dose effect of exposure to endocrine disruptors, which the World Health Organization defined in 2002 as exogenous, or external, substances or mixtures that alter the function of the endocrine system. Disruptors include the obvious players — natural and synthetic hormones — but also plasticizing compounds, fire-retardant chemicals, and some pesticides.

Symposium program chair Jayadev Raju, Ph.D., left, of Health Canada, and Jones shared ideas just before the proceedings commenced. (Photo courtesy of David Josephy)

A new conversation about endocrine disruption

EDCs can have immediate effects, but their most harmful impacts are often masked or delayed — with exposures during sensitive developmental windows triggering alterations that cause disease in later life, especially in vulnerable individuals and sensitive subpopulations. "Low-dose exposures that seem insignificant may have biological meaning if persistent, bioaccumulative, and/or if exposure is continuous or repetitive," Birnbaum said in her conclusion. "It is not only the dose that makes the poison, but also the timing [of the exposure]."

Referring again to the EPA statement on nonmonotonic dose response earlier this year, Birnbaum reinforced her call for action. "It is time to start the conversation between environmental health scientists, toxicologists, and risk assessors," she said, "to determine how our understanding of low-dose effects and nonmonotonic dose responses influence the way risk assessments are performed for chemicals with endocrine-disrupting activities."

During the question-and-answer portion of her presentation, Birnbaum fielded a range of questions about high-throughput screening of potentially bad actors; replacement chemicals, such as bisphenol S; and applications, as well as implications, of the precautionary principle.

Auerbach is a molecular toxicologist in the NTP Biomolecular Screening Branch. His primary role is the analysis and interpretation of multivariate data sets. (Photo courtesy of David Josephy)

Gasiewicz discussed a transcription factor that has a critical role in the body's response to toxic exposures. (Photo courtesy of David Josephy)

Josephy described the question-and-answer session following Birnbaum's talk as lively and stimulating. "We need to consider risk-benefit issues," Birnbaum said, in response to one question. "Do we really need particular compounds [that may pose a risk to health] for particular purposes?" (Photo courtesy of the University of Guelph)

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EPA releases chemical screening data and launches challenges

Researchers gained access to more chemical screening data with the U.S. Environmental Protection Agency (EPA) release of new information Dec. 17 on 1,800 chemicals found in industrial and consumer products, food additives, and drugs.

The data were gathered through advanced techniques, including robotics and high-throughput screening, as part of an ongoing federal collaboration to improve chemical screening. The collaboration, Tox21, includes EPA, NTP, the National Center for Advancing Translational Sciences, and the U.S. Food and Drug Administration.

"Making these data publicly available will help researchers across disciplines to better identify hazardous chemicals," said Raymond Tice, Ph.D., who heads the NTP Biomolecular Screening Branch, in the EPA press release. "We are pleased to be a partner in these collaborative efforts and look forward to further enhancing the amount of Tox21 data available to the public."

The chemical screening data is accessible on EPA's new interactive Chemical Safety for Sustainability or iCSS Dashboard.

That same day, EPA announced a series of challenges inviting the science and technology community to work with the data and provide solutions for how the new chemical screening data can be used to predict the lowest dose that shows adverse effects in animals. Challenge winners will receive awards for their innovative research ideas.

"Today's release marks an important milestone in communicating and improving our understanding of the impact chemicals have on human health and the environment," said Lek Kadeli, acting assistant administrator for EPA's Office of Research and Development.

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NTP shares new toxicology approaches with international colleagues

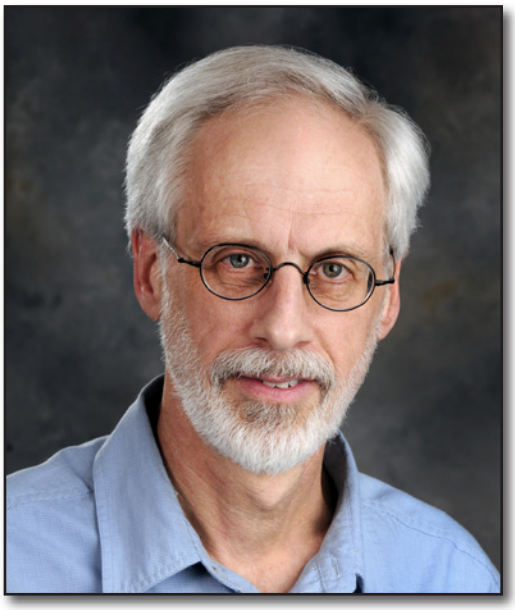
By Robin Mackar

It's not every day scientists get invited to give a presentation in the same place where the Nobel Prize in Physiology or Medicine has been chosen since 1901, but three NTP members did exactly that this fall at the Advancing Risk Assessment of Environmental Agents Conference.

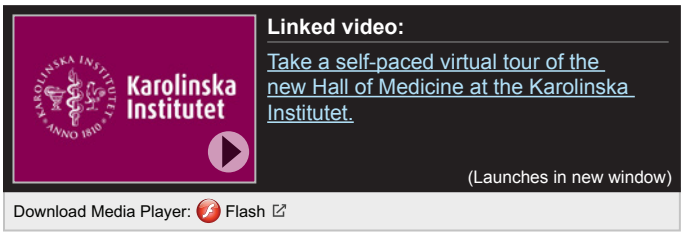
At the request of the Karolinska Institutet in Sweden, NTP Associate Director John Bucher, Ph.D., delivered the keynote lecture Nov. 20 on new approaches in toxicology research and testing and how they are being applied to advance the health risk assessment process in the U.S.

Bucher's talk nicely set the tone for the next session led by two other NTP invited guests, Kristina Thayer, Ph.D., director of the NTP Office of Health Assessment and Translation (OHAT), and Andrew Rooney, Ph.D., OHAT deputy director, who discussed the NTP framework for systematic review.

All of the talks were given in the new state-of-the-art lecture hall, Aula Medica, which, as of Dec. 7, became the venue for scientific presentations by all Nobel laureates in physiology or medicine, before receiving their medals from the King of Sweden. "A very impressive, if not intimidating, environment to give a science talk in," Bucher noted upon his return.



Under Bucher's leadership, NTP has forged several new partnerships, internationally and domestically, including the Tox21 interagency consortium. (Photo courtesy of Steve McCaw)



Advancing risk assessment

The two day conference, "Advancing Risk Assessment of Environmental Agents," was hosted by the Institute of Environmental Medicine (Institutet för Miljömedicin or IMM), an interdisciplinary research department at the Karolinska Institutet. The IMM invited leading researchers from the U.S., Italy, Holland, and Sweden, to discuss how to take research findings and modern technologies and apply them to advance the health risk assessment process.

Anders Ahlbom, Ph.D., director and chair of IMM, kicked off the meeting by welcoming the international speakers, presenting them with a brief overview of health risk assessment at IMM, and laying out some of the major challenges that all government agencies face when conducting environmental health risk assessments.

Thayer leads OHAT efforts to examine emerging issues in toxicology, such as obesity and diabetes, that may be triggered by exposures to endocrine-disrupting chemicals (see story). She also contributes to the NTP and NIEHS green chemistry initiative, which evaluates and compares the relative toxicity of chemicals proposed as safer replacements for other toxic chemicals. (Photo courtesy of Steve McCaw)

One of the major goals of the conference was to figure out how to move forward with the integration of toxicological, epidemiological, and mechanistic research studies into the risk assessment process.

Bucher's talk focused on the proactive approach by NTP to address challenges in environmental health. He discussed the progress made since the release of the NTP Roadmap in 2005, along with recent advances in toxicology testing and analysis approaches.

His discussion set the stage for Thayer and Rooney to illustrate how Roadmap progress has helped NTP establish a systematic review process for health assessments that focuses on the integration of data. OHAT develops literature-based evaluations to frame its investigations of potential human health hazards and examine the state of the science.

In addition to his work on the theoretical framework of systematic review, Rooney was instrumental in the OHAT evaluation of literature on the potential health effects of low-level exposures to lead (see story) in 2011. (Photo courtesy of Steve McCaw)

Learning from one another

Thayer talked about steps 1-4 of the OHAT framework for systematic review, which focuses on the critical aspects of developing the protocol, managing data, and assessing study quality. Thayer also dazzled the 200 attendees with the data entry and analysis tools that NTP is using to conduct the evaluations. All the tools will eventually be made available to the public free of charge.

Rooney focused on steps 5-7, which guide the integration of epidemiological, toxicological, and other relevant data. "There was a lot of interest in the methods we're sharing," Rooney said. "They liked the clear, step-by-step process of how we are integrating different lines of evidence to develop conclusions."

Attendees were especially interested in the data entry tools and the possibility of holding training sessions, so others can use the NTP process, and everyone can begin entering and storing information in the same way, Thayer noted.

"No one has the resources to keep reinventing the wheel," Thayer said. "Other agencies, nationally and internationally, are looking to the NTP to take an active role in developing and evaluating approaches for bringing systematic review methods into environmental health science."

(Robin Mackar is news director in the NIEHS Office of Communications and Public Liaison, and a frequent contributor to the Environmental Factor.)

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Validating civic perspective, grassroots resources for environmental science

By Joe Balintfy

Research by members of the public can meet the rigors of science and help advance environmental health efforts, according to Sara Wylie, Ph.D., assistant professor of anthropology at Northeastern University. Wylie visited NIEHS Dec. 9 and presented "Toward a Civic Science: Putting Tools for Rigorous Research into Public Hands."

"[I'm] really trying to show how changing the tools that we use for science, how building online databases for gathering community experiences, and then how expert analysis of data could produce a responsive network for community-based environmental monitoring," Wylie said.

Wylie outlined what she called a civic approach to environmental health research, with people on the front lines becoming directly involved in doing rigorous science. Using such devices as balloons, modified consumer cameras, and free-source software, the end results of a grassroots approach can include better images of oil spills, more epidemiological data on endocrine-disrupting chemicals, and more thorough monitoring of well water.

Listen as Wylie describes her concept of civic science and the ways today's tools can be modified to encourage popular, grassroots research (01:45)

Read Transcript

Tools in the hands of those closest to the sources

What started as development of an inexpensive way to make satellite-like maps, using helium balloons and digital cameras, evolved into a resource platform called Public Lab, which communities used during the Gulf oil spill to create, archive, and improve documentation of the disaster's environmental impact.

Symma Finn, Ph.D., of the NIEHS Population Health Branch, hosted the presentation. As she explained, balloon mapping validated local concerns that things were not as cleaned up as

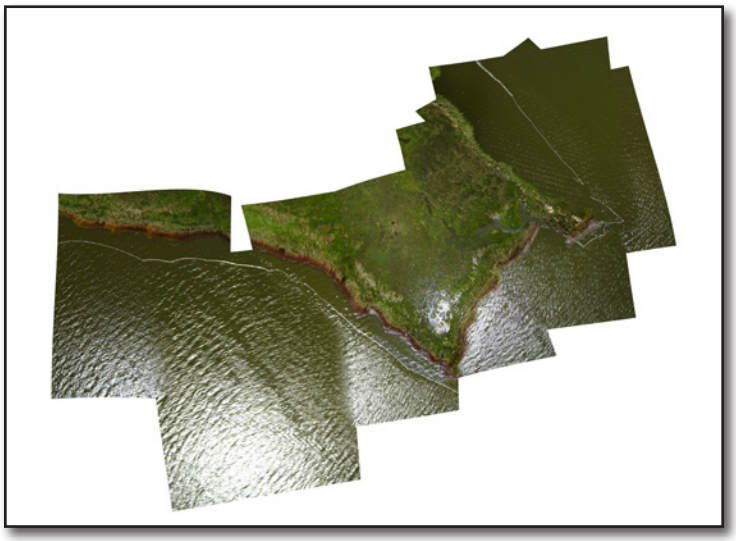
Presentation demonstrates how efforts parallel NIEHS strategic plan

Throughout Wylie's presentation, she made parallels to NIEHS strategic goals, in particular goals 5-8, which include identifying emerging environmental health threats, conducting community-based participatory research, creating collaborations, and teaching environmental health science.

For example, she pointed out that the tools of Public Lab help identify emerging threats, foster community-based research, and encourage scientific literacy. One tool, adapted by FEMA to crowdsource hurricane damage images, resulted in more than 6,000 individuals, making more than 130,000 assessments, showing how a health threat can be monitored, and how the public can be involved and educated. The Endocrine Disruption Exchange, she said, also meets the goal of monitoring health threats, as well as enhancing collaboration, while the public use of do-it-yourself tools and contributing to accessible databases fosters scientific literacy.

Wylie pointed out how her research projects connect to NIEHS strategic goals 5-8. (Photo by Steve McCaw)

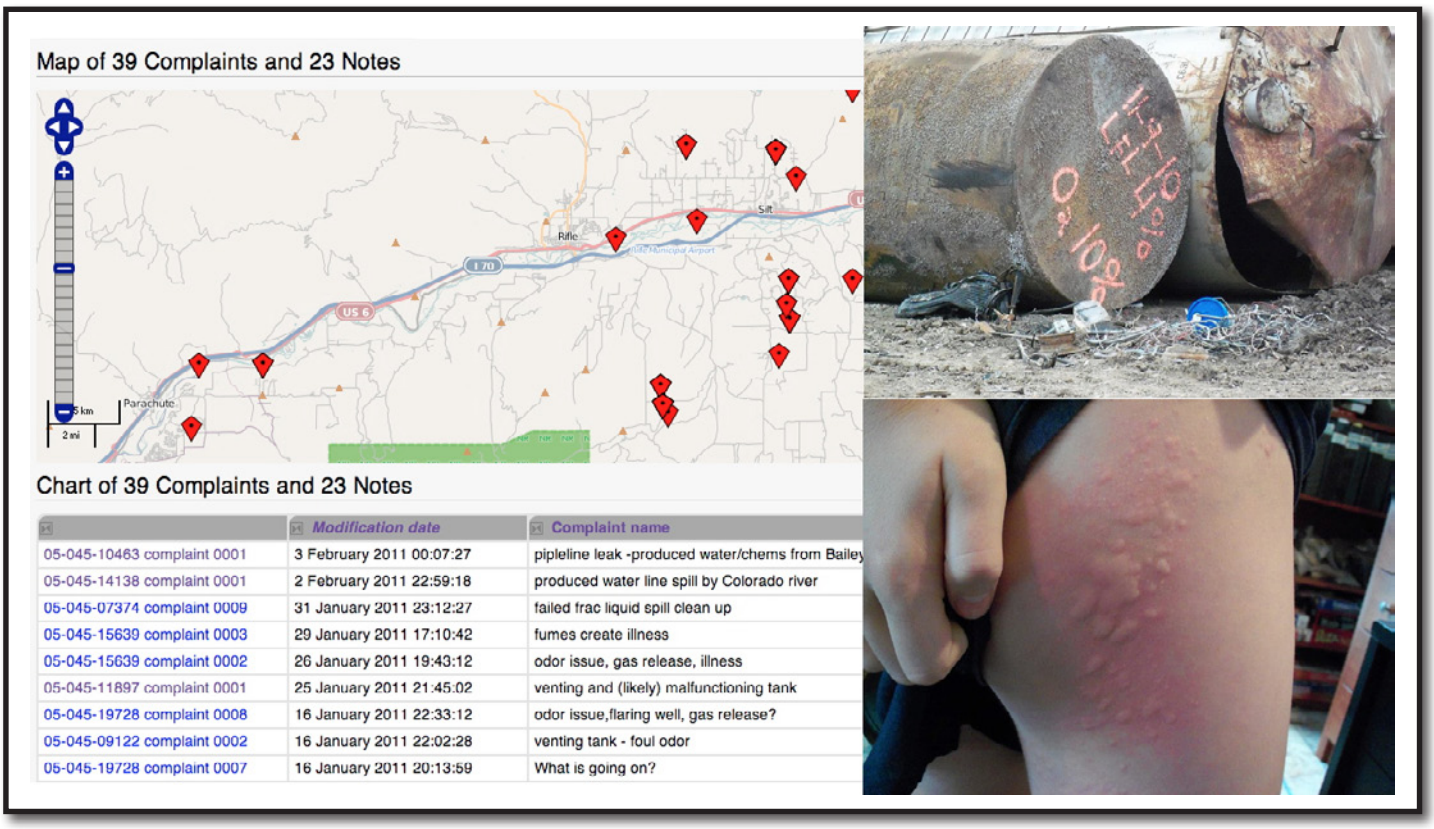
seen in satellite images. “People on the ground understand their communities and their ecosystem a whole lot better than scientists give them credit for,” Finn said, adding that scientists grounded in the grassroots can achieve what Native Americans term local ecological knowledge.



A balloon map of the Gulf oil spill in Wilkinson Bay, La., shows broken booms better than satellite images can. This kind of mapping can also be integrated with satellite images and Google maps. (Photo courtesy of Public Lab)

Balloon mapping, which uses free-source mapping software such as MapKnitter, has since been used to help detect inflows at the Gowanus Canal, a Superfund cleanup site in New York. Wylie said there are many more resources Public Lab offers. “One of the tools that Public Lab has developed is for doing low-cost thermal inspections of your home.”

Wylie emphasized that these tools, along with others in development, can help both communities and government agencies. She pointed to a collaboration that involved Federal Emergency Management Agency (FEMA) use of a Public Lab tool shortly after Hurricane Sandy. “[It] enabled 6,000 people to get online and participate.” People searched images of the New Jersey coast to identify the ones with the most damage, to help FEMA target its response.



The WellWatch website is an extrAct tool that maps complaints and tracks issues, including health outcomes, near oil and gas wells. Wylie said it is a platform for rigorous grassroots mapping and epidemiology that creates support networks across communities. (Photo courtesy of MIT Comparative Media Studies)

Community-based data collection and analysis

Wylie also described civic science data resources, and pointed to The Endocrine Disruption Exchange, which has compiled a database of chemicals and several metals used in natural gas development. Finn echoed Wylie's argument about the potential for data collected by communities to guide research. "It may bring up questions or hypotheses that we would not have thought of [otherwise]," she said.

Another example Wylie shared is extrAct, a suite of publicly accessible databases and maps that help aggregate grassroots efforts. She said the goal of extrAct is to promote communication. "For instance, [extrAct can help] communities dealing with oil and gas extraction find each other and build a community of concern around that industry — particularly for land owners, who tend to experience issues in real isolation — trying to create an infrastructure for people to share their problems and to start attracting researchers to look at their cases."

(Joe Balintfy is a public affairs specialist in the NIEHS Office of Communications and Public Liaison.)

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Newly dedicated facility to house NIEHS-funded labs

By Eddy Ball

Officials at the University of North Carolina Wilmington (UNCW) cut the ribbon Nov. 18 for a new facility that will serve NIEHS-funded labs on the N.C. coast participating in an innovative public-private partnership initiative to advance health research and translation.

Baden stands beside samples in the red tide study culture room. (Photo courtesy of UNCW and Jamie Moncrief)

The ceremony at the new 69,000 square-foot Marine Biotechnology in North Carolina (MARBIONC) building featured comments by UNCW Chancellor Gary Miller, Ph.D., the UNCW Board of Trustees, and Congressman Mike McIntyre, D-N.C. In attendance were NIEHS Program Director Frederick Tyson, Ph.D., grantee Daniel Baden, Ph.D., representatives from the National Institute of Standards and Technology (NIST), National Oceanographic and Atmospheric Administration, and MARBIONC, as well as public-private partners and the general public.

UNCW used 30-year bonds to pay for half of the $30 million building. The other half came from federal stimulus money provided by NIST. The green building is LEED (Leadership in Energy and Environmental Design) silver certified and offers flexible modular lab space for lease.

Scientists in the university-related MARBIONC program have been working toward the goal of turning materials in the marine environment into new products, drugs, and technologies, as well as creating potential spin-off companies and jobs. As its UNCW website proclaims, "MARBIONC is in the business of transforming the mysteries of the deep into the miracles of the marketplace."

Crest Research Park at UNCW is a place for university researchers, private firms, and government agencies to work together to create biotechnology products and solutions. (Aerial photo courtesy of UNCW)

The NIEHS connection

At the hub of the NIEHS connection with the MARBIONC facility is Baden, an expert in the field of marine biology and an NIEHS grantee since 1991, when he was on the faculty at Miami University. Currently, Baden is director of the Center for Marine Science (CMS), which is the UNCW partner in MARBIONC, and administers the non-profit MARBIONC Development Group.

Research underway at CMS is emblematic of the way MARBIONC plans to translate basic research. Baden's work with extremely toxic microorganism blooms that flourish in warm Atlantic waters led to the surprise discovery of a natural antitoxin, brevenal, which is also produced by the organism. It turns out that brevenal has potential for treating patients with cystic fibrosis, chronic obstructive pulmonary disease, and other related lung diseases (see story).

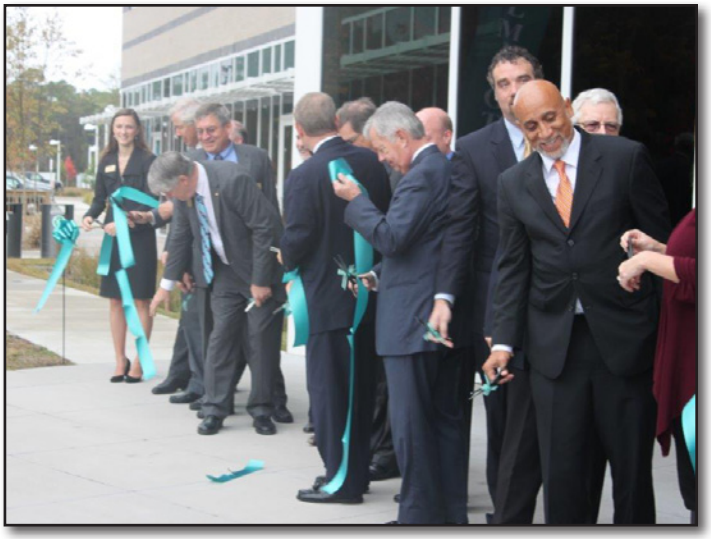
The MARBIONC building's labs are well-lit, spacious, easily adaptable for tenants, and filled with state-of-the-art equipment. (Photo courtesy of Dan Baden)

"The antitoxin itself, known as brevenal, promotes a series of physiologic effects known as mucociliary clearance," Baden explained in an interview with Steve Curwood of the radio program Living on Earth. "And the easy way of saying that is it makes your mucus thinner and it makes it capable of being expulsed from the lung much more readily."

"Put the two together, and it should be a therapy for anything that has thick, ropey mucus that can't be cleared from the lung."

According to Tyson, the opening of the MARBIONC marks a major success coming from the harmful algal bloom research that NIEHS has supported for several decades. "Characterization of the mechanisms of toxicity associated with brevetoxins, and the ultimate identification of brevenal and its therapeutic potential, was the key research driver that led to the establishment of MARBIONC Development Group and the state-of-the-art marine research facility," he said.

Among the dignitaries speaking at the dedication of the new building was McIntyre, who represents the state's seventh congressional district, which includes Wilmington and much of southeastern N.C. (Photo courtesy of Dan Baden)



Tyson, right with orange tie, joined in the ribbon cutting that officially opened the new building. (Photo courtesy of Dan Baden)

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Autism studies build on past investments and guide future research

By Kelly Lenox

Three new NIEHS-funded studies on autism spectrum disorder (ASD) illustrate the broad scope of current research into this significant health concern. Cindy Lawler, Ph.D., chief of the NIEHS Genes, Environment, and Health Branch, which manages the Institute's grants for autism research, discussed these exciting new advances in the quest to better understand and help prevent and treat these disorders.

Results of long-term investment

A study team led by researchers at the University of Southern California (see story) found that the combination of prenatal exposure to air pollutants, combined with a genetic predisposition to ASD, led to a higher risk of the disorder. These results provide an important advance in the field, and also illustrate, as Lawler pointed out, the value of long-term investment. "This story has been a while in the making, but NIEHS has been involved along the trajectory, funding not only this study, but some of those on which it builds."

For Lawler, the Center for Children's Environmental Health at the University of California (UC), Davis, illustrates how consistent funding and interdisciplinary collaboration are moving the field forward. The center gave rise to the CHARGE (Childhood Autism Risks from Genetics and the Environment) study at the UC Davis MIND Institute. "It takes a lot of time to build the infrastructure of a large study. We're now reaping the benefits of investments we made starting more than ten years ago, and the work is beginning to uncover environmental risk factors," she explained. "This is only possible because of the funding that helped build it and is still supporting it."

New directions in research — exploring the mind-gut connection

Another study (see story), examining the incidence of gastrointestinal (GI) symptoms and ASD, is part of a growing interest in chronic co-occurring conditions. "This study confirms what we hear from a lot of parents, especially regarding how some of the comorbidities, such as GI issues, are as important as the core symptoms of autism in terms of the impact on families," explained Lawler.

Lawler is the NIEHS program lead for autism research. (Photo courtesy of Steve McCaw)

However, more research is needed to discover whether the GI symptoms contribute to the core symptoms or the reverse, or whether other changes might be at the root of both groups of symptoms.

Work connecting GI disturbances to ASD also has implications for other studies that are beginning to explore the microbiome, a community of microorganisms inhabiting an area such as the human gut. Very little is known about how the microbiome of children with autism compares to that of typically developing children, or of children with developmental disabilities but not autism.

Lawler pointed out that findings related to GI disturbances may advance knowledge of ASD, as well as lead to treatment approaches that may provide relief for families.

Listening to families

It was information from families, specifically, Somali families in Minneapolis, that led the University of Minnesota to investigate the prevalence of autism among their children (see story).

"The prevalence in Somalis was high, with one in 32 children affected, but it was not different from the prevalence in whites. The rate in Hispanics and black non-Somali children was significantly lower," Lawler observed.

In addition, intellectual disability among children with ASD was found much more often in Somali children than in all other groups, but the reasons are unknown.

"Are there cultural issues related to assessing IQ in the Somali population, or that make it less likely for schools and health service providers to identify ASD in Somali children who have average or above average intelligence?" Lawler continued. "If there is a true difference in the characteristics of Somali children with ASD, further study is needed to determine which factors may be involved, for example, factors related to race, ethnicity, health care practices, socioeconomics, or immigration."

Advancing autism research

From uncovering gene-environment interactions, to expanding understanding of connections between co-occurring symptoms, to breakthroughs in epidemiology, researchers are making wide-ranging discoveries and answering questions that will help guide future autism research.

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New evidence of gene–environment interaction in autism

By Kelly Lenox

Children possessing a specific genetic risk factor appear more likely to develop autism spectrum disorder (ASD) when prenatal exposure to air pollution occurs, according to a study led by NIEHS-funded researchers at the Keck School of Medicine at the University of Southern California (USC). The study, published online in the journal Epidemiology, establishes a synergistic interaction between a genetic risk factor, the MET rs1858830 genotype, and an environmental factor, air pollution.

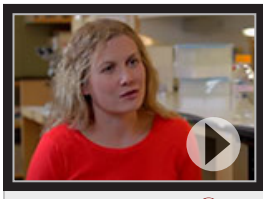
"Our research shows that children with both the risk genotype and exposure to high air pollutant levels were at increased risk of autism spectrum disorder, compared to those without the risk genotype and [with] lower air pollution exposure," explained Heather Volk, Ph.D., assistant professor of research in preventive medicine and pediatrics at USC.

The study builds on earlier research demonstrating independent contributions to autism risk from MET and from exposure to air pollutants.

Campbell noted that much work is yet to be done. "It will be important to replicate this finding and to determine the mechanisms by which these genetic and environmental factors interact to increase the risk for autism." (Photo courtesy Don Milici)

Results may clarify effect of allele on risk of autism

The finding that factors such as air pollution exposure may be involved in ASD is significant, because gene-only analyses have shown inconsistent evidence of a link between common alleles, or alternative forms of a gene, and development of ASD.



Linked video:

Watch Volk discuss her research on how environmental factors work together with genetics to increase risk of disorders such as ASD. (1:32)

(Launches in new window)

Download Media Player: Flash

"Although gene-environment interactions are widely believed to contribute to autism risk, this is the first demonstration of a specific interaction between a well-established genetic risk factor and an environmental factor that independently contribute to autism risk," noted study co-author Daniel Campbell, Ph.D., assistant professor of psychiatry and the behavioral sciences at USC.

The authors call for independent replication of their findings and note the need for a more detailed understanding of the underlying mechanisms.

Volk is lead author of the study finding that children with both a genetic risk factor and prenatal exposure to air pollutants experienced increased risk for ASD. (Photo courtesy of Heather Volk)

Citation: Volk, HE, Kerin T, Lurmann F, Hertz-Picciotto I, McConnell R, Campbell DB. 2014. Autism Spectrum Disorder: Interaction of Air Pollution with the MET Receptor Tyrosine Kinase Gene. Epidemiology 25(1):44-47.

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Parents are right: autistic children experience more GI symptoms

By Kelly Lenox

Researchers at the University of California, Davis (UCD), have found that young children with autism spectrum disorder (ASD) and developmental delays are at least three times more likely to experience frequent gastrointestinal (GI) problems than are typically developing children.

The study findings appeared in the November 2013 issue of the Journal of Autism and Developmental Disorders.

Parents of children with ASD reported frequent occurrence of certain GI symptoms, including diarrhea, constipation, and sensitivity to foods, six to eight times more often than do parents of typically developing children. Furthermore, among all children with ASD, the presence of GI symptoms was associated with behaviors such as irritability, social withdrawal, and hyperactivity.

Hertz-Picciotto, lead researcher on the study, also launched the CHARGE study, the first large, comprehensive population-based study of environmental factors in autism, and the landmark MARBLES study of early environmental and biologic predictors of autism. (Photo courtesy UC Regents)

Results parallel parent reports

"After years of parents raising concerns about such symptoms, the huge differences we see … put to rest the idea that gastrointestinal problems among children with autism spectrum disorder are just an accumulation of case reports," observed Irva Hertz-Picciotto, Ph.D., lead researcher on the study.

Named one of the 2013 Top 10 Advances in Autism Research by Autism Speaks, the study is notable for its involvement of a large, ethnically diverse population. Researchers enrolled nearly 1,000 children, aged 24-60 months, approximately one-half of whom were white, one-third Hispanic, and one-sixth from other racial and ethnic backgrounds.

"GI problems may create behavior problems, and those behavior problems may create or exacerbate GI problems," noted Virginia Chaidez, Ph.D., a postdoctoral researcher at UCD at the time of the study. "One way to try to tease this out would be to begin investigating the effects of various treatments and their effects on both GI symptoms and problem behaviors." (Photo courtesy UC Regents)

Determining causality requires further study

Researchers note that, although mechanisms and contributing factors explaining the differences they found are not yet understood, a chronic problem causing pain, discomfort, or anxiety could plausibly contribute to increased irritability and social withdrawal, especially in a person with deficits in social and communicative skills. Also, because neurotransmitter systems that are active in the brain also function in the gut, further research into GI symptoms could provide insight into neurobiological aspects of the disorder.

Citation: Chaidez V, Hansen RL, Hertz-Picciotto I. 2013. Gastrointestinal problems in children with autism, developmental delays or typical development. J Autism Dev Disord; doi:10.1007/s10803-013-1973-x [Online 6 November 2013].

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Autism in Minneapolis higher among Somalis and whites than other groups

By Kelly Lenox

The prevalence of autism spectrum disorder (ASD) in 2010 among Somali and white children in Minneapolis was greater than among black non-Somali and Hispanic children, according to a new University of Minnesota (UM) project and resulting community report. The project was funded jointly by several NIH institutes, the Centers for Disease Control and Prevention (CDC), and Autism Speaks, as well as others. Somali children with ASD were also more likely to have an intellectual disability than children with ASD in all other racial and ethnic groups.

In the early 1990s, thousands of Somali refugees came to United States after civil war erupted in Somalia, a country located in the horn of Africa. Many were resettled in Minnesota, especially Minneapolis. (Image courtesy of Minneapolis Somali Autism Spectrum Disorder Prevalence Project)

Children with ASD in Minneapolis were typically diagnosed around age 5, which means children are not being diagnosed as early as they could be, according to the researchers. They further report that the overall prevalence of ASD in the city in 2010 was higher than in most other communities studied by the CDC in 2008, though differences in timing and size of communities make comparison difficult.

The Minneapolis Somali Autism Spectrum Disorder Prevalence Project was developed after a 2009 study by the Minnesota Department of Health found higher than expected enrollment of Somali children in the city's preschool special education program for children with ASD. The project was designed to determine whether, among children who live in Minneapolis, the prevalence of ASD was higher in Somali children than in non-Somali children.

Records screening reveals questions for further research

"This project, based on analysis of numerous and varied records, produced fascinating, complex results," noted Cindy Lawler, Ph.D., chief of the NIEHS Genes, Environment, and Health Branch, which administers the Institute's funding for autism research.

Amy Hewitt, Ph.D., director of the Research and Training Center on Community Living at UM, suggests how these results point to next steps. "Future research can and should build upon these findings to better understand how ASD affects Somali and non-Somali children," she said. "This project was not designed to tell us why these differences exist, but its findings support the need for more research on why and how ASD affects Somali and non-Somali children and families differently."

Hewitt directed the project, comparing Somali and non-Somali children in Minneapolis, in response to community concerns about ASD in the Somali community. (Photo courtesy University of Minnesota)

Resources to support earlier diagnosis and intervention

Noting that increased awareness could lower the age of diagnosis and assist families in providing the best possible environment for their child's development, the report concludes by directing families to a wide range of resources, both in the Minneapolis area and online.

Resources include child development trackers, clinics for diagnosis and assessment, intervention programs designed to meet individual needs, and organizations engaged in advocacy and research. One Somali parent quoted in the report shared her child's experience with target intervention. "I have seen my child make tremendous gains when her specific needs were matched to the services she received. What works for one child does not work for another."

NIH funding for the project was provided by NIEHS, along with the Eunice Kennedy Shriver National Institute of Child Health, National Institute of Mental Health, and National Institute on Minority Health and Health Disparities.

Race and ethnicity	Children with ASD identified/ Total population	Prevalence estimate (prevalence per 1,000 children)	95% Confidence interval*
Overall	255 of 12,329	**1 in 48** (20.7 per 1,000)	18.3 – 23.4 per 1,000
Somali	31 of 1,007	**1 in 32** (30.8 per 1,000)	21.6 – 43.8 per 1,000
White	120 of 4,336	**1 in 36** (27.7 per 1,000)	23.1 – 33.1 per 1,000
Black (non-Somali)	53 of 3,312	**1 in 62** (16 per 1,000)	12.2 – 20.9 per 1,000
Hispanic	30 of 2,399	**1 in 80** (12.5 per 1,000)	8.7 – 17.9 per 1,000

Note: We are unable to report on Asian/Pacific Islanders and Native Americans due to their low numbers.
* 95% confidence interval is the range in which an estimate is likely to fall.

ASD prevalence among children aged 7-9 in Minneapolis in 2010, by race and ethnicity (Graphic courtesy of Minneapolis Somali Autism Spectrum Disorder Prevalence Project)

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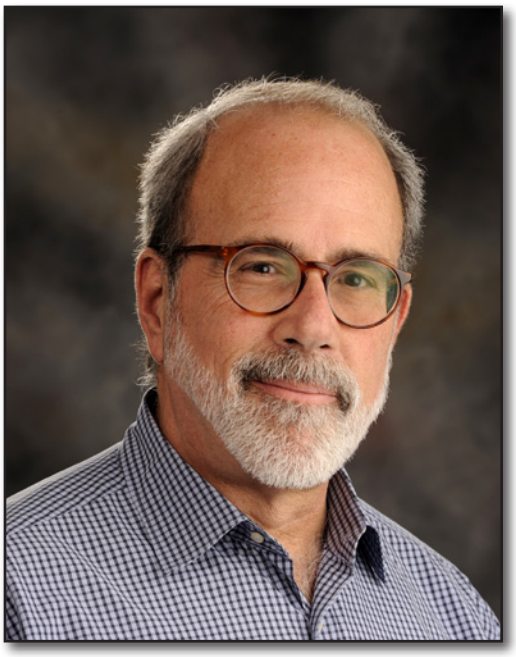
Latoni tapped to head Scientific Review Branch

By Eddy Ball

NIEHS welcomed its new Scientific Review Branch (SRB) chief Nov. 18, as Alfonso Latoni, Ph.D., joined the leadership ranks of the Division of Extramural Research and Training (DERT).

Latoni leads a staff of five scientific review officers (SROs) and four extramural support assistants (ESAs). SRB is a kind of front door for aspiring NIEHS grantees, responsible for the initial scientific and technical merit review of grant applications and contract proposals submitted to NIH for funding.

Latoni and the review officers have a responsibility to the public and to the scientific community, for identifying the most meritorious scientific research to support, and to applicants, for giving every grant application and contract proposal an expert, objective, timely, and fair initial peer review. "We need to show the world that we have the best scientists," he said. "Our [review panel] rosters have to be top notch."



Latoni said his wife, Carmen, who is a financial person with an M.B.A., crunched the numbers during their search for a home near NIEHS. One of his daughters plans to continue her graduate work in early childhood and elementary education in N.C., while his other daughter, Elena, who lives in Puerto Rico, has done work with the U.S. Department of Agriculture as a scientist and soil conservationist. (Photo courtesy of Steve McCaw)

Not just more of the same

A native of Puerto Rico, Latoni received his undergraduate education from the University of Puerto Rico (UPR), and later went on to earn his Ph.D. from Boston College in sociology, with a focus on social economy and social policy. Prior to joining NIH in 2002, he was associate professor at the UPR Mayagüez, from 1986-2000, and director of the Minority Affairs Program at the American Sociological Association in Washington, D.C., from 2000-2002.

During his 11-year career at NIH in Bethesda, Md., Latoni served as an SRO at the National Institute on Aging (NIA), from 2002-2005, and at the Center for Scientific Review, from 2005-2008, before returning to NIA to serve as deputy chief of its Scientific Review Branch, from 2008-2013.

For Latoni, his new position is the natural next step in his career at NIH in scientific peer review. Although, in many ways, the policies and procedures are standardized, he was quick to add, "It's really not just more of the same."

Similar in size to NIEHS, NIA is also one of the biological process, rather than disease-specific or organ-specific institutes, and has a broad portfolio of grants. Still, NIEHS offers even greater challenges, with its more varied interdisciplinary portfolio. Grants at NIEHS deal with just about everything environmental, ranging from basic and clinical research, public health, and environmental justice, to disaster-response worker training, development of personal environmental monitors, and the design of technologies for remediating Superfund sites.

"This [topic range of grants] is new for me," he said. "I'm learning along with them [the SROs and ESAs]. My initial approach is to learn how each of them does the job, as well as to bring my experience, and to build, with them, an exceptional and unique review branch."

Life down south in the slow(er) lane

Latoni laughed, as he talked about his relocation from Bethesda to the N.C. Triangle area, and recalled an earlier discussion of colleges with his younger daughter, Angélica. When she told her parents she wanted to attend the University of North Carolina at Charlotte, he'd asked her, "Why would you want to go to North Carolina?"

Three years later, when Latoni accepted the position at NIEHS, his daughter, who is now at Elon University and has a keen sense of irony, turned the tables by asking him the same question. His answer won't surprise many transplants at NIEHS.

Latoni pointed to quality of life and family — and a three-hour commute each day from suburban Montgomery County, Md., into Bethesda that could easily become four hours if anything went wrong with traffic or the weather.

"I'm still trying to get over that commuting mindset," he said. "I've found myself arriving at meetings here half an hour early, because I'd become so used to getting a head start."

Now, Latoni is measuring his trips from a new home in nearby Cary, N.C., to NIEHS in terms of minutes, and spending the hours he would have wasted behind the wheel in Bethesda with his family instead. "I feel we can make a life here," he said. "There are so many good things happening to me now, and I look forward to our future here."

No one, except Latoni himself, was more excited by his appointment than DERT Deputy Director Pat Mastin, Ph.D., who served as acting chief following the retirement of former branch chief Terry Nesbitt, Ph.D. (Photo courtesy of Steve McCaw)

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Students in NIEHS program WOW! supporters with interactive presentations

By Jacqueline Powell

NIEHS scientists reaped the rewards of teaching and service, as they participated in the Citizen Schools WOW! Event Dec. 12 at Lowe's Grove Middle School in Durham, N.C. After spending 10 weeks learning about cells and DNA in a new learning module designed and taught by NIEHS volunteers, it was time for students to share what they had learned with family, friends, and teachers.

The door to the presentation room was decorated as an ion channel within a cell membrane. Inside, students presented posters and guided adults through hands-on cell biology presentations. One group illustrated cell division, by presenting a poster, making model cells out of clay, and using a light microscope. Another team of students focused on the properties of DNA, by performing a strawberry DNA extraction, while others illustrated cellular structures, by decorating cookies with different types of candy.

A Lowe's Grove Middle School student, right, described two forms of cell division, mitosis and meiosis, before using handmade clay model cells to illustrate the physical changes that accompany different stages of cell division. Visitors were also encouraged to observe cells on a slide, using a light microscope. (Photo courtesy of Steve McCaw)

When one of the middle school students was asked about his favorite part of the apprenticeship, he said, "I don't even know. I liked all of it." He then quickly returned to extracting DNA from mashed up strawberries.

"I think this new curriculum on cell biology really lets [NIEHS scientists] play to their strengths. All of us deal with cells and DNA in some way, so it's easy to pick a topic that you're excited about," said Shannon Whirledge, Ph.D., an Intramural Research Training Award (IRTA) fellow in the NIEHS Molecular Endocrinology Group.

Citizen School apprenticeships depend on volunteer teachers

In the reception preceding the student presentations, Citizen Schools staff emphasized the utility of this academically focused after school program, which depends on community volunteers to teach 21st century skills in a broad range of areas.

Coordinating such an ambitious program required commitment on both sides, and Jesula Charles, Citizen Schools teaching fellow and liaison to NIEHS volunteers, served many important functions. She coordinated the children's after school learning activities, oversaw enrollment, and monitored students' performance to make sure everyone was progressing. NIEHS scientists and staff also made significant contributions, by developing the students' curriculum. Huei-Chen Lao, K-12 science education and outreach coordinator with the NIEHS Office of Science Education and Diversity (OSED), played a crucial role in helping volunteers share the responsibility of teaching.

"While I play a leadership role to keep things moving along, Huei-Chen makes it happen," said Ericka Reid, Ph.D., OSED director. She's great and she really knows the science.

One of the program's students, center, guided visitors through an interactive game that demonstrated his newfound knowledge of DNA. (Photo courtesy of Steve McCaw)

Volunteers for the fall Citizen Schools apprenticeship

- Floyd Adsit
- Sara Andres, Ph.D.
- Miranda Bernhardt, Ph.D.
- Terry Blankenship-Paris, D.V.M.
- Christopher Campos, Ph.D.
- Felicity Davis, Ph.D.
- Bill Fitzgerald
- David Goulding
- Sylvia Hewitt
- Jacqueline Locklear, D.V.M.
- Page Myers
- Palmyra Romeo
- Kirsten Verhein, Ph.D.
- Shannon Whirledge, Ph.D.
- Tanya Whiteside
- Kimberly Wiggins, Ph.D.
- Wipawee (Joy) Winuthayanon, Ph.D.
- Gary ZeRuth, Ph.D.

Another of the students, left, helped a Citizen Schools supporter, right, extract DNA from a strawberry, as volunteer instructor Hewitt, an NIEHS biologist, looked on. (Photo courtesy of Steve McCaw)

Volunteers and OSED staffers showed off the poster for the NIEHS program, "It's a Small World: Cells and DNA." Shown, from left, are Davis, Reid, Hewitt, Lao, Wiggins, and author Powell, a veteran of the spring 2013 outreach effort. (Photo courtesy of Steve McCaw)

(Former NIEHS postdoctoral fellow Jacqueline Powell, Ph.D., is a writer and analyst with Education and Training Systems International [ETSI].)

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Duke brings Superfund research to the Elizabeth River Learning Barge

By Sara Mishamandani

The end of 2013 marks the first full semester of Duke University Superfund Research Program (SRP) field work and outreach on the Elizabeth River Project (ERP) Dominion Virginia Power Learning Barge.

Duke SRP partnered with ERP to develop content and materials for the floating classroom, now in its fifth year of operation, as well as complimentary materials for fourth grade teachers back in the classroom. ERP is a nonprofit organization that leads community efforts to restore the environmental health of the Elizabeth River in southeastern Virginia.

The Learning Barge is the world's first floating wetland classroom, powered by sun and wind, and equipped with live wetlands, an enclosed classroom, composting toilets, and a rainwater filtration system. (Photo courtesy of the Elizabeth River Project)

Hundreds of elementary school students in the Chesapeake Bay area make field trips to the Learning Barge each year, to learn about river environmental stewardship and how to make the polluted Elizabeth River swimmable and fishable by 2020. Members of the Duke SRP Research Translation Core (RTC) developed a new teaching module on adaptation, using Duke SRP research concepts, which launched on the Learning Barge in fall 2013.

Fourth graders on the Learning Barge examine their catch from the Elizabeth River. (Photo courtesy of the Elizabeth River Project)

"Adaptation is part of the fourth grade science curriculum, but something students often test poorly on. It is a difficult concept for students to understand without seeing it firsthand," said Robin Dunbar, the ERP Education Director. "Duke's activity on the Learning Barge helps students make the connection and understand the concept of adaptation in a way that cannot be done in the classroom."

Duke research on the Elizabeth River

Researchers at the Duke Superfund Research Center, led by Richard Di Giulio, Ph.D., are conducting research on the Atlantic Wood Industries Superfund site located on the Elizabeth River (see story). The site, a former wood treatment facility, left the Elizabeth River heavily contaminated with polycyclic aromatic hydrocarbons (PAHs).

Duke investigators are working to better understand how the PAH contamination in the Elizabeth River is affecting fish populations. They have discovered that a small killifish species in the Elizabeth River, the *Fundulus heteroclitus*, or mummichog, has developed resistance to acute toxicity, heart deformities, and cancers that are linked to PAH exposure. Duke researchers continue to study how this adaptation to PAHs may impact killifish health later in life.

Duke researcher Savannah Volkoff, right, sorted mummichogs during a fish collection trip on the Elizabeth River. She worked with Kroeger to develop the Learning Barge activity and materials. (Photo courtesy of Savannah Volkoff)

Translating findings to fourth graders

On the Learning Barge, students attend a fifteen-minute fishing station where they can fish off the side of the barge for samples. Students observe their catch, as they hear about how organisms living in the Elizabeth River, such as the mummichog, have had to adapt to survive, and the costs of that adaptation. The script, developed by Duke RTC, brought in elements of Duke SRP research, while fulfilling specific concepts, in accordance with the Virginia Science Standards of Learning.

"Duke investigators are studying the concept of adaptation at a very high level," said Dunbar. "The Duke RTC was able to take it down to where it begins, so students can understand."

To complement the field trip, Duke SRP staff also developed resources on adaptation for fourth grade teachers, available on the Duke SRP website. Resources include a factsheet on *Fundulus heteroclitus*; a worksheet with a fictional story describing adaptations to PAH exposures and associated fitness costs; and questions for evaluating student understanding.

"The Learning Barge activity was a great opening into working with the Elizabeth River Project, and we hope to have a more active role with the organization in the future," said Gretchen Kroeger, the Duke RTC project coordinator. "It was also a lot of fun to see the learning module in action and to see how the students were really engaged in the activities."

(Sara Mishamandani is a research and communication specialist for MDB Inc., a contractor for the NIEHS Superfund Research Program and Division of Extramural Research and Training.)

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Industry-academic collaboration streamlines decades of data

By Raj Gosavi

Knowing the toxic effects of pharmaceutical drugs is important in drug development as well as in environmental science, but the relevant data are typically scattered throughout the scientific literature and a host of independent databases. Increasingly, scientists are recognizing the compelling need for finding and integrating this information, systematically, using common search terminology to make the data useful and accessible for academic and industrial researchers, as well as the general public.

Shown, above, at a data integration meeting in July 2013 (see story), Mattingly, like many of her colleagues working in big data applications, feels that networking data about chemical-gene and chemical-protein interactions, as well as chemical-disease and gene-disease relationships, can help scientists develop novel hypotheses about the origins of environmentally influenced diseases. (Photo courtesy of Steve McCaw)

As demonstrated by a new study, funded in part by NIEHS, scientists at the pharmaceutical corporation Pfizer Inc. and academic researchers affiliated with the Comparative Toxicogenomics Database (CTD) have made major progress toward that goal, by integrating information about the toxicity of more than 1,200 pharmaceutical drugs.

Molecular toxicologist Carolyn Mattingly, Ph.D., associate professor at North Carolina State University (NCSU) and lead researcher on the study, has been directing development of CTD with NIEHS support since 2001 (see story). "We felt that the information that was lacking were the mechanisms that connect exposure to environmental chemicals with diseases," she said.

CTD is a publicly available research resource developed initially at the Mount Desert Island Biological Laboratory, where Mattingly was affiliated prior to moving to NCSU in January 2012. "This is the only database out there that connects mechanisms of chemical action to potential impacts on human health," Mattingly said. The collaboration with Pfizer, which took CTD biocurators — professional scientists who curate, collect, annotate, and validate information — a year to complete, significantly increased information about pharmaceutical and environmental chemicals.

Mapping hundreds of thousands of chemical-disease interactions

The database provides chemical-gene-disease information and associated functional and pathway data. The Pfizer collaboration specifically added data for chemicals that may be involved in cardiovascular, neurological, kidney, and liver disorders.

Current screening methods for drugs, although advanced, need to be complemented with newer methods for assessing compound toxicity. With the ability to predict toxic side effects of drugs, the database can potentially improve drug development, by allowing generation of new early-stage mechanistic screening of the drugs.

Data collection and integration took advantage of Pfizer's efficient text-mining procedures, focusing on select chemicals, combined with CTD's manual curation of more than 88,000 research articles. The information from the scientific literature was then integrated with the existing database to expand its network of chemical-gene-disease interactions.

Highlighting the common goal of the collaboration with Pfizer, Mattingly said, "It was a win-win situation. What pharmaceutical companies are trying to do is essentially what environmental scientists are trying to do — understand and prevent toxicity."

"The type of partnership demonstrated by the success of this joint Pfizer-CTD effort is essential to translating scientific data and knowledge into meaningful information that can be utilized to advance our understanding of environmental agents and their impacts and ultimately to improving public health," said Allen Dearry, Ph.D., director of the NIEHS Office of Scientific Information Management.

Data from CTD is increasingly cited and integrated with other biological databases, thereby enhancing access to the information. As she looked forward to further developments in CTD, Mattingly observed, "We continue to work closely with collaborators to develop additional tools and data modules to improve the utility of the database."

Citation: Davis AP, Wiegers TC, Roberts PM, King BL, Lay JM, Lennon-Hopkins K, Sciaky D, Johnson R, Keating H, Greene N, Hernandez R, McConnell KJ, Enayetallah AE, Mattingly CJ. 2013. A CTD-Pfizer collaboration: manual curation of 88,000 scientific articles text mined for drug-disease and drug-phenotype interactions. Database 2013; doi:10.1093/database/bat080.

(Raj Gosavi, Ph.D., is a research fellow in the NIEHS Structure and Function Research Group.)

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Science Notebook

2013 papers of the year

From the more than 2,800 NIEHS-funded studies published in 2013, leaders of the Institute's three research divisions selected 30 for special recognition as Papers of the Year.

Research funded by grants (click title for abstract)

- Flame retardant Firemaster 550 confirmed as endocrine disruptor
- Tributyltin linked to transgenerational obesity
- Reducing air pollution continues to increase life expectancy
- Early pregnancy inflammation could increase autism risk
- Cost of mercury pollution
- BPA exposure in the NICU
- Global cost of childhood lead exposure
- Using membrane transporters to improve crops for sustainable food production
- Metabolomics reveals early changes in metabolic pathways for Alzheimer's disease
- Epigenetic effects of DDT lead to obesity in later generations
- Mechanism for amyloid-beta accumulation in Alzheimer's disease
- DNA methylation in the human genome
- Graphene sheets pierce and enter cells
- Improving health for low-income workers
- Father's obesity could have epigenetic effects

In-house research (click title for abstract)

- Consequences of ribonucleotide removal by topoisomerase 1
- The role of p53 during bacterial pneumonia
- DNA methylation could predict breast cancer risk
- Observing a DNA polymerase using time-resolved crystallography
- Early mouse development influences norepinephrine neuron diversity
- APOBEC cytidine deaminases generate many mutations in human cancers
- Tanning gene linked to increased risk of testicular cancer

- Assessing cockroach allergen exposure via its structure
- Probiotics use during pregnancy may reduce diseases in children
- Identification of novel immune regulatory elements and epigenetic plasticity in memory lymphocytes

National Toxicology Program research (click title for abstract)

- Spontaneous mesotheliomas in F344/N rats are characterized by dysregulation of cellular growth and immune function pathways
- Chronic exposure of renal stem cells to inorganic arsenic induces a cancer phenotype
- RNASeq profiling reveals novel hepatic gene expression pattern in aflatoxin B1 treated rats
- Recruitment of normal stem cells to an oncogenic phenotype by noncontiguous carcinogen-transformed epithelia depends on the transforming carcinogen
- Bisphenol A affects androgen receptor function via multiple mechanisms

Research funded by grants

Flame retardant Firemaster 550 confirmed as endocrine disruptor

An animal study conducted by NIEHS grantees shows that perinatal exposure to the flame retardant Firemaster 550, commonly found in house dust, is associated with endocrine disrupting effects. The findings reveal the need for more research on the mechanisms involved and the health effects of humans exposed to the product.

The researchers evaluated the effects of Firemaster 550 in rats and found that its components accumulated in tissues of exposed mice and their offspring. The offspring exhibited advanced female puberty, weight gain, male cardiac hypertrophy, and problems with exploratory behaviors. The results suggest that Firemaster 550 may affect growth and neurodevelopment.

Citation: Patisaul HB, Roberts SC, Mabrey N, McCaffrey KA, Gear RB, Braun J, Belcher SM, Stapleton HM. 2013. Accumulation and endocrine disrupting effects of the flame retardant mixture Firemaster 550 in rats: an exploratory assessment. J Biochem Mol Toxicol 27(2):124-136. [Synopsis]

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Tributyltin linked to transgenerational obesity

An NIEHS-supported study showed that mice prenatally exposed to the endocrine disruptor tributyltin (TBT) were more likely to be obese, and the effects persisted in subsequent generations not directly exposed to TBT. The findings hold important implications for understanding obesity in people.

TBT is used as an antifungal agent in some paints, certain plastics, and consumer products. Researchers exposed pregnant mice to doses of TBT that were comparable to the established human tolerable daily intake. The offspring of the pregnant mice exposed to TBT, and the subsequent two generations of mice, had a greater number of fat cells, larger fat cells, and heavier fat depots. If TBT has similar effects on people, then exposure

to it and other endocrine disruptors could reprogram the metabolism of exposed individuals, as well as future generations, predisposing them to weight gain.

Citation: Chamorro-Garcia R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B. 2013. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. Environ Health Perspect 121(3):359-366. [Synopsis]

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Reducing air pollution continues to increase life expectancy

NIEHS grantees report that air pollution reductions occurring from 2000 to 2007 were associated with improved life expectancy. These results show that the last decade of air pollution control continues to positively affect public health.

From 2000 to 2007, U.S. levels of particulate matter less than 2.5 micrometers in diameter (PM2.5) have declined, but at a slower rate than between 1980 and 2000. To find out if these levels continued to improve life expectancy, the researchers looked at yearly average PM2.5 and life expectancy data for 545 rural and urban U.S. counties from 2000 to 2007. Controlling for socioeconomic status, smoking prevalence, and demographic characteristics, they found that the average life expectancy increased by 0.35 years for every 10 micrograms per meter decrease in PM2.5 concentration. The association between life expectancy and air pollution levels was stronger in more urban and densely populated counties.

Citation: Correia AW, Pope CA 3rd, Dockery DW, Wang Y, Ezzati M, Dominici F. 2013. Effect of air pollution control on life expectancy in the United States: an analysis of 545 U.S. counties for the period from 2000 to 2007. Epidemiology 24(1):23-31. [Synopsis]

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Early pregnancy inflammation could increase autism risk

Inflammation during pregnancy could be associated with an increased risk for autism, according to research that was partially funded by NIEHS. The researchers looked at an inflammatory biomarker called gestational C-reactive protein (CRP) in the Finnish Maternity Cohort, which contains an archive of serum samples collected from approximately 810,000 pregnant women in Finland.

Analysis of serum corresponding to 677 childhood autism cases, and an equal number of matched controls, revealed that the risk of autism among children in the study increased by 43 percent among mothers with CRP levels in the top 20th percentile, and by 80 percent for maternal CRP in the top 10th percentile. These findings could not be explained by maternal age, paternal age, gender, previous births, socioeconomic status, preterm birth, or birth weight. The researchers caution that the results should be viewed in perspective, since the prevalence of inflammation during pregnancy is substantially higher than the prevalence of autism.

Citation: Brown AS, Sourander A, Hinkka-Yli-Salomäki S, McKeague IW, Sundvall J, Surcel HM. 2013. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol Psychiatry; doi:10.1038/mp.2012.197 [Online 22 January 2013]. [Synopsis]

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Cost of mercury pollution

According to a study supported in part by NIEHS, each year in Europe, more than 1.8 million children are born with unsafe prenatal methylmercury exposures. Exposure to methylmercury typically occurs from eating fish, which bioconcentrate the contaminant. Methylmercury affects brain development leading to a lower IQ and, thus, lower earning potential.

To calculate the costs associated with this exposure, the researchers examined mercury concentrations in hair samples from the DEMOCOPHES study of exposure to environmental chemicals, as well as other studies. They assumed that mercury levels below 0.58 micrograms per gram of hair would have little adverse effect. The researchers estimated that preventing exposure within the European Union would bring an annual benefit equivalent to 600,000 IQ points per year, corresponding to the estimated annual economic benefit of 8-9 billion euro. Prevention would have the most impact in southern Europe, where hair-mercury concentrations were the highest.

Citation: Bellanger M, Pichery C, Aerts D, Berglund M, Castano A, Cejchanova M, Crettaz P, Davidson F, Esteban M, Fischer ME, Gurzau AE, Halzlova K, Katsonouri A, Knudsen LE, Kolossa-Gehring M, Koppen G, Ligocka D, Miklavcic A, Reis MF, Rudnai P, Tratnik JS, Weihe P, Budtz-Jorgensen E, Grandjean P. 2013. Economic benefits of methylmercury exposure control in Europe: Monetary value of neurotoxicity prevention. Environ Health 12:3. [Synopsis]

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BPA exposure in the NICU

A study, supported in part by NIEHS, identified medical devices as a potential source of exposure to bisphenol A (BPA) among premature infants in neonatal intensive care units (NICU). BPA is used to manufacture polycarbonate plastics, which are used in medical devices such as intravenous administration sets, syringes, and catheters.

The researchers examined urinary BPA concentration for 55 infants and categorized each infant's medical device use as low or high, based on the number and invasiveness of devices. They found that the median urinary total BPA concentration of infants who had required four or more medical devices for three previous days was significantly higher, 36.6 micrograms per liter, than for the infants requiring three or fewer devices, 13.9 micrograms per liter. The researchers also collected and analyzed breast milk or formula samples and found that the increased BPA concentration was not associated with the infants' nutritional intake.

Citation: Duty SM, Mendonca K, Hauser R, Calafat AM, Ye X, Meeker JD, Ackerman R, Cullinane J, Faller J, Ringer S. 2013. Potential sources of bisphenol A in the neonatal intensive care unit. Pediatrics 131(3):483-489. [Synopsis]

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Global cost of childhood lead exposure

According to research supported by NIEHS, low-income and middle-income countries experience the largest burden of lead exposure, with cost measured in what are known as international dollars. An international dollar is a hypothetical currency used to compare costs from various countries. It has the same purchasing power as a dollar would have in the U.S.

The researchers calculated lead-associated loss by developing a regression model to estimate average blood lead levels and estimating the lead-attributable economic costs with an environmentally attributable fraction model. They examined only the neurodevelopmental effects of lead, which were assessed using IQ points. The investigators estimate that the total lead-associated economic loss ranges from $728.6 billion to $1.1625 trillion international dollars, including $134.7 billion in Africa, $142.3 billion in Latin America and the Caribbean, and $699.9 billion in Asia.

Citation: Attina TM, Trasande L. 2013. Economic costs of childhood lead exposure in low- and middle-income countries. Environ Health Perspect 121(9):1097-1102. [Synopsis]

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Using membrane transporters to improve crops for sustainable food production

NIEHS grantees published findings that indicate understanding the biology of plant membrane transporters may be a key contributor to the goal of global food security. With the global population predicted to grow by at least 25 percent by 2050, the need for sustainable production of nutritious foods will be crucial for human and environmental health.

The authors discuss examples where fundamental research is currently being translated into practical applications, such as showing how specialized plant membrane transporters can be used to enhance yields of staple crops, increase nutrient content, and increase resistance to key stresses, including salinity, pathogens, and aluminum toxicity. They also examine potential applications linked to breakthroughs in basic research that are yet to be applied to crop plants.

Citation: Schroeder JI, Delhaize E, Frommer WB, Guerinot ML, Harrison MJ, Herrera-Estrella L, Horie T, Kochian LV, Munns R, Nishizawa NK, Tsay YF, Sanders D. 2013. Using membrane transporters to improve crops for sustainable food production. Nature 497(7447):60-66. [Story]

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Metabolomics reveals early changes in metabolic pathways for Alzheimer's disease

With funding from NIEHS, researchers found changes in metabolic pathways that were detectable in blood plasma of Alzheimer's patients.. The findings suggest that it might be possible to identify plasma biomarkers for early Alzheimer's disease diagnosis, monitoring disease progression, and evaluating therapeutic approaches.

The researchers used a nontargeted metabolomics approach based on liquid chromatography and mass spectrometry to analyze cerebrospinal fluid and plasma samples from 45 people in the Mayo Clinic Study on Aging, and Mayo Clinic Alzheimer's Disease Research Center. In total, the investigators found 342 plasma and 351 cerebrospinal fluid significantly altered metabolites. Patients with mild cognitive impairment and Alzheimer's disease showed significant impairment in energy metabolism and other physiological functions. The researchers say that additional research, using targeted metabolomics, could identify specific panels of biomarkers.

Citation: Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. 2013. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One 8(5):e63644. [Synopsis]

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Epigenetic effects of DDT lead to obesity in later generations

A mouse study, supported in part by NIEHS, indicates that ancestral exposure to the insecticide dichlorodiphenyltrichloroethane (DDT) can promote obesity and associated disease in later generations. The findings imply that environmental exposures experienced several generations ago might influence today's rates of obesity, although the degree of involvement is not known.

The researchers found differential DNA methylation regions, which were epigenetic changes, in sperm of the third generation. Genes associated with these regions were previously shown to be associated with obesity. Although banned in the U.S., DDT is used to control malaria in other parts of the world. The researchers stressed that long-term health and economic effects of DDT exposure on future generations should be considered in areas where DDT is used.

Citation: Skinner MK, Manikkam M, Tracey R, Guerrero-Bosagna C, Haque M, Nilsson EE. 2013. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. BMC Med 11:228. [Synopsis]

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Mechanism for amyloid-beta accumulation in Alzheimer's disease

An NIEHS grantee and his colleagues report that low levels of copper can accumulate in the brain, leading to amyloid-beta plaques that are the hallmark of Alzheimer's disease. The research provides useful information for developing preventative or therapeutic approaches for controlling neurotoxic amyloid-beta levels in the aging brain.

The researchers used normal mice, a mouse model of Alzheimer's disease, and human brain endothelial cells in the study. In normal aging mice, they found that copper accumulated in brain capillaries, which was associated with a reduction in a protein that removes amyloid-beta from the brain called low-density lipoprotein receptor-related protein 1 (LRP1). The same LRP1 decrease appeared in human cells. In the mouse model of Alzheimer's disease, copper accumulated in brain capillaries and the parenchyma, unlike normal aging mice. Overall, this work demonstrated that copper's effect on amyloid-beta homeostasis in the brain depends on whether it accumulates in capillaries or in the parenchyma.

Citation: Singh I, Sagare AP, Coma M, Perlmutter D, Gelein R, Bell RD, Deane RJ, Zhong E, Parisi M, Ciszewski J, Kasper RT, Deane R. 2013. Low levels of copper disrupt brain amyloid-beta homeostasis by altering its production and clearance. Proc Natl Acad Sci U S A 110(36):14771-14776. [Synopsis]

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DNA methylation in the human genome

In mammals, 70-80 percent of all cytidine-phosphateguanosine (CpG) dinucleotides are methylated, but NIEHS-supported researchers report that only a fraction of these CpGs likely participate in genome regulation in a developmental context. The DNA methylation signatures, called differentially methylated regions, may be used to guide new, more effective approaches that examine the most informative portion of CpGs.

Genome-wide association studies showed that differentially methylated regions often contained single nucleotide polymorphisms associated with cancer and Alzheimer's disease. The investigators used their set of differentially methylated regions to correctly identify an unknown tissue sample and to classify the types of cells present in a heterogeneous sample.

Citation: Ziller MJ, Gu H, Muller F, Donaghey J, Tsai LT, Kohlbacher O, De Jager PL, Rosen ED, Bennett DA, Bernstein BE, Gnirke A, Meissner A. 2013. Charting a dynamic DNA methylation landscape of the human genome. Nature 500(7463):477-481. [Synopsis]

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Graphene sheets pierce and enter cells

NIEHS grantees report that graphene materials with micrometer-scale dimensions, known as graphene microsheets, can enter cells when their sharp protrusions pierce the cell membrane. Understanding how these graphene sheets interact with cells can help scientists develop materials that are not harmful to the body.

Confocal fluorescence and electron microscopy confirmed that graphene's rough edges and corners could pierce primary human keratinocytes, human lung epithelial cells, and murine macrophages. The imaging also showed that cells could completely internalize graphene sheets with lateral dimensions of 0.5-10 micrometers. More research is needed to understand how the microsheets affect cells, but the researchers say that microsheets might disrupt cytoskeleton and cell motility and cause problems with epithelial barriers.

Citation: Li Y, Yuan H, von dem Bussche A, Creighton M, Hurt RH, Kane AB, Gao H. 2013. Graphene microsheets enter cells through spontaneous membrane penetration at edge asperities and corner sites. Proc Natl Acad Sci U S A 110(30):12295-12300. [Synopsis]

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Improving health for low-income workers

An NIEHS grantee co-authored a paper that calls for improving the health of low-income workers by integrating health protection and health promotion programs that can be delivered at worksites, state and local health departments, community health centers, and community-based organizations. Low-income workers experience overlapping occupational and nonoccupational risks that can be worsened by limited resources and societal racism.

The authors provide six broad recommendations for reducing health inequities among low-income workers — improve access and quality of work-related data, integrate work environmental factors into care at community

health centers, improve the exchange of information and ideas, increase the integration of health and occupational health education and training, test and evaluate new approaches, and improve worker and community engagement.

Citation: Baron SL, Beard S, Davis LK, Delp L, Forst L, Kidd-Taylor A, Liebman AK, Linnan L, Punnett L, Welch LS. 2013. Promoting integrated approaches to reducing health inequities among low-income workers: applying a social ecological framework. Am J Ind Med; doi:10.1002/ajim.22174 [Online 26 March 2013]. [Synopsis]

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Father's obesity could have epigenetic effects

A study, partially supported by NIEHS, found that newborns with obese fathers had significantly less DNA methylation of the insulin-like growth factor 2 (IGF2) gene. Since reduced DNA methylation of this gene is associated with a higher risk of developing certain cancers, the study findings suggest that a father's obesity could influence his child's future health.

They examined DNA from 79 newborns whose mothers participated in the Newborn Epigenetics Study during pregnancy and also gathered information about both parents using questionnaires and medical records. Even after adjusting for several maternal and newborn characteristics, they observed a persistent inverse association between DNA methylation in the offspring and paternal obesity. The researchers say that the changes in DNA methylation could result from obesity-related factors, such as diet or having diabetes, that were not measured in the study.

Citation: Soubry A, Schildkraut JM, Murtha A, Wang F, Huang Z, Bernal A, Kurtzberg J, Jirtle RL, Murphy SK, Hoyo C. 2013. Paternal obesity is associated with IGF2 hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort. BMC Med 11:29. [Synopsis]

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In-house research

Consequences of ribonucleotide removal by topoisomerase 1

NIEHS researchers, together with collaborators at Umea University in Sweden, have determined that topoisomerase 1 (Top1), an enzyme important for uncoiling DNA during replication and transcription, can also remove ribonucleotides incorporated into DNA during replication.

Using yeast as a model system, the scientists studied strains that were genetically engineered to incorporate a large number of ribonucleotides into DNA, and were defective in RER, Top1, or both. In the absence of RER, Top1 incised the DNA backbone where ribonucleotides were present, thereby initiating the removal of about 5,000 ribonucleotides from the genome. Because the enzymes involved in processing ribonucleotides in DNA are conserved, the authors suggest that the genome instability resulting from Top1 cleavage at ribonucleotides in DNA may be relevant to Aicardi-Goutieres syndrome, and possibly to other autoinflammatory disorders.

Citation: Williams JS, Smith DJ, Marjavaara L, Lujan SA, Chabes A, Kunkel TA. 2013. Topoisomerase 1-mediated removal of ribonucleotides from nascent leading-strand DNA. Mol Cell 49(5):1010-1015. [Synopsis]

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The role of p53 during bacterial pneumonia

NIEHS researchers recently discovered that the transcription factor p53 modulates host defense through regulating microbicidal function and fate of phagocytes during bacterial pneumonia. This study revealed a fundamental link between defense of genome and host during environmental insult.

The authors used mice that had their p53 genes deleted (p53-/-) or in which p53 was pharmacologically inhibited. Both sets of mice displayed enhanced clearance of extracellular bacteria during pneumonia. The lungs of p53-/- mice displayed genome-wide induction of NF-kappaB response element-enriched proinflammatory genes in the steady state, and enhanced induction of cytokines upon infection. Despite enhanced bacterial clearance, infected p53-/- mice suffered increased mortality from pneumonia, likely due to aggravated lung injury from an overexuberant immune response.

Citation: Madenspacher JH, Azzam KM, Gowdy KM, Malcolm KC, Nick JA, Dixon D, Aloor JJ, Draper DW, Guardiola JJ, Shatz M, Menendez D, Lowe J, Lu J, Bushel P, Li L, Merrick BA, Resnick MA, Fessler MB. 2013. p53 integrates host defense and cell fate during bacterial pneumonia. J Exp Med 210(5):891-904. [Synopsis]

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DNA methylation could predict breast cancer risk

NIEHS scientists have discovered DNA methylation in blood could prove to be an effective indicator of who will develop breast cancer. Using the NIEHS Sister Study, a nationwide cohort of women, ages 35-74, whose sisters had breast cancer, researchers used DNA extracted from white blood cell samples and assessed methylation at 27,000 sites across the genome.

The team found evidence that women who subsequently develop breast cancer have different blood methylation profiles than women who remain cancer free, and that these methylation differences are detectable months to years before the clinical diagnosis of breast cancer. The scientists also found that epigenetic modifications were significantly more accurate in predicting who will develop breast cancer than the known risk factors and polymorphisms, although they caution their test is not yet accurate enough for clinical use.

Citation: Xu Z, Bolick SC, DeRoo LA, Weinberg CR, Sandler DP, Taylor JA. 2013. Epigenome-wide association study of breast cancer using prospectively collected Sister Study samples. J Natl Cancer Inst 105(10):694-700. [Synopsis] [Story]

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Observing a DNA polymerase using time-resolved crystallography

NIEHS scientists utilized a technique called time-resolved crystallography to examine how a model human DNA polymerase beta (pol beta) chooses a nucleotide during DNA synthesis. The technique confirmed features of the computational results the researchers had generated earlier, but also revealed pol beta changes its shape, depending on whether it incorporates a complementary base pair or correct nucleotide.

The study also found that pol beta forms a third metal binding site during correct, but not incorrect, nucleotide insertion, and pyrophosphate more easily dissociates after incorrect nucleotide insertion. Prior to this evidence, researchers believed that only two metal ion-binding sites were used by all polymerases in their mechanism of action and pyrophosphate was released instantly. The researchers hope this information will lead to a better understanding of the potential causes of disease.

Citation: Freudenthal BD, Beard WA, Shock DD, Wilson SH. 2013. Observing a DNA polymerase choose right from wrong. Cell 154(1):157-168. [Synopsis] [Story]

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Early mouse development influences norepinephrine neuron diversity

Applying a technique known as intersectional genetic fate mapping to the developing mouse brain, NIEHS researchers characterized neurons that produce and release norepinephrine (NE), a hormone and neurotransmitter. As a result, they were the first to develop a map of communication pathways in the growing mouse brain. Since NE neurons are involved in several physiological processes, such as food intake and sleep, and are lost in Parkinson's and Alzheimer's diseases, understanding NE neurons will lead to improvements in human health.

The scientists found that NE neurons derived from a specific rhombomere, or segment in the developing hindbrain, shared common features in the adult brain. The group also determined that these various subgroups of NE neurons were talking to many different parts of the adult mouse brain. Prior to this study, neuroscientists believed only one group of NE neurons communicated with the cerebral cortex.

Citation: Robertson SD, Plummer NW, de Marchena J, Jensen P. 2013. Developmental origins of central norepinephrine neuron diversity. Nat Neurosci 16(8):1016-1023. [Synopsis] [Story]

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APOBEC cytidine deaminases generate many mutations in human cancers

NIEHS researchers and colleagues at the Broad Institute of MIT and Harvard in Cambridge, Mass., report that a set of proteins, known to protect against retroviruses and retrotransposons, can cause mutations that are widespread in human cancers.

These mutations, which have a characteristic mutation signature, are produced by apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases. Scientists from the team developed an analysis to detect and statistically evaluate the prevalence of APOBEC mutations in 2,680 tumor samples, collected from 14 types of cancer. Using this strategy, they discovered that APOBEC enzymes produce the majority of mutations in some bladder, cervical, breast, head and neck, and lung tumors. They also found APOBEC signature mutations specifically in genes that have been implicated in cancer development and progression, highlighting a potential link between APOBEC enzymes and carcinogenesis.

Citation: Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, Kiezun A, Kryukov GV, Carter SL, Saksena G, Harris S, Shah RR, Resnick MA, Getz G, Gordenin DA. 2013. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 45(9):970-976. [Synopsis] [Story]

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Tanning gene linked to increased risk of testicular cancer

Collaborative efforts between NIEHS and the University of Oxford have found that a variant in a gene that promotes skin tanning is associated with an increased risk of testicular cancer. This variant, also known as a single-nucleotide polymorphism (SNP), is located in the KITLG gene, a gene that is controlled by the tumor suppressor p53, the most commonly mutated gene in human cancers.

The SNP confers protection to the skin against sun damage, by increasing production of pigmented cells called melanocytes. The authors suggest that during human evolution, this SNP has become more common in populations with light skin for this beneficial reason. However, in testicular cells, the situation appears reversed. The researchers hypothesize that increased cell division driven by the KITLG SNP may permit the growth of tumor cells, increasing cancer risk for those that carry the gene variant.

Citation: Zeron-Medina J, Wang X, Repapi E, Campbell MR, Su D, Castro-Giner F, Davies B, Peterse EF, Sacilotto N, Walker GJ, Terzian T, Tomlinson IP, Box NF, Meinshausen N, De Val S, Bell DA, Bond GL. 2013. A polymorphic p53 response element in KIT ligand influences cancer risk and has undergone natural selection. Cell 155(2):410-422. [Synopsis] [Story]

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Assessing cockroach allergen exposure via its structure

NIEHS scientists and their collaborators have obtained the crystal structure of the cockroach allergen Bla g 1. It is the first structure to be solved for this group of insect proteins. Determining the structure of Bla g 1 allows for the standardization of assays that measure it in absolute units.

The X-ray crystallography revealed that Bla g 1 had a novel fold with the capacity to bind various lipids. Further analysis using mass spectrometry and nuclear magnetic resonance indicated that Bla g 1 could accommodate a variety of hydrophobic ligands that could be important for a variety of physiological purposes, including sensitizing humans. Overall, the authors believe that this work would allow a better assessment of Bla g 1 exposure, which is important in evaluating new intervention strategies. Additionally, the structure may be useful in designing new immunotherapies.

Citation: Mueller GA, Pedersen LC, Lih FB, Glesner J, Moon AF, Chapman MD, Tomer KB, London RE, Pomes A. 2013. The novel structure of the cockroach allergen Bla g 1 has implications for allergenicity and exposure assessment. J Allergy Clin Immunol 132(6):1420-1426. [Synopsis]

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Probiotics use during pregnancy may reduce diseases in children

Based on data from the Norwegian Mother and Child Cohort Study (MoBa), a research team led by NIEHS scientists have determined that, for the general population, probiotics consumed during pregnancy and infancy may help prevent atopic eczema and rhinoconjunctivitis in early childhood.

The researchers used data from more than 40,000 mother and children pairs participating in the MoBa study, which recruited Norwegian women between 1999 and 2008. Thirty-seven percent of pregnant mothers in the study consumed these probiotic milk and yogurt products, and 18 percent of them also gave their child probiotic milk products after 6 months of age. The researchers found that maternal probiotic milk consumption in pregnancy, as well as consumption during early childhood, was associated with a slightly reduced relative risk of atopic eczema at 6 months, and rhinoconjunctivitis symptoms between 18-36 months.

Citation: Bertelsen RJ, Brantsaeter AL, Magnus MC, Haugen M, Myhre R, Jacobsson B, Longnecker MP, Meltzer HM, London SJ. 2013. Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases. J Allergy Clin Immunol; doi:10.1016/j.jaci.2013.07.032 [Online 10 September 2013]. [Synopsis]

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Identification of novel immune regulatory elements and epigenetic plasticity in memory lymphocytes

A collaborative team, led by NIEHS investigators, determined that the global reprogramming of the epigenome, or the epigenetic modifications of DNA, permits adult somatic cells to differentiate into diverse cell types.

To investigate the function of DNA methylation changes associated with immune activation, the authors determined whether activation-induced differentially methylated regions were enriched for regulatory elements and also found that Alu elements display differential methylation patterns, correlated with decreased expression of the de novo methyltansferase DNMT3A in GC B cells. The authors propose that the loss of DNA methylation during the naive to GC B cell transition permits these cells to differentiate toward memory or plasma cell fates, and to generate the differential response to antigenic challenge.

Citation: Lai AY, Mav D, Shah R, Grimm SA, Phadke D, Hatzi K, Melnick A, Geigerman C, Sobol SE, Jaye DL, Wade PA. 2013. DNA methylation profiling in human B cells reveals immune regulatory elements and epigenetic plasticity at Alu elements during B-cell activation. Genome Res 23(12):2030-2041. [Synopsis]

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National Toxicology Program research

Spontaneous mesotheliomas in F344/N rats are characterized by dysregulation of cellular growth and immune function pathways

Scientists wanted to understand why aged male Fischer 344/N rats are prone to developing spontaneous peritoneal mesotheliomas that arise predominantly from the tunica vaginalis of the testes.

Global gene expression profiles of spontaneous mesotheliomas from these rats were taken from 2-year National Toxicology Program (NTP) carcinogenicity bioassays. The analyses determined that these tumors were associated with upregulation of various growth factors, oncogenes, cytokines, pattern recognition response receptors, and pathogen-associated molecular patterns receptors, and the production of reactive oxygen and nitrogen species, as well as downregulation of apoptosis pathways. Alterations in these pathways, in turn, trigger molecular responses that stimulate cell proliferation and promote tumor survival and progression.

Citation: Blackshear PE, Pandiri AR, Ton TV, Clayton NP, Shockley KR, Peddada SD, Gerrish KE, Sills RC, Hoenerhoff MJ. 2013. Spontaneous mesotheliomas in F344/N rats are characterized by dysregulation of cellular growth and immune function pathways. Toxicol Pathol; doi:10.1177/0192623313501894 [Online 26 August 2013].

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Chronic exposure of renal stem cells to inorganic arsenic induces a cancer phenotype

Evidence indicated that developmental arsenic exposure impacts renal carcinogenesis in humans and mice, and since emerging theory suggested cancer may be a disease of stem cells (SCs), the researchers wanted to see if inorganic arsenic targets SCs, or partially differentiated progenitor cells (PCs), for oncogenic transformation.

Scientists chronically exposed the rat kidney SC/PC cell line, RIMM-18, to low-level arsenite (500 nM) for up to 28 weeks, and found that dysregulation of SC maintenance genes and signaling pathways are common during oncogenesis. During arsenite exposure, expression of several genes associated with normal kidney development and SC regulation and differentiation, such as Wt-1, Wnt-4, and Bmp-7 were aberrantly altered. These data reveal that multipotent SCs may be targets of arsenic during renal carcinogenesis.

Citation: Tokar EJ, Person RJ, Sun Y, Perantoni A, Waalkes MP. 2013. Chronic exposure of renal stem cells to inorganic arsenic induces a cancer phenotype. Chem Res Toxicol 26(1):96-105.

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RNASeq profiling reveals novel hepatic gene expression pattern in aflatoxin B1 treated rats

Researchers used deep sequencing (DESeq) to investigate the subchronic effects of 1 ppm aflatoxin B1 (AFB1), a potent hepatocarcinogen, on the male rat liver transcriptome prior to onset of histopathological lesions or tumors.

DESeq analysis identified 1,026 differentially expressed transcripts at greater than two-fold change compared to 626 transcripts by microarray due to base pair resolution of transcripts by RNA-Seq, probe placement within transcripts or an absence of probes to detect novel transcripts, splice variants and exons. They found that the rat transcriptome contains many previously unidentified, AFB1-responsive exons and transcripts supporting RNA-Seq's capabilities to provide new insights into AFB1-mediated gene expression leading to hepatocellular carcinoma.

Citation: Merrick BA, Phadke DP, Auerbach SS, May D, Stiegelmeyer SM, Shah RR, Tice RR. 2013. RNA-Seq profiling reveals novel hepatic gene expression pattern in aflatoxin B1 treated rats. PLoS One 8(4):e61768.

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Recruitment of normal stem cells to an oncogenic phenotype by noncontiguous carcinogen-transformed epithelia depends on the transforming carcinogen

NTP scientists wanted to know whether the recruitment of normal stem cell (NSCs) into cancer stem cells (CSCs) by noncontact co-culture was specific to arsenic-transformed isogenic malignant epithelial cells (MECs). Therefore, they utilized co-culture to examine the effects of neighboring noncontiguous cadmium-transformed MECs (Cd-MECs) and N-methyl-N-nitrosourea-transformed MECs (MNU-MECs) on NSCs.

They found that Cd-MECs can recruit nearby NSCs into a CSC-like phenotype, but MNU-MECs do not. Thus, the recruitment of NSCs into CSCs by nearby MECs is dependent on the carcinogen originally used to malignantly transform the MECs.

Citation: Xu Y, Tokar EJ, Person RJ, Orihuela RG, Ngalame NN, Waalkes MP. 2013. Recruitment of normal stem cells to an oncogenic phenotype by noncontiguous carcinogen-transformed epithelia depends on the transforming carcinogen. Environ Health Perspect 121(8):944-950.

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Bisphenol A affects androgen receptor function via multiple mechanisms

BPA is an endocrine disrupting compound that affects the development and function of the female and male reproductive system. Since its mechanism of action is unclear, the research team initiated the study to investigate how BPA may affect ten different nuclear receptors.

The scientists examined stable cell lines that contained an individual nuclear receptor ligand binding domain-linked to the beta-Gal reporter using a quantitative high throughput screening format in the Tox21 screening program. The results showed that two receptors, estrogen receptor alpha (ERalpha) and androgen receptor (AR), are affected by BPA in opposite direction. The team also performed transient transfection experiments with full-length receptors and their corresponding response elements linked to luciferase reporters, as well as evaluating BPA effects in nuclear translocation assays using EGPF-tagged receptors. The authors found that BPA enhanced ERalpha nuclear foci formation, but at a 100-fold higher concentration. BPA was unable to activate AR.

Citation: Teng C, Goodwin B, Shockley K, Xia M, Huang R, Norris J, Merrick BA, Jetten AM, Austin CP, Tice RR. 2013. BisphenolA affects androgen receptor function via multiple mechanisms. Chem Biol Interact 203(3):556-564.

The contributing writer for the NIEHS grantee research summaries was Nancy Lamontagne, a science writer with MDB Inc., a contractor for the NIEHS Division of Extramural Research and Training, Superfund Research Program, and Worker Education and Training Program.

NIEHS research summaries were written by Aleksandra Adomas, Ph.D., a research fellow in the NIEHS Laboratory of Molecular Carcinogenesis Eukaryotic Transcriptional Regulation Group; Jacqueline Powell, Ph.D., a former NIEHS postdoctoral fellow and a writer and analyst with Education and Training Systems International; Monica Frazier, Ph.D., an IRTA fellow in the NIEHS Laboratory of Molecular Genetics Mechanisms of Mutation Group; Melissa Kerr, a chemistry student at North Carolina Central University and intern in the NIEHS Office of Communications and Public Liaison; Mallikarjuna Metukuri, Ph.D., a research fellow in the Metabolism, Genes, and Environment Group of the NIEHS Laboratory of Signal Transduction; Bailey Schug, a health promotion and nutrition student at Appalachian State University and an intern in the NIEHS Office of Communications and Public Liaison; Deepa Singh, Ph.D., a visiting fellow in the NIEHS Laboratory of Molecular Genetics Mechanisms of Mutation Group.

NTP research summaries were contributed by NTP staff and submitted by their branch chiefs.

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Lewis Cantley discusses cancer metabolism in Rodbell Lecture

By Sheila Yong

The prestigious Dr. Martin Rodbell Lecture Series Seminar Dec. 10 featured Lewis Cantley, Ph.D., discussing his research into phosphoinositide 3-kinase (PI3K) signaling and its role in cancer progression. In his talk, "PI3K and cancer metabolism," Cantley presented exciting discoveries he and his colleagues have made over the past several decades in PI3K signaling, and how they have translated their findings into new treatment strategies for various cancers and diseases.

Cantley's research has spurred drug development efforts for treating various cancers and hereditary familial syndromes. One significant success is everolimus, a drug used to treat lymphangioleiomyomatosis (LAM), a rare lung disease that affects mostly women. "In the past, LAM patients would have to undergo lung transplant. Unfortunately, the transplanted lung would eventually fail and the patients would die anyway," Cantley explained. With everolimus, LAM patients can now lead normal lives, without relying on supplemental oxygen or undergoing lung transplant. (Photo courtesy of Steve McCaw)

Cantley is director of the Cancer Center at Weill Cornell Medical College and New York-Presbyterian Hospital, and the Margaret and Herman Sokol Professor in Oncology Research at Weill Cornell.

James Putney, Ph.D., head of the NIEHS Calcium Regulation Group in the Laboratory of Signal Transduction, and a longtime friend, compared Cantley's scientific career to Rodbell's. "Lew is an especially appropriate speaker for the Rodbell

lecture, because both he and Marty initially conducted experiments that yielded unexpected results. While most people would have moved on to something else, they realized that these results were actually telling them something important."

Cantley's scientific instinct and dedication led him to the discovery of PI3K signaling in 1988, and subsequent advances in cancer research.

Researchers from across many disciplines attended Cantley's lecture, many of whom were seated in the front row. Shown, right to left, are senior scientists Jerrel Yakel, Ph.D., and Serena Dudek, Ph.D., from the NIEHS Laboratory of Neurobiology; Division of Intramural Research and Division of NTP senior scientist Richard Paules, Ph.D.; NTP Associate Director John Bucher, Ph.D.; biologist Wendy Jefferson, Ph.D., from the NIEHS Reproductive Medicine Group; and NIEHS Scientific Director Darryl Zeldin, M.D. (Photo courtesy of Steve McCaw)

Crossing the line between normal cell growth and cancer

Cantley began his lecture with a description of the insulin signaling pathway, which controls glucose metabolism. Upon insulin stimulation, insulin receptors undergo phosphorylation and become active. This phenomenon triggers a signaling cascade mediated by class IA PI3Ks, which generate phosphatidylinositol (3,4,5)-triphosphate (PIP3) from phosphatidylinositol (4,5)-bisphosphate (PIP2), a phospholipid found in the cell membrane. PIP3 serves as a docking site for several downstream proteins, including protein kinases AKT and PDK1.

These proteins, in turn, become activated and translocate to other locations in the cell to facilitate various downstream processes, thus promoting survival and growth. Produced by oncogenes, these proteins cause cancer when their functions are misregulated.

On the other hand, several proteins along the pathway, such as PTEN and tuberin, serve as brakes to halt cell growth when nutrients and growth factors are low. This regulatory mechanism ensures that the pathway is active only when conditions are favorable. These tumor suppressors protect the cells from cancer.

Cancer forms when cells grow, even when nutrients or growth factors are absent, due to continuous activation of the growth pathway. "Mutations or amplification of these oncogenes, and the loss of function of these tumor suppressor genes, account for an incredible fraction of human cancers," Cantley emphasized, citing the Cancer Genome Database.

According to Cantley, 70-95 percent of common human cancers present with at least one mutation in the PI3K signaling network, especially women's cancers. Cantley now leads a team of prominent researchers funded by a $15 million grant from Stand Up To Cancer to design clinical trials that will determine which patients are likely to benefit from PI3K inhibitors.

Cantley's lecture appealed to people across the Institute, such as Sean Chiou, Ph.D., contractor for the NIEHS Computer Technology Branch. A former bench scientist, Chiou performed research on insulin signaling during his graduate school days. (Photo courtesy of Steve McCaw)

A new player in cancer metabolism

Ten years after discovering PI3K, Cantley's group identified phosphatidylinositol 5-phosphate 4-kinase (PI5P4K), which converts phosphatidylinositol 5-phosphate to PIP2. "It has been very frustrating to figure out what this enzyme does," Cantley said. Mice that lack either the alpha or beta isoform of PI5P4K exhibit little to no phenotype, while mice lacking both isoforms are not viable. Interestingly, mice that lack both copies of p53, while retaining one copy of PI5P4K-beta, are protected from cancer.

p53 is among the most heavily studied tumor suppressors, and is frequently lost in many types of cancers. Not surprisingly, many of these tumors also exhibit high levels of PI5P4K expression. Through detailed experimentation, using mouse models and human cancer cell lines, Cantley's group determined that both p53 and PI5P4K provide alternative pathways for regulating glucose metabolism and suppressing oxidative stress.

When cells lose p53, the PI5P4K pathway becomes essential in combating oxidative stress. Therefore, cancer cells lacking p53 upregulate the PI5P4K pathway to promote cell survival. "We believe that PI5P4K is a good target for treating tumors that lack p53 while sparing normal tissues," he concluded.

Speakers in the Rodbell Lecture Series receive a statue depicting the hand of Nobel laureate and former NIEHS Scientific Director, Martin Rodbell, Ph.D., holding the three key elements involved in cell signaling. Sculptor Carl Regutti, who created the statue and attended the Rodbell lecture in 2012 as a family guest, died in April 2013. (Photo courtesy of Steve McCaw)

(Sheila Yong, Ph.D., is a visiting fellow in the NIEHS Inositol Signaling Group.)

With his Rodbell statue in hand, Cantley joined, left to right, NIEHS and NTP Director Linda Birnbaum, Ph.D., Barbara Rodbell, and Putney for a group photo. Barbara, Rodbell's widow and honored guest, has attended every Rodbell lecture, since Rodbell gave the first talk in the series in 1998. (Photo courtesy of Steve McCaw)

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Seminar showcases Toxicogenetics Challenge winners

By Ernie Hood

Some 60 scientists gathered Dec. 2 at NIEHS for an informative seminar, "Crowdsourcing Tox21 Qualitative High Throughput Screening Data," focusing on the NIEHS–NCATS–UNC DREAM Toxicogenetics Challenge.

Speakers described the Tox21 project that generated the data used in the Challenge, the innovative approaches employed by the teams that won the two subchallenges, and the value of crowdsourcing to advance and accelerate scientific knowledge.

The June 11-Sept. 15 Challenge was co-sponsored by NIEHS, the National Center for Advancing Translational Sciences (NCATS), the Carolina Center for Computational Toxicology (CCCT) at the University of North Carolina at Chapel Hill (UNC), DREAM (Dialogue for Reverse Engineering Assessments and Methods), and Sage Bionetworks. NIEHS and NTP Director Linda Birnbaum, Ph.D., moderated the seminar. "The Challenge represents a groundbreaking new direction for toxicity testing," she said.

The bigger picture of challenges — the strength of crowdsourcing

DREAM representative Gustavo Stolovitzky, Ph.D., from the IBM Computational Biology Center, called into the seminar to discuss "Data Re-use and the Wisdom of Crowds." He provided a history of crowdsourcing in computational biology, and explained why it makes sense to conduct challenges and leverage the wisdom of crowds to advance biomedical research. He pointed out that data sets multiply their impact by becoming accessible to a wide segment of the scientific community, and noted that after a competition is complete, the crowd's wisdom can be further tapped by fostering collaborative research.

The challenge data source

Nour Abdo, a doctoral student in the Department of Environmental Sciences and Engineering at UNC, spoke about a Tox21 project known as the 1000 genomes toxicity screening project, which utilizes the power of human genome variation for population-scale *in vitro* testing.

The project provided the raw data supplied to the Challenge competitors. Cytotoxicity data was generated on 1,086 human lymphoblast cell lines, representing nine populations from five continents, in an assay with 179 environmental chemicals at eight concentrations. The study generated roughly 2.6 million data points — the largest-scale experiment to date, in terms of the number of cell lines used and chemicals screened.

Abdo presented data from the 1000 genomes toxicity screening project. She said that the current paradigm in toxicity screening is based on using in vivo data to derive a human reference dose for individual chemicals, but the future paradigm being developed will use in vitro data for human reference dose determination. (Photo courtesy of Steve McCaw)

As Abdo explained, the population-based approach appears to be far more powerful than traditional *in vitro* toxicity testing. "The current toxicity testing approaches usually include model systems with very homogeneous genetics, so only the hazard can be evaluated. Our approach incorporates genetic diversity, allowing testing of not only hazards, but also the variability in the population," she said.

The population-based approach allows quantitative assessment of both hazard and interindividual variability in chemical toxicity, as well as identification of susceptible sub-populations, new understanding of the genetic determinants of interindividual variability, and generation of testable hypotheses about toxicity pathways. The data can also be used to build predictive *in silico* models.

Among the many attentive listeners at the challenge seminar were, from right, NTP Associate Director John Bucher, Ph.D., and Health Scientist Administrator Jerry Heindel, Ph.D. (Photo courtesy of Steve McCaw)

The challenge winners

The challenge included two subchallenges. Subchallenge 1, which generated 99 submissions from 34 teams, involved using the supplied data to accurately predict individual responses to compound exposure, based on genomic information. Subchallenge 2, with 85 submissions from 24 teams, called for development of a model to accurately predict how a particular population would respond to certain types of chemicals. Teams from the same institution, the Quantitative Biomedical Research Center (QBRC) at the University of Texas Southwestern Medical Center, were named best performer in both of the subchallenges.

Joined at the podium by Birnbaum, Tang, right, was clearly delighted with the response to her presentation. (Photo courtesy of Steve McCaw)

Hao Tang, Ph.D., lead scientist for the subchallenge 2 team and a member of the subchallenge 1 team, described the methods the teams employed successfully to come in first in both segments of the competition. In subchallenge 1, the key to robust toxicity predictivity in the model was the use of fine mapping based on the geographic area where a cell line originated. For subchallenge 2, the model was enhanced, by incorporating supervised feature selection and multimodal distribution-based analysis into standard quantitative structure-activity relationship modeling.

"Those approaches, along with others contributed by many of the other challenge teams, will undoubtedly improve our ability to use both genomic data and chemical data to accurately predict cytotoxicity *in vitro*," said NTP Biomolecular Screening Branch Chief Raymond Tice, Ph.D. "The Challenge accomplished exactly what we had hoped it would."

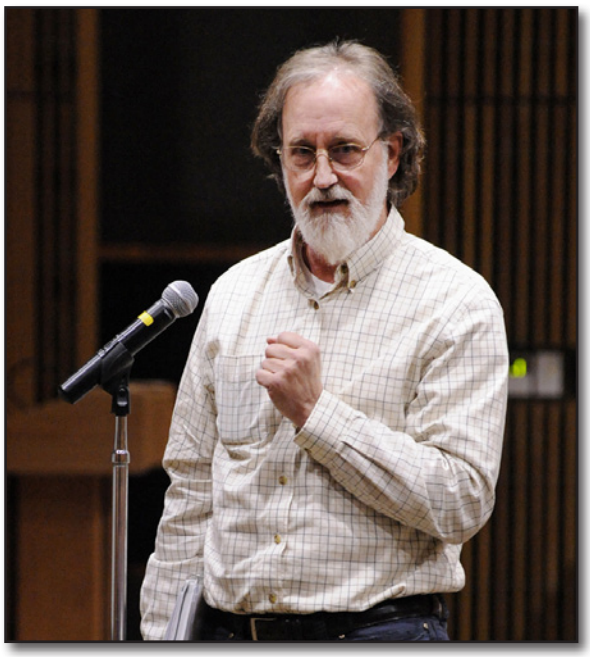
Results of the challenge will be published in the journal Nature Biotechnology.

(Ernie Hood is a contract writer with the NIEHS Office of Communications and Public Liaison.)

Stolovitzky said that the wisdom of crowds means that the aggregate results from multiteam competitions are more robust and more predictive than any individual results. (Photo courtesy of Gustavo Stolovitzky)

Biostatistician Fred Wright, Ph.D., from CCCT, said the 1000 genomes toxicity screening project has shown that, while there is much evidence of heritability in susceptibility to environmental exposures, it can be difficult to isolate. "What Nour has shown is that, somehow, there is signal lurking there, but it's spread out amongst a lot of small effect variants throughout the genome," he said, in response to a question from the audience. (Photo courtesy of Steve McCaw)

Ivan Rusyn, M.D., Ph.D., also from CCCT, elaborated on a point made by Tang, as he responded to a question from an attendee. "To me, as a toxicologist, one of the most important outcomes of these challenges was that it's not necessarily that we need to collect more data, but what we can do with the data that we have." (Photo courtesy of Steve McCaw)



Environmental Genomics Group lead Douglas Bell, Ph.D., asked about the relative merits of the array data versus RNA-Seq data, as used in the challenge. (Photo courtesy of Steve McCaw)

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NTP panel peer reviews substances for potential listing as carcinogens

By Ernie Hood

A panel of experts peer-reviewed the two most recent NTP draft Report on Carcinogens (RoC) documents, known as monographs, at a public meeting Dec. 12-13 at NIEHS.

The panel was charged with reviewing the draft monographs for *ortho*-Toluidine and pentachlorophenol and by-products of its synthesis. The monographs contain the rationale and background information to support NTP listing recommendations for inclusion in the RoC. The panel voted on whether the scientific evidence supports the listing recommendations, which can characterize a substance as either "known to be human carcinogens" or as "reasonably anticipated to be human carcinogens."

After thoughtful discussions, the panel chaired by Kenneth McMartin, Ph.D., a professor in the Department of Pharmacology, Toxicology, and Neuroscience at Louisiana State University Health Sciences Center, recommended listing *ortho*-Toluidine as a known human carcinogen and pentachlorophenol and by-products of its synthesis as reasonably anticipated to be a human carcinogen.

Ruth Lunn, Dr.P.H., director of the Office of the Report on Carcinogens, briefed the peer review panel on the process for preparing the draft monographs, and presented much of the information on ortho-Toluidine. (Photo courtesy of Steve McCaw)

Monograph on ortho-Toluidine

The substance *ortho*-Toluidine is used to make dyes, rubber chemicals, herbicides, and the local anesthetic prilocaine. It has been listed in the RoC since 1983 as reasonably anticipated to be a human carcinogen. Since then, several cancer studies have been published in peer-reviewed literature, and the International Agency for Research on Cancer (IARC) has concluded that the compound is carcinogenic to humans. For these reasons, it was selected for a reevaluation and a possible change in RoC listing status.

NTP staff highlighted studies, presented in the draft monograph, showing credible evidence of an association between urinary-bladder cancer and exposure to *ortho*-Toluidine, based on consistent findings across human studies. Evidence from studies in experimental animals and on mechanisms of carcinogenicity also supports that finding. The peer-review panel unanimously concurred with the NTP's preliminary listing recommendation that *ortho*-Toluidine is known to be a human carcinogen.

NTP Health Scientist Gloria Jahnke, D.V.M., presented most of the information on pentachlorophenol and by-products of its synthesis to the panel. (Photo courtesy of Steve McCaw)

Monograph on pentachlorophenol and by-products of its synthesis

Studies evaluating the carcinogenicity of pentachlorophenol and by-products of its synthesis were discussed in great length and

detail among the peer-review panel and RoC staff, to determine if there was sufficient evidence to support NTP's preliminary listing recommendation of known to be a human carcinogen, with non-Hodgkin's lymphoma as the primary cancer endpoint of concern in these studies.

Pentachlorophenol is a chlorinated aromatic compound that was used in the U.S. as a commercial and residential wood preservative and multipurpose biocide, until it was restricted in the mid-1980s to non-residential use. Today, it is limited to commercial wood preservation in items such as utility poles, fence posts, and railroad ties.

The panel members stated that overall the evidence of carcinogenicity from the studies in human cancer was limited. They agreed that there was one very good study that found an association between exposure to pentachlorophenol and by-products of its synthesis, but thought the evidence from the other studies was much more limited, due to their small size and potential for confounding factors.

Following the robust discussion of pentachlorophenol and the by-products of its synthesis, NTP Associate Director John Bucher, Ph.D., right, praised the panel's work. "This has been one of the more detailed and very careful reviews that I've seen any of our expert panels engage in," he said. "The effort you've put in on this is quite remarkable." Next to Bucher is Mary Wolfe, Ph.D., director of the NTP Office of Liaison Policy and Review, who briefed the panel on the scientific issues raised by public comments regarding both substances under review. (Photo courtesy of Steve McCaw)

The panel also struggled over whether the candidate substance should be pentachlorophenol itself or pentachlorophenol and the byproducts of its synthesis. Technical-grade pentachlorophenol is a mixture, with up to 10 percent of the substance being by-products of its synthesis, many of which are dioxin-like compounds that may contribute to its carcinogenicity.

At the end of the day, the panel voted to recommend changing the NTP preliminary listing decision of known to be human carcinogen, to reasonably anticipated to be a human carcinogen, based on limited evidence from studies in humans. The listing recommendation also took into consideration the sufficient evidence of carcinogenicity of pentachlorophenol, and pentachlorophenol and by-products of its synthesis, from studies in experimental animals and supporting mechanistic evidence.

Panel member Laura Beane Freeman, Ph.D., tenure track researcher at the National Cancer Institute, agreed with the panel's recommendation to change the pentachlorophenol and the by-products of its synthesis listing. "I don't view the whole body of epidemiologic evidence as being supportive of pentachlorophenol being listed as a carcinogen," she said. (Photo courtesy of Steve McCaw)

Next Steps

The NTP will consider the panel's comments and public comments, as it makes revisions to the draft monographs. The updated monographs will be presented to the NTP Board of Scientific Counselors at the next meeting in April 2014.

(Ernie Hood is a contract writer with the NIEHS Office of Communications and Public Liaison.)

Panelist Allan Smith, M.D., Ph.D., of the University of California, Berkeley, center, contributed many thoughtful remarks during the discussions about the two draft monographs. (Photo courtesy of Steve McCaw)

Peer-review member Gabriele Sabbioni, Ph.D., of Tulane University, asked several pointed questions during the course of the proceedings on the review of ortho-Toluidine. (Photo courtesy of Steve McCaw)

Stephen Nesnow, Ph.D., a retired senior scientist at the National Health and Environmental Effects Research Laboratory at the U.S. Environmental Protection Agency in Research Triangle Park, N.C., paid close attention to the data being presented on the two substances under review. (Photo courtesy of Steve McCaw)

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Preterm birth linked with maternal phthalate exposure during pregnancy

By Nancy Lamontagne

Researchers led by NIEHS Superfund Research Program grantee John Meeker, Sc.D., from the University of Michigan (UM), report that women with the highest levels of phthalate exposure during pregnancy had up to five times the odds of preterm birth, compared to women with the lowest exposure. Phthalates can be found in food stored or processed in plastic; plastic products, such as shower curtains and vinyl flooring; and personal care products, such as deodorants and lotions.

"Preterm birth is a serious public health challenge, and rates are significantly higher than they were 20 to 30 years ago," Meeker explained. "Explanations for this increase remain elusive. If environmental factors, such as exposure to phthalates, are indeed a cause of preterm birth it may represent a modifiable risk factor that could lead to effective interventions."

Meeker is an associate professor of environmental health sciences and associate dean for research at the UM School of Public Health. (Photo courtesy of UM School of Public Health)

Adding evidence

Meeker previously published results from a small pilot study that showed a link between phthalate exposure and preterm birth. The new study, published in JAMA Pediatrics and co-authored by UM doctoral student Kelly Ferguson and Thomas McElrath, M.D., Ph.D., of Brigham and Women's Hospital, builds on the pilot study, by using a nested case control design to study a much larger group of women. They also analyzed urine samples from up to three time points during pregnancy, to better determine overall phthalate exposure.

Ferguson, a doctoral student in environmental health sciences at UM, is first author of the new paper. (Photo courtesy of Kelly Ferguson)

The study included 130 mothers who had delivered prior to 37 weeks of completed gestation, and 352 control mothers who delivered at or after 37 weeks. The researchers examined the nine phthalate metabolites of four phthalates and found that, depending on the individual phthalate, women with the highest levels of exposure to four of the phthalates during pregnancy had two- to five-times the odds of preterm birth compared to those with the lowest exposure.

The researchers found the strongest dose-dependent associations with maternal levels of the two di-2-ethylhexyl phthalate (DEHP) metabolites, mono-(2-ethyl)-hexyl phthalate (MEHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and the summed levels of all the DEHP metabolites.

Subset of preterm births shows stronger association

The researchers also analyzed a subset of 57 mothers with preterm deliveries preceded by spontaneous preterm labor or preterm premature rupture of the membranes. When this group was examined alone, the odds ratios increased for all the phthalate metabolites. This finding indicates a stronger association between phthalate exposure and spontaneous preterm birth, than the association with all preterm births.

"One of the limitations to studying factors associated with preterm birth has been that it is a heterogeneous outcome with a number of different underlying conditions or biological mechanisms that are possible," Meeker said. "Restricting cases to those with spontaneous preterm birth was important to try and narrow down a potentially more homogeneous group. Our hypothesis was that phthalates may cause biological responses, such as altered endocrine function, inflammation, or oxidative stress, which may lead to a spontaneous preterm birth."

The researchers caution that, before implementing interventions aimed at decreasing phthalate exposure, more studies are needed to confirm these findings, to examine the sources of the phthalates, and to better understand the mechanisms involved. Phthalate exposure might be reduced by eating fresh foods that have undergone less processing and packaging, and by purchasing phthalate-free plastics and personal care products, but Meeker noted that research on the effectiveness of these activities in reducing exposure remains limited.

The researchers are continuing to explore biological mechanisms that may be involved in the associations they observed, and whether certain populations may be particularly susceptible to environmental influences on preterm birth. They are also examining sources, pathways, and routes of exposure for pregnant women, to help inform interventions.

Citation: Ferguson KK, McElrath TF, Meeker JD. 2013. Environmental phthalate exposure and preterm birth. JAMA Pediatr; doi:10.1001/jamapediatrics.2013.3699 [Online 18 November 2013].

(Nancy Lamontagne is a science writer with MDB Inc., a contractor for the NIEHS Division of Extramural Research and Training.)

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LSB speaker discusses the role of mobile DNA elements in nature

By Deepa Singh

Understanding the significance of mobile DNA elements is important, because half of human genome is derived from them, according to Phoebe Rice, Ph.D. Rice presented her work on DNA transposition and site-specific recombination, both processes that involve movement of DNA from one location to another, in a seminar Dec. 12 sponsored by the NIEHS Laboratory of Structural Biology.

Rice, left, joined Williams and host Schellenberg, right, following her talk. (Photo courtesy of Steve McCaw)

Mobile DNA elements, also known as the transposons or transposable elements, represent a potent force for change within both prokaryotic and eukaryotic genomes, and can be a major source of mutation. "In bacteria, it can facilitate evolution and antibiotic resistance, and often provide enzymes that make useful biotech tools," explained Rice, pointing to the translational potential of her research.

The host of Rice's seminar was Matthew Schellenberg, Ph.D., a visiting fellow in the Genome Stability Structural Biology Group headed by Scott Williams, Ph.D. Rice is a professor in the Department of Biochemistry and Molecular Biology at the University of Chicago. Her group combines biochemistry and X-ray crystallography to study different stages of protein-DNA interactions during transposition and recombination.

Rice played with a handmade stuffed DNA prop she used during her talk to illustrate the structure of recombinases in complex with DNA. (Photo courtesy of Steve McCaw)

Convergent and divergent evolution of bacteriophage Mu transposome

For DNA transposition, Rice used the example of bacteriophage Mu, a bacterial virus that propagates itself by repeated transposition and integration into its host genome. Cancer-causing retroviruses, such as HIV, use a similar mechanism to integrate into their host genomes. Therefore, understanding the mechanism of Mu transposition is important in efforts to prevent and cure retroviral diseases.

Structural studies of DNA transposase MuA, in complex with bacteriophage DNA ends and the target DNA, determined intertwined networks of protein-protein and protein-DNA contacts. MuA, in the absence of DNA, consists of a single subunit that has five domains and is usually represented or cartooned as beads on a string. But in the presence of DNA, four subunits of MuA assemble on the DNA, while the same domains on different subunits have different functions.

According to Rice, "The structure is quite compact, but is made up of a tangled mess of three DNA segments and four proteins."

Compared to other DNA transposases and retroviral integrases, the MuA complex has a different arrangement of the DNA-binding domains and regulatory domains, but the domain containing the catalytic site is quite similar. In her conclusion, Rice noted that all DNA transposases diverged a long time ago from a common catalytic domain, but have all converged back to transcatalysis, since they all recognize one end of DNA and engage chemically on the other end.

Proposed model for the site-specific recombination of serine recombinases

In the second part of her talk, Rice focused on site-specific recombination that moves mobile DNA elements between nonhomologous sites within a genome and produces genetic variants upon which evolution depends. The recombinases are often classified into serine-recombinase families and tyrosine-recombinase families, based on their active site nucleophile.

Many serine recombinases function as resolvases. "The resolvases are required to clean up after some DNA transposases and also after replication," explained Rice. They resolve the original host DNA that is still fused to the target DNA with a transposon at the junction, and convert large DNA molecules into smaller ones.

The structure of the several recombinases in complex with DNA is known, but there is still confusion about how they exploit DNA topology to regulate recombination. After comparing several different recombinases, Rice's lab proposed a divergent-convergent evolution, since they all have similar sequence, structure, and topology, but the details of how they exploit that topology are very different.

Concluding her talk, Rice described her latest work on a different pair of serine recombinases that are encoded by the mobile genetic element that turns garden-variety Staphylococcus aureus into the methicillin-resistant form of the bacterium, MRSA. Infection is increasingly common in crowded settings, such as hospitals, posing a significant public health challenge, due to the difficulty of treating new strains, as they evolve, with currently available medications.

(Deepa Singh, Ph.D., is a visiting fellow in the NIEHS Mechanisms of Mutation Group.)

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Researchers' work translated into positive results for public health

By Keith Herrell and Joe Balintfy

An NIEHS-funded study of chemicals in the blood of 6-8 year-old girls in the Cincinnati-Northern Kentucky metropolitan area, also known as Greater Cincinnati, and San Francisco Bay area, collected between 2005 and 2009, showed a direct relationship between the duration of being breast and the children's blood levels of certain polyfluoroalkyl chemicals (PFCs). The study was published in the scientific journal Environmental Pollution.

PFCs are in a number of household products, and these chemicals can find their way into the water supply, eventually ending up in breast milk. Their health effects are still being studied, but other researchers have found serious concerns that include increased risk of cancer.

"This project represents a success story of research findings being translated to public health," said Susan Pinney, Ph.D., professor of environmental health and deputy director of the Center for Environmental Genetics at the University of Cincinnati (UC).

High levels of PFCs in Greater Cincinnati area

Early in the study, a preliminary list of chemicals to be tested was shared with Antonia Calafat, Ph.D., head of the Personal Care Products Laboratory at the Centers for Disease Control and Prevention (CDC), who suggested that PFCs and other relevant environmental chemicals be added to the list. It was subsequently discovered that about half of the girls in one particular area of Greater Cincinnati had significant concentrations of perfluorooctanoic acid (PFOA), a type of PFC, in their blood.

At that point, NIEHS, the National Cancer Institute (NCI), which co-funded the study, and CDC agreed to measure the blood concentration in all of the study participants from the Northern Kentucky area.

Study results lead to changes in water intake filtration

Using preliminary data from these measurements, in 2007, study investigators visited officials from the Greater Cincinnati Water Works (GCWW) and Northern Kentucky Water District (NKWD) to inform them of the findings. A meeting with the parents of study participants from Northern Kentucky was also held.

Over the next five years, the NKWD and GCWW considered the study results in determining the appropriate frequency for reactivating its granulated activated carbon filters. NKWD draws its water from the Ohio River and Licking River, while GCWW receives its water from the Ohio River and the Great Miami Aquifer.

Prior to publication of the study, the researchers again met with the leadership of the water departments, sharing findings that had been presented at the International Society for Environmental Epidemiology August meeting in Basel, Switzerland.

"The message is not that you shouldn't breast feed," Pinney said. "The message is that if we don't take care of getting these chemicals out of the water, not only are the persons themselves exposed, but the exposure carries through to children who are breast fed."

Among the findings

- Granular activated carbon filtration can reduce PFOA exposure through drinking water sources.
- Reduction of PFOA exposure through drinking water treatment also reduces internal exposure in breast fed girls.

Pinney said it's especially rewarding when research points to effective strategies for primary prevention. (Photo courtesy of Cincinnati Children's Hospital Medical Center)

Second author Frank Biro, M.D., is director of research, adolescent and transition medicine, and professor in the UC Department of Pediatrics. (Photo courtesy of Cincinnati Children's Hospital Medical Center)

Citation: Pinney SM, Biro FM, Windham GC, Herrick RL, Yaghjyan L, Calafat AM, Succop P, Sucharew H, Ball KM, Kato K, Kushi LH, Bornschein R. 2014. Serum biomarkers of polyfluoroalkyl compound exposure in young girls in Greater Cincinnati and the San Francisco Bay Area, USA. Environ Pollut 184:327-334.

(This story was adapted from an article by Keith Herrell, a public information officer with healthNEWS, the UC Academic Health Center Public Relations and Communications publication. Joe Balintfy is a public affairs specialist in the NIEHS Office of Communications and Public Liaison.)

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New study finds toenails help trace arsenic exposure from foods

By John Cramer and Joe Balintfy

Diet alone can be a significant source of arsenic exposure, regardless of arsenic concentrations in drinking and cooking water, according to a study led by Dartmouth College, funded in part by NIEHS, and published in Nutrition Journal. The study also confirms that toenail clippings are a good biomarker, or indicator, of long-term exposure to arsenic from consuming alcohol, Brussels sprouts, and dark meat fish.

Cottingham said some high-arsenic foods are important as part of a healthy diet — they just shouldn't be consumed every day or multiple times a day. (Photo courtesy of Kathryn Cottingham)

Listen as Cottingham describes the study findings and their public health significance (01:52)

Read Transcript

Previous studies have shown that diet can be an important source of total arsenic exposure, but this new study also took into account arsenic in drinking and cooking water. Household water is thought to be the most significant source of arsenic exposure in regions where water arsenic concentrations are elevated. Exposure to arsenic has been linked to a variety of health problems.

"It is obviously a known carcinogen and has effects on the cardiovascular system and lung functioning," said William Suk, Ph.D., head of the NIEHS Hazardous Substances Research Branch. "Because there is no known mechanism, it is hard to determine how low a dose can be tolerated."

Foods found to contain arsenic

Researchers asked 852 study participants about their average consumption, over the previous year, of 120 different foods, including dairy, fruits, vegetables, eggs, meat, breads, beverages, and baked goods. They found arsenic in toenail clippings is most strongly linked with consumption of Brussels sprouts and alcohol, especially beer for men and white wine for women. Those who drank more alcohol and ate more Brussels sprouts had more arsenic in their toenail clippings, which makes sense because alcoholic beverages can have higher arsenic content and are known to interfere with the metabolic pathways that detoxify arsenic.

"With Brussels sprouts, there is some emerging evidence that shows vegetables in this particular group contain sulfur molecules that help the plants detoxify arsenic," explained Dartmouth Professor Kathryn Cottingham, Ph.D., the study's lead author. "We think that Brussels sprouts are high in arsenic because the compounds that give them their characteristic smell also cause them to have potentially higher arsenic concentrations."

Researchers also found increased toenail arsenic in people who eat dark meat fish, which includes tuna steaks, mackerel, salmon, sardines, bluefish, and swordfish. Fish generally contain a form of arsenic that is thought to safely pass through the human body without being metabolized, but dark meat fish also contains arsenic compounds that can be metabolized.

Water is still a main source

Both Cottingham and Suk emphasized that it is important to limit arsenic exposure, in particular inorganic arsenic, which is found in water. "Filtering water that is contaminated does work," said Suk. Cottingham encouraged everyone who has a private well to get it tested, because, as she noted, everyone drinks water every day.

The study included researchers from Dartmouth College, Stony Brook University, University of North Carolina at Chapel Hill, Geisel School of Medicine at Dartmouth, and University of Missouri. The research was conducted by the Children's Environmental Health and Disease Prevention Research Center at Dartmouth, one of 14 centers whose aim is to develop strategies to prevent, detect, and treat environmentally related health conditions and communicate these strategies to families, health professionals, and policymakers. The centers are funded by NIEHS and EPA. This study also received support from the National Cancer Institute.

"We continue to see new emerging science on other sources of arsenic in our food supply," said Kimberly Gray, Ph.D., NIEHS program lead for the centers. "This validates the need for continued monitoring." She added that more research is needed to fully understand the health impacts, and that it is time to consider safety standards for arsenic in food.

Citation: Cottingham KL, Karimi R, Gruber JF, Zens MS, Sayarath V, Folt CL, Punshon T, Morris JS, Karagas MR. 2013. Diet and toenail arsenic concentrations in a New Hampshire population with arsenic-containing water. Nutr J 12(1):149.

(This story was adapted from an article by John Cramer, associate director for media relations at Dartmouth College. Joe Balintfy is a public affairs specialist in the NIEHS Office of Communications and Public Liaison.)

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Researchers find elevated flame retardant exposure among U.S. gymnasts

By Sara Mishamandani

Gymnasts may be ingesting or inhaling dust created by foam blocks that contain hormone-disrupting flame retardant chemicals, according to a new study from the Boston University (BU) School of Public Health, supported in part by NIEHS.

"Our results suggest that the study gymnasts are highly exposed, but it's unclear what health risks, if any, they would face as a result of this exposure," said Courtney Carignan, Ph.D., lead author of the study published online Dec. 3 in the journal Environmental Science and Technology.

The study detected 4-6.5 times more flame retardants in the blood of collegiate gymnasts than in the general U.S. population.

Carignan graduated from BU this spring with her Ph.D. She is now training as a postdoctoral researcher at the Children's Environmental Health and Disease Prevention Center at Dartmouth College. (Photo courtesy of Courtney Carignan)

Measuring exposure to flame retardants

NIEHS-funded researchers affiliated with the BU and Duke University Superfund Research Program (SRP) Centers collected blood and samples wiped from hands of 11 female gymnasts, aged 18-22, who were training at a collegiate gym in the eastern United States. Each participant filled out a questionnaire about her personal characteristics, gymnastics history, gym use, and habits, including hand washing, transportation, and diet. All participants reported practicing gymnastics for at least 12 years and averaging 19 hours a week in the gym at the time of the study.

The gymnasts' blood contained the compound bromodiphenyl ether (BDE)-153, a component of the flame retardant PentaBDE, at levels comparable to groups with high occupational exposure, such as U.S. foam recyclers and carpet installers.

PentaBDE, which was widely used in polyurethane foam for furniture, was voluntarily phased out by U.S. manufacturers in 2005, and is now banned in 172 countries.. However, PentaBDE, which can accumulate in living organisms, is a long-lasting chemical in the environment and is still present in U.S. products manufactured before 2005.

Previous studies showed that PentaBDE can disrupt endocrine activity and affect thyroid regulation and brain development. Early exposure to PentaBDE has been linked to low birth weight, lowered IQ, and impaired motor and behavioral development in children.

Getting to the source of exposure

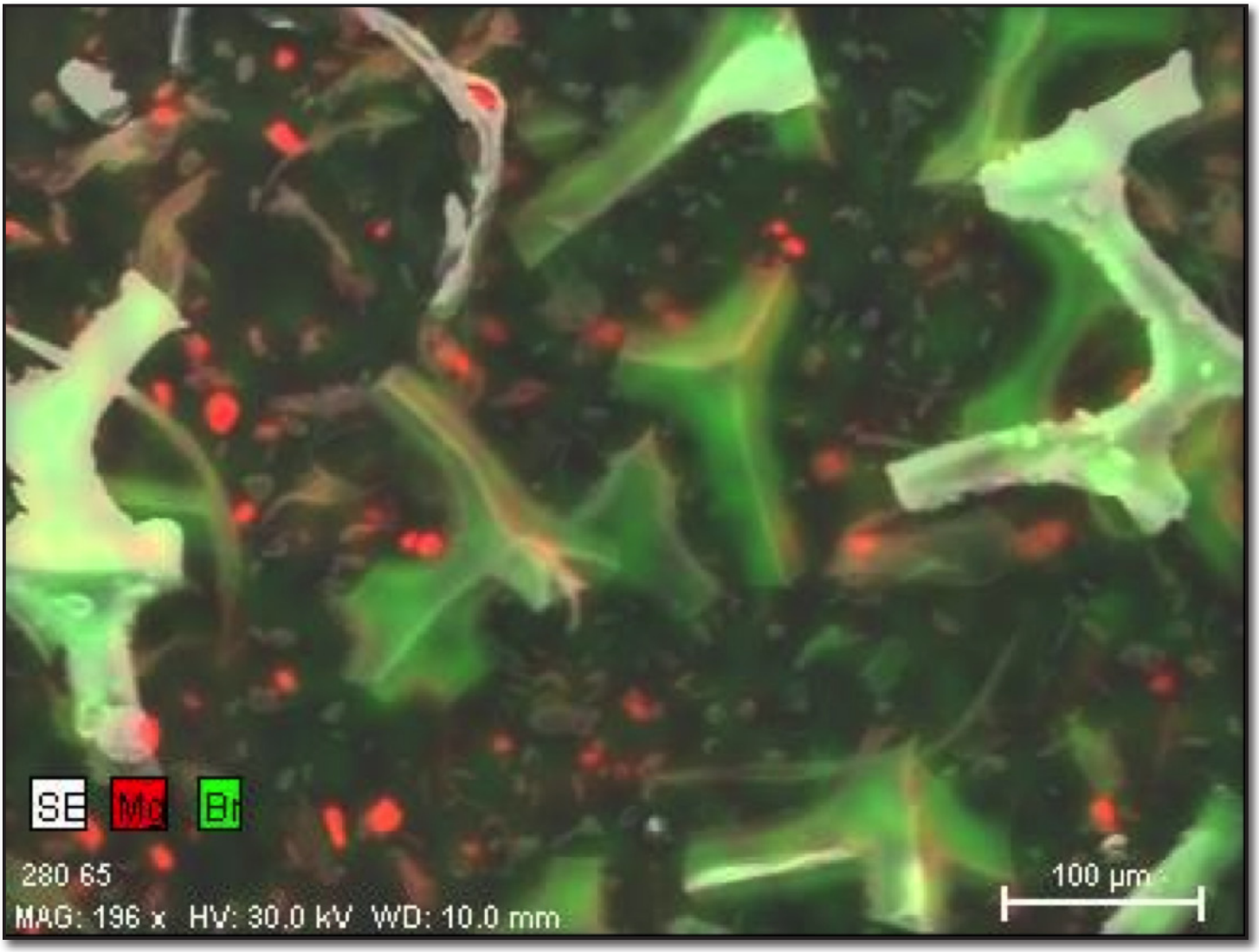
Most gyms contain a large pit filled with hundreds of foam polyurethane blocks, which provide a soft landing for gymnasts learning new acrobatic moves. Samples of dust and foam taken from the study participants' gym, as well as two other U.S. gyms, suggest that the foam blocks, some of which were up to 20 years old, were the likely source of exposure. Handwipe samples from the gymnasts after practice contained 2-3 times more flame retardants than before practice.

To reduce exposure, Carignan recommends washing hands after touching equipment at the gym and showering after leaving the gym. Carignan also started the Gymnast Flame Retardant Collaborative, which provides information about flame retardants and gymnastics through a website.

The study was funded by NIEHS individual research grants and an NIEHS training grant. SRP grantees Wendy Heiger-Bernays, Ph.D., Heather Stapleton, Ph.D., Thomas Webster, Sc.D., and Michael McClean, Sc.D., are principal researchers on the grants and study authors.

Citation: Carignan CC, Heiger-Bernays W, McClean MD, Roberts SC, Stapleton HM, Sjödin A, Webster TF. 2013. Flame retardant exposure among collegiate United States gymnasts. Environ Sci Technol 47(23):13848-13856.

(Sara Mishamandani is a research and communication specialist for MDB Inc., a contractor for the NIEHS Superfund Research Program and Division of Extramural Research and Training.)



Researchers used a scanning electron microscope with energy-dispersive X-ray spectroscopy to create an image of the dust from a gymnastics foam pit. The presence of brominated flame retardants in fragments of foam are indicated in green. Red indicates magnesium, presumably from gym chalk. (Photo courtesy of Simon C. Roberts, Duke University)

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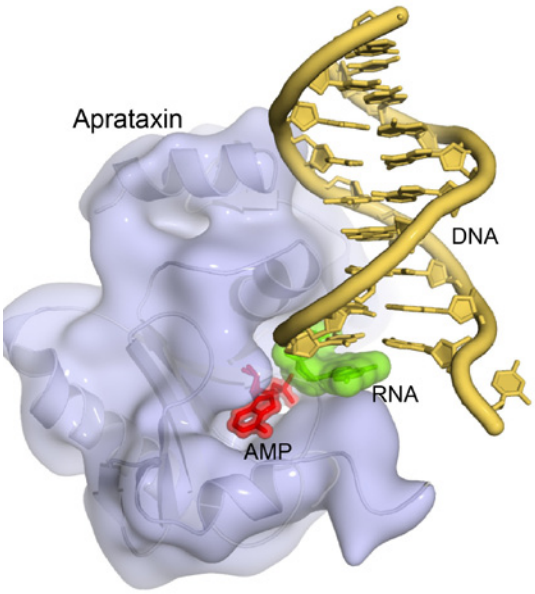
NIEHS study identifies protein involved in DNA damage removal

By Robin Arnette

For years, scientists thought that the majority of DNA damage occurred as a result of radiation, toxicant exposures, or some other environmental insult, but NIEHS research has determined that the insertion of RNA into DNA may be an underappreciated source of many more unknown lesions. In the genetic arms race, organisms from yeast to humans have a protein weapon called aprataxin that counters the RNA-triggered onslaught.

The team, led by Scott Williams, Ph.D., head of the NIEHS Genome Stability Structural Biology Group, published the results online Dec. 22 in Nature. The scientists used X-ray crystallography to visualize how human aprataxin handles RNA-DNA damage, and are the first to identify an enzyme removing a lesion arising from RNA-DNA. They call this process the RNA-DNA damage response, and its discovery may help uncover some of the genetic mishaps that contribute to neurodegenerative diseases, as well as lead to potential new therapies for cancer patients.



The molecular structure depicts how human aprataxin processes an RNA-DNA lesion. Aprataxin (blue) binds to the lesion (red) that results from the insertion of RNA (green) into DNA (yellow). (Graphic courtesy of Scott Williams)

Targeting RNA

Scott Williams said RNA incorporation into DNA happens by accident during replication and has largely unknown consequences. He explained why removing RNA from DNA was so important.

"When you have RNA in place of DNA, DNA ligase, the enzyme that seals DNA ends, stops part way through its reaction," he said. "Rather than protecting the genome, it leaves a lesion that makes the damage worse."

The lesions prevent the DNA from making copies of itself, so the cell has to address the injury. As one of the proteins involved in DNA repair pathways, aprataxin spots these RNA-DNA lesions and removes them. However, mutations in the human aprataxin protein affect its ability to bind and eliminate RNA-DNA damage, and result in a rare, heritable cerebellar-wasting disease known as ataxia with aculomotor apraxia 1, as well as other disorders, including multiple system atrophy, a Parkinson's-like condition.

Scott Williams reiterated that RNA-triggered lesions were a new idea in the field of DNA repair. (Photo courtesy of Steve McCaw)

This study also furthers the work done by Thomas Kunkel, Ph.D., head of the NIEHS DNA Replication Fidelity Group, and research fellow Jessica Williams, Ph.D., who are co-authors on the paper. Kunkel and Jessica Williams study DNA polymerases, the enzymes that replicate DNA and also incorrectly insert RNA into DNA. RNA incorporation is a major cause of genetic instability and actually happens quite frequently.

"Current estimates for the number of ribonucleotides incorporated per round of DNA synthesis are 13,000 for yeast and greater than 1,000,000 for a mammalian cell," said Jessica Williams. "This number exceeds the total of all other DNA lesions studied in the DNA repair field and provides an enormous potential to create toxic lesions that require aprataxin for repair."

Designing cancer therapies

This recent aprataxin work brings scientists closer to figuring out causes of neurodegenerative disease, but may also benefit cancer patients. Percy Tumbale, Ph.D., an NIEHS Intramural Research Training Award fellow in Scott Williams' group and co-first author on the paper, said that, under the right circumstances, blocking aprataxin function might cause cancer cell death.

"We are exploring the possibility that we can use aprataxin's atomic structure to develop inhibitors of this enzyme," Tumbale said. "When combined with agents that create DNA damage or block other DNA damage response pathways, aprataxin inhibitors may have utility in cancer therapy."

Citation: Tumbale P, Williams JS, Schellenberg MJ, Kunkel TA, Williams RS. 2013. Aprataxin resolves adenylated RNA-DNA junctions to maintain genome integrity. Nature; doi: 10.1038/nature12824 [Online 22 December 2013].

Thanks to Tumbale's expertise in X-ray crystallography, the Scott Williams group was able to crystallize mutant forms of aprataxin engaged with RNA-DNA lesions. (Photo courtesy of Steve McCaw)

Jessica Williams' work with DNA polymerase epsilon was essential to the research. (Photo courtesy of Steve McCaw)

Matthew Schellenberg, Ph.D., is a visiting fellow in the Scott Williams group. He, Tumbale, and Jessica Williams share first authorship of the article. (Photo courtesy of Steve McCaw)

Kunkel is a leading authority on DNA replication fidelity. About five years ago, his group found that RNA is transiently incorporated into human genomes and removed. (Photo courtesy of Steve McCaw)

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NIEHS research helps unlock one of the secrets of stem cells

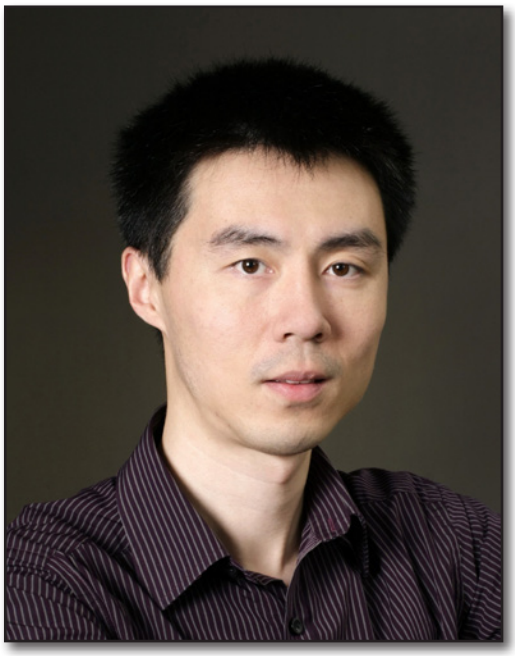
By Ernie Hood

What makes stem cells so special? The quest to answer that seemingly simple question has spawned thousands of basic scientific studies at labs across the world in recent years. Last month, the NIEHS Stem Cell Biology Group in the Laboratory of Molecular Carcinogenesis published the results of a new study that help explain the molecular basis of self-renewal and differentiation in embryonic stem cells (ESCs).

The paper, "The THO complex regulates pluripotency gene mRNA export and controls embryonic stem cell self-renewal and somatic cell reprogramming," appeared in the Dec. 5 edition of the journal Cell Stem Cell.

ESCs can give rise to many different cell types commonly found in the adult body. Because of that potential, they are of great interest to both biomolecular researchers and regenerative medicine scientists. At any given time, ESCs face a choice between differentiation, when they respond to specific developmental cues to transform into specific cell types, and self-renewal, when they divide but retain their stem cell status.

Although the transcriptional regulation of ESC self-renewal and pluripotency — the capability to differentiate into one of many cell types — has been extensively investigated, post-transcriptional mechanisms have been poorly understood, until now. Using advanced experimental technologies, the NIEHS group led by Guang Hu, Ph.D., discovered a protein complex that apparently exerts post-transcriptional regulation of gene expression in ESCs.



"ESCs need to respond to developmental cues in the proper way, but the exact connection between which cue goes through which pathway, to link to which particular mechanism, to flip on the switch or the rheostat, is a key question that most people are still trying to answer," said Hu. "We have not uncovered that particular link in the connection with the THO complex yet, but we are actively searching for it." (Photo courtesy of Steve McCaw)

The THO complex as a rheostat

The THO protein complex has long been known as a housekeeping complex involved in mRNA export, but its role in regulating ESCs has only now come to light, after Hu and his team assayed genes across the entire mouse genome. "In addition to this general housekeeping role, we showed that the THO complex also has a regulatory function in that it preferentially regulates a subset of genes that are uniquely important for ESCs," said Hu.

As Hu explained, transcriptional control is like a set of light switches, where genes can be turned on or off. However, a post-transcriptional regulator, such as the THO complex, acts as a rheostat, or variable resistor, allowing refined control without fully turning on or off the switch. "The THO complex provides a non-committal way to allow the cells to respond to environmental cues, so they can either choose the fate of differentiating to a particular cell type or maintain the stem cell state without fully committing, as the complex regulates the mRNA export without impacting transcription itself," he said.

Potential applications

Aside from the significant contribution this discovery makes to the basic understanding of mechanistic processes in ESCs, it may eventually lead to new cell-based therapies, based on manipulating the THO complex to generate induced pluripotent stem cells. It could also spawn new approaches in regenerative medicine, by fostering the manipulation of cell fate in the culture dish, to make various cell types for specific therapeutic purposes.

Williams is also a lead researcher in the Laboratory of Reproductive and Developmental Toxicology. She has been at NIEHS since 2007. (Photo courtesy of Steve McCaw)

Characterizing the network or pathway is another key objective. "At this moment, we don't know why the THO complex can specifically recognize and regulate these ESC-specific genes, but you would imagine that, at some point, this post-transcription regulation must talk to the transcription regulation, so that there is no conflict between the two," said Hu.

Mouse experiments confirm relevance

The NIEHS Reproductive Medicine Group, led by Carmen Williams, M.D., Ph.D., also contributed to the research, by conducting experiments involving mouse preimplantation embryos, which showed that THO proteins are needed for proper embryo development to the blastocyst stage, when the embryo is beginning to generate pluripotent stem cells. "This finding means that Guang's experiments in ES cells are actually relevant to the physiological situation in vivo, rather than applying only to cultured cells," she explained.

Citation: Wang L, Miao YL, Zheng X, Lackford B, Zhou B, Han L, Yao C, Ward JM, Burkholder A, Lipchina I, Fargo DC, Hochedlinger K, Shi Y, Williams CJ, Hu G. 2013. The THO complex regulates pluripotency gene mRNA export and controls embryonic stem cell self-renewal and somatic cell reprogramming. Cell Stem Cell 13(6):676-690.

(Ernie Hood is a contract writer with the NIEHS Office of Communications and Public Liaison.)

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NTP seminar explores functional ecology

By Eddy Ball

An ongoing lecture series sponsored by NTP made an unexpected turn into animal ecology Dec. 10 with a talk at NIEHS by zoologist Vladimir Vershinin, D.Sc.

Hosted by Biomolecular Screening Branch Chief Ray Tice, Ph.D., the talk, on "Amphibian Populations in Urban and Natural Environment in Urals," explored the impact of urban pollution and ecological changes, in and around the industrial city of Yekaterinburg, Russia, to frogs, newts, and salamandrella.

Tice, who leads the Tox21 predictive toxicology initiative for NTP, said afterwards, "This was a different kind of talk — one we're not used to hearing." But, he added that seeing these effects, on what many consider a sentinel species, may offer insight into the kinds of changes in biochemistry, morphology, life span, and reproduction that chemicals may produce in human populations.

Studying amphibians for 36 years

Vershinin is a professor and department chair of zoology at Ural Federal University in Yekaterinburg, whose group has gathered and analyzed data on amphibian populations at 26 geographical locations along the land border between Europe and Asia since 1977. He is also head of the Department of Functional Ecology of Terrestrial Animals at the Institute of Plant and Animal Ecology of the Russian Academy of Sciences.

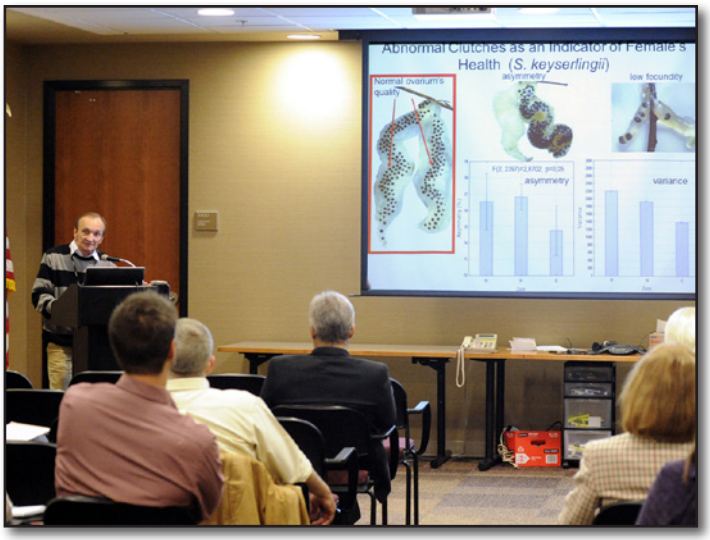
"The Urals represent a complicated biochemical situation," Vershinin said at the beginning of his talk. "Yekaterinburg is one of the Uralian cities that appeared as a settlement around [metallurgical and heavy machinery] factories … which are some of the complicated sources of pollution." (Photo courtesy of Steve McCaw)

Early in his talk, Vershinin described a central premise behind these studies, saying, "Amphibians are a prototype of terrestrial vertebrates… [whose] development is strongly affected by environment." He described the associations between the amphibian's skeletal structure, fertility, rate of physical abnormalities, and cardiac function over the past three decades with increased urbanization.

The down side of urbanization

Vershinin's group divided Yekaterinburg, Russia's third largest city, into four zones, by levels of development and density, ranging from the city center to forestland. The team analyzed water chemistry, average temperature, and a broad range of responses and outcomes over time in these areas.

The researchers collected data on changes in relative liver mass, sexual differentiation, skin permeability, and cardiac function, specifically isometrical myocardial force and myocardial contraction. They also observed changes in reproductive strategy, finding changes in onset of puberty, body length, and size, as well as fertility and egg size. A few of the changes helped amphibians adapt to a more threatening environment, but most were almost exclusively negative in terms of impact on population survival.



Among his many slides of dramatic changes in the amphibian population was the one above, which shows anomalies in egg clutches among the outlying population of Siberian salamanders living in and around Yekaterinburg. (Photo courtesy of Steve McCaw)

Some of Vershinin's most dramatic examples involved external and internal abnormalities that reduced the quality of the population. Dramatic changes in egg clutches and ovarian symmetry contributed to low fertility. The researchers observed significant changes in eye pigmentation, the number and form of fingers and toes, and internal skeletal formation.

The amphibians' relationships with other organisms in their environment also changed, with an increase in cysts and a higher percentage of eosinophils, or white blood cells, reflecting a rise in parasite infection.

Detailed observations, but few clear associations

The strength of Vershinin's data collection was its long-term comprehensive monitoring. While the NTP scientists in the audience were impressed by the collection of large amounts of data in the studies, naturally as toxicologists, they wanted more.

Vershinin pointed to likely causes, such as lead and a strong association between some of the abnormalities and radioactivity. When he was questioned about effects of some of the chemicals that are of interest in several NTP studies, such as endocrine-disrupting compounds, Vershinin conceded the limitations of his observational approach and how much work remains to be performed.

"We still have some gaps in our knowledge on the evolution of amphibian populations," he told the audience. But, as people at the talk seemed to agree, Vershinin's group has created a model for gathering and analyzing the data — the important first step in conducting a comprehensive study of population to ultimately tease out the health effects of exposure to a mixture of multiple chemicals and other environmental factors.

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"This is one of those kinds of seminars that occur because somebody knows somebody who happens to be visiting here," Tice said in his introduction. In Vershinin's case that somebody was NIEHS grantee Alexander Kinev, Ph.D., of Creative Scientist Inc. Vershinin was a visiting scholar at NESCent, the National Evolutionary Synthesis Center, in Durham, N.C., presenting talks on his research and future plans for building a publicly accessible database. (Photo courtesy of Steve McCaw)

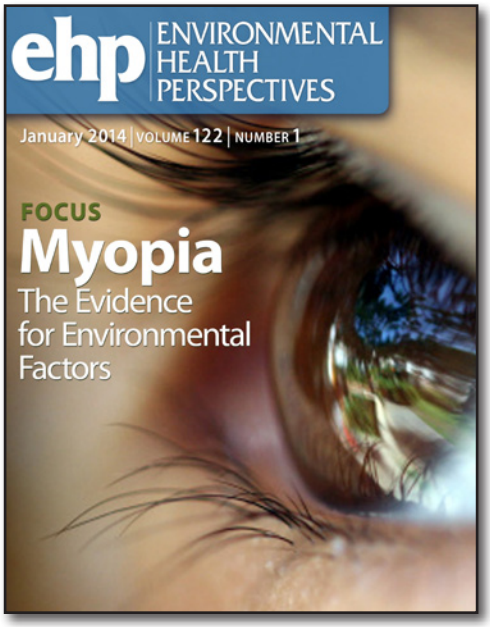
This month in EHP

The January issue of Environmental Health Perspectives (EHP) highlights the role of environmental factors in myopia and the unique qualities of wind turbine noise.

http://twitter.com/ehponline

Myopia: The Evidence for Environmental Factors

Myopia, or nearsightedness, was long blamed on genetics and behavior, but dramatic increases in prevalence, in recent decades, point to environmental factors as well. Moreover, time spent outdoors appears to have a protective effect against myopia, although investigators still aren't sure why.



Wind Turbines: A Different Breed of Noise?

Since at least 1930, researchers have amassed a body of evidence on the adverse nonauditory health effects of environmental noise. More recently, a new source of noise has entered the scene — wind turbines. As wind farms become more common near populated areas, researchers are investigating the unique qualities of the noise they produce.

Featured research and related news articles this month include:

- **Maternal Blood, Plasma, and Breast Milk Lead: Lactational Transfer and Contribution to Infant Exposure** — Lead Transfer During Breastfeeding: A Start Toward Filling in the Data Gaps
- **Pollutant Exposures From Natural Gas Cooking Burners: A Simulation-based Assessment for Southern California** — Cooking Up Indoor Air Pollution: Emissions From Natural Gas Stoves
- **Maternal Concentrations of Persistent Organochlorine Pollutants and the Risk of Asthma in Offspring: Results From a Prospective Cohort With 20 Years of Follow-up** — A Long-Term Risk? Prenatal POPs Exposure and Asthma in Young Adults
- **Air Pollution–mediated Susceptibility to Inflammation and Insulin Resistance: Influence of CCR2 Pathways in Mice** — Toxicity Beyond the Lung: Connecting PM2.5, Inflammation, and Diabetes

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Extramural papers of the month

By Nancy Lamontagne

- Epigenetic changes associated with pancreatic cancer might lead to early detection
- Phthalate exposure linked to preterm birth
- A 3-D map of chromatin interactions
- Girls are reaching puberty earlier

Read the current Superfund Research Program Research Brief. New issues are published on the first Wednesday of each month.

Epigenetic changes associated with pancreatic cancer might lead to early detection

In work funded in part by the NIEHS, researchers identified epigenetic modifications in BNC1 and ADAMTS1 genes that were detectable in people with early-stage pancreatic cancer, but not in cancer-free people. The findings from this small preliminary study point to the possibility of a blood test that might detect early-stage pancreatic cancer. Pancreatic cancer is almost always fatal, because it isn't usually discovered until it has spread.

The researchers used methylation on beads technology, a recently developed approach that uses nanotechnology to capture and analyze very small amounts of DNA. Using this method, they detected methylation changes in DNA circulating in 42 serum samples from patients with pancreatic cancer. For the BNC1 gene promoter, the researchers achieved a sensitivity of 79 percent and specificity of 89 percent, and for the ADAMTS1 gene, the sensitivity was 48 percent and specificity 92 percent. When using both markers, the approach achieved an overall sensitivity of 81 percent (95 percent confidence interval [CI], 69-93 percent) and specificity of 85 percent (95 percent CI, 71-99 percent).

The researchers say that, although larger studies are needed, their findings strongly suggest that BNC1 and ADAMTS1 promoter methylation could be used as biomarkers for identifying individuals at risk for pancreatic cancer.

Citation: Yi JM, Guzzetta AA, Bailey VJ, Downing SR, Van Neste L, Chiappinelli KB, Keeley BP, Stark A, Herrera A, Wolfgang C, Pappou EP, Iacobuzio-Donahue CA, Goggins MG, Herman JG, Wang TH, Baylin SB, Ahuja N. 2013. Novel methylation biomarker panel for the early detection of pancreatic cancer. Clin Cancer Res 19(23):6544-6555.

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Phthalate exposure linked to preterm birth

NIEHS-supported research has found that, depending on the phthalate examined, women with the highest levels of exposure during pregnancy had 2-5 times the odds of preterm birth, compared to women with the lowest exposure. The findings point to phthalate exposure as a potentially preventable contributing factor to premature birth.

Using a nested case control study design, the researchers examined associations between average levels of phthalate exposure during pregnancy and preterm birth in 130 mothers who had delivered prior to 37 weeks of completed gestation. The study also included 352 control mothers who delivered at or after 37 weeks. To determine overall phthalate exposure, the researchers used multiple urine samples during pregnancy. They found that preterm birth showed the strongest dose-dependent associations with maternal levels of the two di-2-ethylhexyl phthalate (DEHP) metabolites, mono-(2-ethyl)-hexyl phthalate (MEHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and the summed levels of all the DEHP metabolites.

The researchers also analyzed a subset of 57 mothers with preterm deliveries preceded by spontaneous preterm labor or preterm premature rupture of the membranes. When this group was examined alone, the odds ratios increased for all the phthalate metabolites. This finding indicates a stronger association between phthalate exposure and spontaneous preterm birth than the association with all preterm births.

Phthalate exposure can occur from food, plastics, and personal care products, such as deodorants and lotions. The researchers caution that, before implementing interventions aimed at decreasing phthalate exposure, more studies are needed to confirm these findings, to examine the sources of the phthalates, and to better understand the mechanisms involved.

Citation: Ferguson KK, McElrath TF, Meeker JD. 2013. Environmental phthalate exposure and preterm birth. JAMA Pediatr; doi:10.1001/jamapediatrics.2013.3699 [Online 18 November 2013]. (Story)

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A 3-D map of chromatin interactions

An NIEHS grantee and colleagues generated a high-resolution map of three-dimensional (3-D) chromatin interactions in human cells. The map suggests that the looping structure of chromatin is relatively stable, once established in a cell type.

DNA contains areas known as cis-regulatory sequences where transcription factors can bind to regulate gene expression. The 3-D loops of chromatin help control gene expression, by bringing cis-regulatory DNA sequences close to their target genes. Although scientists have identified a large number of cis-regulatory sequences in the human genome, many of the target genes for these sequences are unknown. To identify these target genes, the researchers mapped the 3-D interactions of cis-regulatory sequences in human fibroblast cells, using a genome-wide chromosome conformation capture analysis method.

The researchers determined more than 1 million long-range chromatin interactions at a resolution of 5-20 kilobases. One of their findings was that DNA sequences, activating gene expression after treatment of cells with the tumor necrosis factor alpha, are already in contact with their target promoters before signaling.

This unexpected discovery suggests that enhancer-promoter interactions form before signaling and change little when activated during transcription. Thus, the looping structure of chromatin is likely cell-type specific, and could influence the selection or activation of target genes by a ubiquitous transcription activator.

Citation: Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, Yen CA, Schmitt AD, Espinoza CA, Ren B. 2013. A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature 503(7475):290-294.

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Girls are reaching puberty earlier

Research that is part of the NIEHS Breast Cancer and the Environment Research Program, a cohort of more than 1200 girls, found that breast development is occurring at an earlier age, and that earlier development was strongly associated with greater body mass index (BMI). Earlier than average maturation is a risk factor for obesity and hypertension, as well as breast, ovarian, and endometrial cancer.

From 2004 to 2011, the researchers followed girls in the San Francisco Bay Area, Greater Cincinnati, and New York City who were 6-8 years old when enrolled in the study. At regular intervals, trained and certified staff used Tanner staging to assess sexual maturity. They found that the age at onset of breast development (stage 2) varied by race/ethnicity, BMI at baseline, and geographical site. For African-American, Hispanic, white non-Hispanic, and Asian participants, the median age at onset of breast stage 2 was 8.8, 9.3, 9.7, and 9.7 years, respectively. Girls with BMIs greater than the 85th percentile reached breast stage 2 at younger ages.

Compared to data from studies conducted in the 1990s, white non-Hispanic girls are now maturing at younger ages, while the maturation age for African-American girls is similar to that found in the earlier studies.

Citation: Biro FM, Greenspan LC, Galvez MP, Pinney SM, Teitelbaum S, Windham GC, Deardorff J, Herrick RL, Succop PA, Hiatt RA, Kushi LH, Wolff MS. 2013. Onset of breast development in a longitudinal cohort. Pediatrics 132(6):1019-1027.

(Nancy Lamontagne is a science writer with MDB Inc., a contractor for the NIEHS Division of Extramural Research and Training.)

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Intramural papers of the month

By Kelly Lenox, Jacqueline Powell, Bailey Schug, and Deepa Singh

- tssRNAs associated with paused Poll II serve as scaffold for transcription factors
- Asthmatic reaction is dependent on dose and timing of endotoxins
- Modest changes in dNTP levels affect cell's ability to repair mutations
- Distinct features of RNA binding protein make it unique among its family

tssRNAs associated with paused Poll II serve as scaffold for transcription factors

Researchers from NIEHS have found that nascent transcription start site-associated (tss) RNAs, produced and stably bound by RNA polymerase II (Pol II) that has paused during early elongation, could provide a target for the recruitment of factors that modulate gene expression. Since transcription is a critical step in the creation of proteins from information within the genome, this work may provide insight into environmentally responsive gene expression and identify novel approaches for treating disease.

The scientists developed a highly sensitive method of characterizing the dynamics of promoter-associated Pol II and tssRNAs generated during early elongation in fruit fly, or *Drosophila*, cells. They performed a biochemical fractionation procedure that allowed separation of the short, capped RNA species, and identified their origins genome-wide using high-throughput sequencing. They found that paused Pol II and associated tssRNAs were very stable, long-lived species, remaining near gene promoters for tens of minutes before resuming transcription elongation.

The researchers propose that tssRNAs provide a physical framework on which transcription factors that regulate productive elongation and promoter chromatin could bind to release paused Pol II into productive elongation, thereby modulating gene expression. **(KL)**

Citation: Henriques T, Gilchrist DA, Nechaev S, Bern M, Muse GW, Burkholder A, Fargo DC, Adelman K. 2013. Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. Mol Cell 52(4):517-528.

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Asthmatic reaction is dependent on dose and timing of endotoxins

NIEHS scientists showed that immune responses to inhaled allergens are dependent on quantity and time of exposure to endotoxins. Two different arms of the adaptive immune response contribute to allergic asthma — T helper (Th) 2 cells and Th17 cells. These two arms of the immune response, which respond differently to factors in the environment, account for some of the heterogeneity seen in asthma. This knowledge offers potential opportunities for therapies that disrupt specific pathways associated with one or the other of these types of asthma.

Allergic sensitization, which is the biological basis for allergic asthma, is caused by allergens found in plants, insects, and animals. However, it is also caused by adjuvants, or ingredients that increase the immune response, such as those found in microbial products, including lipopolysaccharide (LPS). The researchers used a mouse model to demonstrate that immune responses to inhaled allergens are highly dependent on the doses of inhaled LPS, including the amounts found naturally in the environment. They discovered that low doses of LPS promote classical, Th2-driven allergic responses to inhaled allergens, whereas moderate doses of endotoxin induce stronger Th17 responses and associated neutrophilia.

The researchers also demonstrate that inhalation of moderate doses of LPS during sensitization induces regulatory responses that, after multiple allergen exposures, limit the severity and longevity of asthma-like features. **(BS)**

Citation: Whitehead GS, Thomas SY, Cook DN. 2013. Modulation of distinct asthmatic phenotypes in mice by dose-dependent inhalation of microbial products. Environ Health Perspect; doi:10.1289/ehp.1307280 [Online 29 October 2013].

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Modest changes in dNTP levels affect cell's ability to repair mutations

Recent studies by NIEHS researchers have indicated that the cellular levels of deoxynucleoside-5'-triphosphates (dNTPs), the building blocks for DNA, have to be strictly regulated to maintain low mutation rates. The main enzyme responsible for controlling dNTPs is ribonucleotide reductase (RNR) and mutations in RNR have a direct bearing on the rate of DNA mutations.

The researchers isolated *Escherichia coli* mutant RNRs and found two new alleles in which dGTP and dATP concentrations were affected almost twofold, up versus down, respectively. Interestingly, despite these modest dNTP changes, the mutation rate in the *rpoB* gene encoding the beta subunit of bacterial RNA polymerase was increased more than 3,000-fold. They hypothesized the change could be due, in part, to collapse of the mismatch repair (MMR) system that normally removes most errors post-DNA replication.

The scientists used several approaches to explain the high mutability of these two RNR mutants. They measured mutation frequencies in MMR-proficient and deficient backgrounds, and observed that the MMR is no longer active in these two mutants. Similarly, overproduction of one of the components of the MMR system (*mutL*) or the inclusion of a *dnaQ926* antimutator allele lowered the mutation frequencies, indicating the restoration of an active MMR system.

Overall, these studies indicate that abnormal levels of dNTPs can saturate MMR and can lead to hypermutability and error catastrophe. **(DS)**

Citation: Ahluwalia D, Schaaper RM. 2013. Hypermutability and error catastrophe due to defects in ribonucleotide reductase. Proc Natl Acad Sci U S A 110(46):18596-18601.

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Distinct features of RNA binding protein make it unique among its family

NIEHS scientists and their collaborators recently uncovered unique structural features of the tandem zinc finger (TZF) domain of tristetraprolin (TTP), an RNA-binding protein. TTP is the best-known member of the TTP family of tandem zinc finger proteins that promote the degradation of target mRNAs.

While TTP family proteins have highly conserved TZF domains that are critical for RNA binding, researchers performed a cross-species TZF domain sequence alignment and uncovered seven residues that were highly specific to TTP. By generating a structural model of TTP based on the known structure of another TTP family protein, ZFP36L2, they discovered that the second zinc finger of TTP was structurally distinct from the first zinc finger of TTP and both zinc fingers of ZFP36L2. These differences were mainly observed in the C-x8-C intervals, where six of the seven TTP-specific residues were located. Researchers then mutated residues within the TZF domain of TTP to examine their role in RNA binding. Mutating sequence equivalent residues in the first and second zinc fingers of TTP often had different effects on RNA binding, indicating that the two zinc fingers of TTP contain functionally distinct residues.

This research suggests that although the TZF domain of TTP family proteins is highly conserved, they may bind to RNA differently, based on subtle structural differences. **(JP)**

Citation: Lai WS, Perera L, Hicks SN, Blackshear PJ. 2013. Mutational and structural analysis of the tandem zinc finger domain of tristetraprolin. J Biol Chem; doi:10.1074/jbc.M113.466326 [Online 19 November 2013].

(Kelly Lenox is editor of the Environmental Factor. Former NIEHS postdoctoral fellow Jacqueline Powell, Ph.D., is a writer and analyst with Education and Training Systems International. (ETSI). Bailey Schug studies health promotion and nutrition at Appalachian State University and is an intern in the NIEHS Office of Communications and Public Liaison. Deepa Singh, Ph.D., is a visiting fellow in the NIEHS Mechanisms of Mutation Group.)

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Inside the Institute

Employees make the 2013 Giving Tree a success

By Kelly Lenox

The 2013 Giving Tree, sponsored by the Diversity Council, gave NIEHS employees the opportunity to brighten Christmas for some area children. "It's been an amazing job of partnering with the Salvation Army to share our riches with a lot of people this holiday season," observed Joellen Austin, NIEHS associate director for management.

At the Dec. 5 celebration, the success of the Giving Tree effort was evident. Bill Quattlebaum recalled, "Last year we did 160 tags. We got in 120, and, in the last few days, had to have some more brought over to us. This year, it has been harder to plan, with the furlough and late Thanksgiving, so we have been really pleased with the response."

The NIEHS Diversity Council offered thanks to all. "We are so proud of the way the NIEHS community stepped up to make the holidays brighter for 150 deserving children."

Diversity Council Members

- Brad Collins
- Veronica Godfrey Robinson
- Bill (qb) Quattlebaum
- Cynthia Radford
- Jenn Evans
- Symma Finn
- Bill Jirles
- Myra Westmoreland
- Cheryl Thompson
- Gerard Roman
- Ericka Reid

Gifts under the tree represented just a few of the many donations. (Photo courtesy of Steve McCaw)

All of this year's 150 tags were taken, and some employees went the extra mile. "Gregory Solomon and some of his elves offered to assemble the bicycles that had been donated," Quattlebaum said. Solomon has already renewed the offer for next year. "We don't want the assembly to keep anyone from buying a bike."

Along with admiring the bicycles and other gifts, large and small, the gathered employees used the time to sample the festive treats, while getting better acquainted with one another.

Austin and Collins welcomed employees to the Giving Tree celebration. (Photo courtesy of Steve McCaw)

Diversity Council members Westmoreland, left, and Robinson joined in the festivities. (Photo courtesy of Steve McCaw)

Steve Johnson, left, and William Ragland enjoyed a break from their hectic preholiday schedules. (Photo courtesy of Steve McCaw)

Dick Sloane, right, gestured as he described the size of the pile of gifts over at the NIEHS Keystone building to Rick Woychik, Ph.D., NIEHS deputy director. (Photo courtesy of Steve McCaw)

Sophia Harlid, Ph.D., left, and Michelle Campbell quenched their thirst brought on by the plentiful treats. (Photo courtesy of Steve McCaw)

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NIEHS scientists join other volunteers to teach children about nutrition

Thanks to volunteers from NIEHS and public service groups, six children in a Durham, N.C., program learned important lessons about healthy lifestyle and diet.

The children, who may not have associated sugar in soda with weight before, certainly did after this graphic illustration. (Photo courtesy of Renaldo Dorsey)

NIEHS scientists Sharon Beard and Elena Braithwaite, Ph.D., joined fellow members of the Durham Alumnae Chapter of Delta Sigma Theta Sorority, as well as volunteers from the Durham Alumni Chapter of Kappa Alpha Psi Fraternity and the Durham Chapter of the Links Inc.

Children living temporarily at Genesis Home, a nonprofit organization that works to end homelessness for families with children, participated in a hands-on science lesson Dec. 2 about the importance of fruits and vegetables, a healthy diet, and exercise. The lessons included some graphic illustrations, such as one involving cans of regular soda, which sank in water because of the density of the 30-50 grams of sugar they contain, and cans of diet soda, which floated because of their lower sugar content, weight, and density.

Braithwaite is a regular volunteer in the Durham Alumnae Chapter of Delta Sigma Theta Sorority Science and Everyday Experiences (SEE) summer camp held each year in Durham, N.C. (see story). She is a staff scientist in the NIEHS Comparative Genomics Group and has also volunteered in the Citizen Schools program. (Photo courtesy of Steve McCaw)

Why is diet so important?

Childhood obesity rates in America have tripled in the past thirty years and today, in the U.S., approximately 30 percent of all children, and 40 percent of African-American and Hispanic children, are overweight or obese. Since obesity can lead to numerous health complications, including diabetes, heart disease, high blood pressure, cancer, and asthma, it is important to try to reverse this trend. Some factors that contribute to the increase in obesity rates among children are diet, portion size, and lack of exercise.

Beard started the discussion by talking about the five food groups that serve as the building blocks for a healthy diet. In her presentation, she emphasized the importance of consuming a variety of fruits and vegetables.

As Beard said, “Teachable moments like these are essential to help eliminate minority health disparities, as children learn in a fun environment. We hope they will internalize these healthy concepts. Evidence indicates that ingestion of at least 2 1/2 cups of fruit and vegetables per day is associated with a reduced risk of cardiovascular disease.”

However, Beard noted, according to the CDC's 2013 State Indicator Report on Fruits and Vegetables, more than 40 percent of adults and adolescents in N.C. consume less than one helping of fruits and vegetables each day. She said it continues to be important to remind the general public of this nutritional recommendation.

Bringing the healthy diet message up close and personal

Braithwaite continued the discussion by teaching the participants about nutrition labels and healthy food options, and emphasized the importance of exercise. Students calculated the total calories in a typical meal they would order for lunch from popular fast food restaurants. Many were surprised to see that this one meal contained almost half the calories they should consume in an entire day.

To encourage a balanced and nutritious diet, Braithwaite led a discussion of healthy alternatives for popular items and emphasized the importance of exercise. The students then joined in an exercise where they were asked to do jumping jacks for one minute.

"Many of the participants were surprised to see how much exercise is required to equal the number of calories in a serving of tomatoes on a sandwich," said Braithwaite, who noted that inactivity is also a big problem in N.C. According to the 2013 Annual Report on America's Health Rankings by the United Health Foundation, 24.9 percent, or approximately 1.8 million, adults in N.C. are physically inactive, and 29.6 percent are estimated to be obese.

"Our hope is that this knowledge will assist the participants in making better nutritional choices and promote a healthier lifestyle," Braithwaite said of the program.

Beard is the coordinator of her sorority's SEE camp each summer. At NIEHS, she is a program manager and industrial hygienist in the Worker Education and Training Program. (Photo courtesy of Steve McCaw)

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