Division of Intramural Research

NAEHS Council Update

September 2013

DIR RECRUITMENTS

Director, Clinical Research Program

The NIEHS is searching for a senior investigator to direct its Clinical Research Program. The Director, Clinical Research Program, is responsible for the development, administration, coordination and oversight of investigator-initiated clinical research; provides general advice to the Director and Scientific Director, NIEHS, on matters relating to human and clinical studies; supervises the Office of Research Compliance; and develops policies and programs for the execution of clinical research at NIEHS. The Clinical Director is responsible for creating and maintaining a research environment in which clinical findings influence the direction of laboratory studies and laboratory findings are applied back to the clinical and clinical research communities. The incumbent will facilitate intramural clinical research by identifying opportunities for translating basic science into clinical studies. The Clinical Director will ensure that Institute research reflects the highest standards of scientific excellence and ethical conduct for the protection of human subjects. The incumbent will review matters pertaining to the provision of patient care in research protocols and oversee research allocation, scientific review, and recruitment of staff. The Clinical Director will provide advice and training on the conduct of clinical studies, facilitate clinical research collaborations between intramural and extramural investigators, and develop long-range clinical research goals and objectives relevant to the mission of NIEHS. It is expected that the successful candidate will oversee a personal clinical research program that will involve some combination of outpatient oriented studies within the Clinical Research Unit, epidemiological studies, basic laboratory studies, or inpatient studies at the Clinical Research Center in Bethesda. Emphasis will be placed upon investigators with a primary research interest in clinical research; however, the selected candidate may have a modest independent basic laboratory research program, particularly if the basic research intersects with the candidate's clinical studies. Dr. Carter Van Waes, Clinical Director, National Institute on Deafness and Other Communication Disorders, is chair of the search committee. A Candidate has not yet been identified.

DIR RESEARCH UPDATE

Finding the molecular links between house dust and asthma

Donald N. Cook, Ph.D.

Immunogenetics Group
Laboratory of Respiratory Biology, DIR, NIEHS

Allergic asthma is a debilitating disease, and its prevalence has been increasing over the last several decades. Currently, 12% of adults have been diagnosed with asthma at some point in their life and the annual cost of this disease in the U.S. is estimated at $60 billion. Inhaled corticosteroids have been the standard of care for asthma for many years, but not all asthmatics respond to this treatment, probably because allergic responses in the lung are heterogeneous and involve many different types of immune cells, including dendritic cells, airway epithelial cells, various types of T cells, neutrophils and eosinophils. This presentation will discuss how these various cell types respond to specific environmental stimuli and can trigger maladaptive responses that lead ultimately to allergic asthma. An improved understanding of these cells and the signaling pathways they comprise offers the potential for developing therapeutic strategies that target specific types of asthma, including steroid-resistant asthma. Such strategies might prevent or reverse the course of events that lead to asthma.

BSC REVIEW OF THE EPIDEMIOLOGY BRANCH AND DR. FRED MILLER

The DIR Board of Scientific Counselors reviewed the Epidemiology Branch and Dr. Fred Miller June 2-4, 2013.

Members of the Board of Scientific Counselors that Attended:

- Kenneth B. Adler, Ph.D., BSC Chair, Professor, Dept. of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
- Juan C. Celedón M.D., Dr.P.H., Neil K. Jerne Professor, Dept. of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.
- Jay I. Goodman, Ph.D., Professor, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI
- Monica Justice, Ph.D., Professor, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX
- Serrine S. Lau, Ph.D., Professor and Director, Southwest Environmental Health Sciences Center, Dept. of Pharmacology & Toxicology, University of Arizona College of Pharmacy, Tucson, AZ
- José E. Manautou, Ph.D., Associate Professor, Department of Pharmaceutical Sciences, University of Connecticut School of Pharmacy, Storrs, CT
- Donald P. McDonnell, Ph.D., Glaxo-Wellcome Professor and Chairman of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC
- Karen M. Vasquez, Ph.D., Professor, Division of Pharmacology and Toxicology, Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX
- Roland A. Owens, Ph.D., Ex-Officio BSC Member, Assistant Director, Office of Intramural Research, NIH, Bethesda, MD

Ad Hoc Reviewers that Attended:

- Anthony A. Amato, M.D., Professor, Dept. of Neurology, Harvard Medical School, Brigham & Women's Hospital, Boston, MA
- Christopher I. Amos, Ph.D., Professor, Dept. of Community and Family Medicine, Associate Director for Population Sciences, Dartmouth-Hitchcock Norris Cotton Cancer Center (NCCC) and Geisel School of Medicine, Lebanon, NH
- John R. Balmes, M.D. , Professor, Dept. of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA
- Kim N. Dietrich, Ph.D., Professor, Dept. of Environmental Health Sciences, Director, Division of Epidemiology and Biostatistics, University of Cincinnati College of Medicine, Cincinnati, OH
- Marlene B. Goldman, M.S., Sc.D., Professor and Director of Clinical Research, Dept. of Obstetrics and Gynecology, Geisel School of Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH
- Bernard L. Harlow, Ph.D., Mayo Professor & Division Head, Dept. of Epidemiology & Community Health, University of Minnesota School of Public Health, Minneapolis, MN
- Margaret A. Honein, Ph.D., M.P.H., Distinguished Consultant and Chief, Birth Defects Branch, Division of Birth Defects and Developmental Disabilities, National Center on

Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA
- Claire Infante-Rivard, M.D., Ph.D., James McGill Professor, Dept. of Epidemiology, Biostatistics and Occupational Health, McGill University Faculty of Medicine, Montréal, Quebec, Canada
- Elan D. Louis, M.D., Professor, Dept. of Epidemiology, Professor and Associate Chairman for Academic Affairs and Faculty Development of Neurology, Columbia University Mailman School of Public Health, New York, NY
- John R. McLaughlin, Ph.D., Professor and Senior Investigator, Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute, University of Toronto Dalla Lana School of Public Health, Mount Sinai Hospital, Toronto, Canada
- Westley H. Reeves, M.D., Marcia Whitney Schott Professor, Dept. of Medicine, Chief, Division of Rheumatology and Clinical Immunology, University of Florida School of Medicine, Gainesville, FL
- Beate Ritz, M.D., Ph.D., Professor and Chair, Dept. of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, CA
- John Vena, Ph.D., Department Head & UGA Foundation Professor , Dept. of Epidemiology & Biostatistics, University of Georgia College of Public Health, Athens, GA
- Philip R. Taylor, M.D., Sc.D., Senior Investigator, Genetic Epidemiology Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute, Bethesda, MD
- Martha M. Werler, D.Sc., M.P.H., Professor, Dept. of Epidemiology, Slone Epidemiology Center, Boston University School of Public Health, Boston MA
- Zuo-Feng Zhang, M.D., Ph.D., Professor, Dept. of Epidemiology, Head, Cancer Epidemiology Program, UCLA School of Public Health, Los Angeles, CA

Agenda:

Sunday, June 2: DoubleTree Suites by Hilton Raleigh-Durham, Closed Session
Closed Evening Session

7:00 - 8:00 p.m.	Welcome and Discussion of Past Board Reviews, Drs. Linda Birnbaum, Dale Sandler, Stavros Garantziotis and Darryl Zeldin
8:00 - 10:00	BSC Discussion of Review, Dr. Kenneth Adler; BSC Chair and panel

Monday, June 3: NIEHS Rodbell Conference Rooms 101 ABC
Morning Session

8:30 - 8:45 a.m.	Welcome, Drs. Birnbaum and Zeldin
8:45 - 9:05	Branch Overview, Dale Sandler, Ph.D., Chief
9:05 - 9:55	Chronic Disease Epidemiology Group, Dale Sandler, Ph.D
9:55 - 10:45	Molecular & Genetic Epidemiology Group, Jack Taylor, M.D., Ph.D.
10:45 - 11:00	Break

11:00 - 11:50	Biomarker-based Epidemiology Group, Matt Longnecker, Ph.D
11:50 - 12:35	Closed Sessions with Investigators, Drs. Sandler, Taylor and Longnecker
12:35 - 1:30	Closed – BSC Executive Session and Working Lunch

Afternoon Session

1:30 - 3:00	Poster Session, Fellows and Staff Scientists
3:00 - 3:15	Break
3:15 - 3:45	Closed Sessions with Fellows and Staff Scientists
3:45 - 4:35	Aging & Neuroepidemiology Group, Honglei Chen, M.D., Ph.D.
4:35 - 5:25	Women's Health Group, Donna Baird, Ph.D.
5:30 - 6:00	Closed Sessions with Investigators, Drs. Chen and Baird
6:00	Return to Doubletree Hotel
6:00 - 8:00	Dinner and unassigned time

Closed Evening Session

8:00 - 10:00	BSC Discussion and completion of individual review assignments by each member, All BSC reviewers

Tuesday, June 4: Rodbell Conference Rooms 101 ABC

Morning Session

8:30 - 9:20 a.m.	Genetics, Environment & Respiratory Disease Group, Stephanie London, M.D., Dr.P.H.
9:20 - 10:10	Reproductive Epidemiology Group, Allen Wilcox, M.D., Ph.D.
10:10 - 10:25	Break
10:25 - 11:15	Environmental Autoimmunity Group, Fred Miller, M.D., Ph.D.
11:15 - 12:00	Closed Session with Investigators, Drs. London, Wilcox and Miller
12:00 - 2:00	Closed – BSC Executive Session and Working Lunch
2:00 - 3:30	Closed Session – Debriefing to NIEHS/DIR Leadership, Drs. Birnbaum, Zeldin, Schrader
3:30	Adjournment

DIR LEADERSHIP RETREAT

DIR Council Principals, made up of Laboratory/Branch chiefs, held a half-day retreat at the home of Dr. Dale Sandler on Thursday, July 25, 2013. Dr. Nigel Walker (DNTP) and Dr. Kevin Coray (Coray Gurnitz Consulting) served as facilitators. In light of the reduced size of DIR and the likelihood of continued flat budgets for the foreseeable future, DIR leadership discussed the organization of DIR with the primary goal of identifying a structure that would result in enhancing scientific interactions and collaborations among DIR investigators. Discussions also considered whether reorganization would also result in enhanced administrative efficiencies and potential cost savings.

DIR RESEARCH ACCOMPLISHMENTS

NIEHS team responds to Public Health Concerns in Wake of Deepwater Horizon Disaster in Gulf of Mexico

In April 2010 the Deepwater Horizon Drilling Rig exploded in the Gulf of Mexico leaving behind the largest ever oil spill in terms of size, gallons of oil leaked, affected coastline (in Texas, Louisiana, Mississippi, Alabama, and the Florida Panhandle), and size of the affected population. More than 100,000 persons were involved in some aspect of oil spill clean-up in the wake of this disaster. Investigators from NIEHS recognized that very little is known about the long-term health effects of oil spill exposures despite the fact that there have been as many as 50 major oil tanker spills to date. Clean-up workers had the greatest potential for exposure to the oil and dispersants used in the oil clean-up and are the group in which health effects, if any, should be most salient. The NIEHS team proposed, planned and carried out a longitudinal study designed to evaluate the short-and long-term physical and mental health effects associated with the oil spill. Currently, preparation for potential health research is not part of disaster response. Nonetheless, the team was able to respond quickly, and began interviewing clean-up workers in February 2011, less than a year after the initial explosion in the Gulf. Between February 2011 and March 2013, the GuLF STUDY (Gulf Long-term Follow-up study) surveyed nearly 33,000 persons who participated in some aspect of clean-up including "controls" who trained for clean-up work but were not hired and others who were not directly exposed because they worked in support activities. The team has assembled all of the exposure monitoring data, information on boats, and rosters of workers, and other information that will allow them to fully characterize the exposures of the workers. A subgroup of 11,200 participants completed in-home clinical exams including pulmonary function testing, and two-year health follow-up interviews have begun. A comprehensive clinical exam with about 4,000 persons from Alabama and Louisiana that assesse neurological and respiratory function in greater detail will commence in Fall 2013. This is the largest and most comprehensive study of the potential health effects of an oil spill ever conducted.

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 sister had breast cancer, researchers used DNA extracted from white blood cell samples and assessed methylation at 27,000 sites across the genome. The team also examined known risk factors for breast cancer and genotyped women for nine common polymorphisms associated with breast cancer risk. 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. These findings hold promise for breast cancer detection and risk prediction through methylation profiling of blood.

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

Proteins involved in immunity potentially cause cancer.
NIEHS investigators have determined that a set of proteins involved in the body's natural defenses produces a large number of mutations in human DNA. The finding suggests that these naturally-produced mutations are just as powerful as recognized cancer-causing agents in generating tumors. These proteins are known as "apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like" (APOBEC) cytidine deaminases and the mutations they create can even outnumber all other mutations in some cancers. Investigators looked for signs of genome-wide APOBEC mutagenesis in cancers listed in The Cancer Genome Atlas and found that in many individual bladder, cervical, breast, head and neck, and lung tumors, APOBEC mutagenesis could account for over two-thirds of all mutations in a given tumor.

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. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nat. Genet.,* Epup ahead of print, doi: 10.1038/ng.2702.

Observing a DNA polymerase choose right from wrong
Accurate replication and repair of the genome is vital to genomic stability in all cells, and DNA polymerases provide many of the DNA synthesis and other functions that are essential in the replication and repair processes. To understand the speed and accuracy of DNA polymerases, NIEHS scientists applied time-lapse crystallography to study human DNA polymerase beta. This provided snapshots of structural intermediates of the enzyme as it passed through the catalytic cycle. Conformational adjustments in the polymerase and substrates that hasten correct and deter incorrect nucleotide insertion into DNA were visualized for the first time. Importantly, the structures revealed that the product release step is much slower than other steps. The slow product release step appears to be coupled to downstream events in DNA repair. In summary, this work utilized a new technique to discover novel steps as a polymerase replicates DNA. These results have important implications for understanding the consequences of environmental stressors on genomic stability.

Freudenthal BD, Beard WA, Shock DD, Wilson SH. Observing a DNA polymerase choose right from wrong. *Cell*, 154: 157-168, 2013.

Shining light on p53's role in testicular cancer
Mutations in the p53 gene are common in most types of cancer but are almost never found in testicular tumors. A functional p53 signaling pathway appears to be necessary both for sun tanning and to drive testicular germ cell cancer in light-skinned individuals. Investigators performed an integrated analysis of genome-wide data sets and carried out laboratory experiments to determine that among millions of inherited genetic variations (polymorphisms) in the human genome there is one that leads to p53-dependent up-regulation of the KIT ligand gene (also called Stem Cell Factor) and it imparts a highly significant ($p< 10^{-20}$) increased risk for testicular germ cell cancer. Why is this negative trait common in human populations? Evolutionary analysis suggests that this genetic variant in KIT ligand is strongly linked with p53-dependent tanning in people with light skin color and laboratory studies indicate it may help to prevent skin cancer. Thus over recent human evolution this trait became common in light skinned people through natural selection because it was beneficial but unfortunately, there is a

downside. In modern society this trait is associated with risk of testicular cancer, presumably because upregulation of KIT ligand stimulates testicular stem cells to divide in the presence of DNA damage.

Zeron-Medina J, Wang X, Repapi E, Campbell MR, Su D, Castro-Giner F, Davies B, Peterse EFP, Sacilotto N, Tomlinson IP, Meinshausen N, DeVal S, Bell DA, Bond GL. A Polymorphic p53 Enhancer in the KIT Ligand Oncogene Influences Cancer Risk and Has Undergone Natural Selection. *Cell*, in press.

Bacterial protein in house dust linked to allergic asthma.
NIEHS scientists found that the bacterial protein, flagellin, can be detected in some samples of house dust and can promote allergic responses to inhaled allergens. Mice exposed to house dust showed symptoms of allergic asthma, such as inflammation, mucous production and increased airway obstruction. However, genetically altered mice that are unable to respond to flagellin had reduced levels of these symptoms. They also found that asthmatic subjects had higher levels of antibodies against flagellin than did healthy subjects. Together, these findings suggest that although it is not an allergen, flagellin might promote allergic asthma be strengthening allergic responses to allergens present in common house dust.

Wilson RH, Maruoka S, Whitehead GS, Foley JF, Flake GP, Sever ML, Zeldin DC, Kraft M, Garantziotis S, Nakano H, Cook DN. The Toll-like receptor 5 ligand flagellin promotes asthma by priming allergic responses to indoor allergens. *Nat. Med.*, 18: 1705-1710, 2012.

Developmental origins of central norepinephrine neuron diversity.
Central norepinephrine-producing neurons comprise a diverse population of cells differing in anatomical location, connectivity, function and response to disease and environmental insult. NIEHS scientists determined how differences in early gene expression in the embryonic hindbrain contribute to diversity in the adult mouse brain. They identified four genetically separable subpopulations of mature norepinephrine neurons differing in their anatomical location, axon morphology and efferent projection pattern. These findings provide, for the first time, multiple molecular points of entry for future study of individual norepinephrine circuits in complex behavioral and physiological processes including arousal, attention, mood, memory, appetite and homeostasis.

Robertson S, Plummer N, de Marchena J, Jensen P. Developmental Origins of Central Noradrenergic Neuron Diversity. *Nat. Neurosci.*16: 1016-1023, 2013

The tumor suppressor gene p53 controls the lung's response to bacterial infection.
The transcription factor p53 has been studied for decades as a critical gene that prevents cancer in humans. Although it is expressed by immune cells and activated by a broad array of environmental stressors, its role in the innate immune response to infection is unknown. In this report, the investigators showed that mice with a deleted p53 gene or mice treated with a p53 inhibitor cleared bacterial infection in the lung more effectively than controls. Enhanced pathogen clearance was found to arise from enhanced function of macrophages and neutrophils,

together suggesting that p53 is a common link between defense of the genome and defense of the host against environmental insult.

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. p53 Integrates host defense and cell fate during bacterial pneumonia. *J. Exp. Med.*, 210: 891-904, 2013.

H1N1 flu shots are safe for pregnant women

Some pregnant women fear that a vaccination could harm their fetus. This study showed that pregnant women who received a vaccine against the 2009 H1N1 influenza virus had no increase in fetal loss, while pregnant women who experienced influenza during pregnancy had more miscarriages and still births. The study suggests that pregnant women may more effectively protect their fetus by having an influenza vaccine than by avoiding one.

Håberg SE, Trogstad L, Gunnes N, Wilcox AJ, Gjessing HK, Samuelsen SO, Skrondal A, Cappelen I, Engeland A, Aavitsland P, Madsen S, Buajordet I, Furu K, Nafstad P, Vollset SE, Feiring B, Nøkleby H, Magnus P, Stoltenberg C. Risk of fetal death after pandemic influenza virus infection or vaccination. *N. Engl. J. Med.*, 368: 333-340; 2013.

Atopy is inversely related to nonfatal myocardial infarction in the U.S. population.

Rodent studies indicate that atherosclerosis is driven by a T helper (Th)1 immune program, and reduced by the Th2 immune program that is responsible for allergic disease. Whether this holds in humans is unclear. In this report, the investigators evaluated U.S. national survey data from the National Health and Nutrition Examination Survey, and found that allergen-specific IgE, an objective measure of atopy, is inversely related to self-reported history of myocardial infarction (MI). Participants reporting a history of MI were less likely to have 1 or more positive allergen-specific IgE tests; the strongest inverse relationship was noted for sensitization to house dust mite, and was independent of a long list of established coronary risk factors. These findings suggest that atopy may possibly be protective against MI in humans.

Jaramillo R, Cohn RD, Crockett PW, Gowdy KM, Zeldin DC, Fessler MB. Relation between objective measures of atopy and myocardial infarction in the United States. *J. Allergy Clin. Immunol.*, 131: 405-11.e1-11, 2013.

When RNA meets DNA

Ribonucleotides are the normal building blocks for RNA, but if present in DNA, they sensitize the DNA backbone to cleavage that destabilizes the genome. We previously discovered that ribonucleotides are frequently incorporated into DNA during replication, and that these ribonucleotides are efficiently removed by a dedicated DNA repair process termed RER (for Ribonucleotide Excision Repair). This year, we discovered that when RER is defect (a situation leading to an autoimmunie disease), a second type of ribonucleotide repair is initiated by topoisomerase 1. This is one more step towards understanding the complex biology at the interface between the DNA and RNA worlds.

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

When the "wrong" sugar is good

The "correct" sugar in the sugar-phosphate backbone of RNA is a ribose, whereas the "correct" sugar in the sugar-phosphate backbone of DNA is a d*eoxy*ribose that contains one less oxygen atom. Thus a ribose can be considered the "wrong" sugar in DNA. This year we provided compelling evidence that the presence of this "wrong" sugar in DNA can have a beneficial consequence; it improves the cell's ability to "spell check" replication errors and thereby enhances genome integrity. This is important because defects in spell checking are associated with cancer.

Lujan SA, Williams JS, Clausen AR, Clark AB, Kunkel TA. Ribonucleotides are signals for mismatch repair of leading strand replication errors. *Mol. Cell,* 50: 437-443, 2013.

Molecular mechanism of anticancer drug resistance

DNA topoisomerase II (topo II) plays critical roles in cellular DNA replication and transcription. However, stressors including environmental toxicants and topoisomerase targeted cancer chemotherapeutic drugs can interrupt the topo II-DNA processing reactions to generate genotoxic protein-linked topo II-DNA adducts that block the replication and transcription machinery. Tdp2 initiates a protein-DNA conjugate repair pathway by recognizing and reversing covalent topo II-DNA adducts, functions in cellular topo II drug resistance, and mediates mutant p53 gain of function phenotypes. To shed light onto how Tdp2 acts to maintain the integrity of our genomes, NIEHS scientists interrogated the mechanism of Tdp2 action with structure-activity studies and, used a high-resolution molecular imaging technique (X-ray crystallography) to visualize the Tdp2 in 3 DNA lesion bound states. This work provides insights to the mechanism of Tdp2-linked cancer chemotherapeutic resistance, and establishes a framework for the development of Tdp2 inhibitors that could be employed as adjuvants for commonly employed chemotherapeutic topo II poisons (e.g Etoposide).

Schellenberg MJ, Appel CD, Adhikari S, Robertson PD, Ramsden DA, Williams RS. Mechanism of repair of 5'-topoisomerase II-DNA adducts by mammalian tyrosyl-DNA phosphodiesterase 2. *Nat. Struct. Mol. Biol.*, 19: 1363-1371, 2012.

Sun Exposure May Be Associated with Certain Forms of Autoimmune Muscle Diseases in Children.

This study found that among girls who develop myositis, the odds for developing dermatomyositis (as opposed to polymyositis) are increased about 1.2-fold for each additional unit of ambient UV radiation exposure in the month before illness onset, based on geographic residential location at onset. The odds of having the anti-p155/140 autoantibody, the most common myositis-autoantibody in children with myositis which also happens to be associated with photosensitive skin rashes, was also increased with UV exposure at illness onset by about 1.3 fold, and this was greatest in white boys. In contrast, there was no relationship between UV radiation and another common myositis-autoantibody in children called anti-MJ, or in those patients without a myositis autoantibody. Across the 9 geoclimatic regions of the US, the mean

UV index was associated with increasing odds of having juvenile dermatomyositis and anti-p155/140 autoantibodies, but decreasing odds of having anti-MJ autoantibodies. This is one of the first non-infectious environmental agents associated with the onset of myositis in children. UV radiation may modulate the type of myositis that children develop.

Shah M, Targoff IN, Rice MM, Miller FW, Rider LG, with the Childhood Myositis Heterogeneity Collaborative Study Group. Ultraviolet Radiation Exposure Is Associated With Clinical and Autoantibody Phenotypes in Juvenile Myositis. *Arthritis Rheum.,* 65: 1934–1941, 2013.

Exposure to maternal smoking before birth increases subsequent risk of gestational diabetes in adulthood.

Mothers who smoke while pregnant have offspring who experience a number of health problems, such as those related to small birth weight, and decreased lung function. However, what is now becoming clear is that when those exposed children become adults, a number of health conditions are more common. The link between exposure to maternal smoking before birth, and increased risk of gestational diabetes (i.e., 30 years after exposure), has now been confirmed in two separate studies.

Mattsson K, Källén K, Longnecker MP, Rignell-Hydbom A, Rylander L. Maternal smoking during pregnancy and daughters' risk of gestational diabetes and obesity. *Diabetologia*, 56: 1689-1695, 2013.

Inhibition of Epidermal Growth Factor Receptor Signaling due to Phenobarbital Binding Leads to Activation of the Constitutive Active Androstane Receptor.

Phenobarbital, a central nervous system depressant, has been used as an anticonvulsant for over a century. It has been implicated in the elevated activity of liver metabolic enzymes. Phenobarbital indirectly activates constitutively active androstane nuclear receptor (CAR) that, when active, promotes drug and energy metabolism, along with cell growth and death in the liver. Findings from this study reveals that the activation of CAR by phenobarbital is achieved by the direct binding of phenobarbital to epidermal growth factor receptor (EGFR) and thereby, inhibiting EGFR's signaling.

Mutoh S, Sobhany M, Moore R, Perera L, Pedersen L, Sueyoshi T, Negishi M. Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling. *Sci. Signal.*, 6: ra31, 2013.

Glucocorticoids Alter the Effectiveness of GPCR-Based Drugs

NIEHS scientists discovered a mechanism by which glucocorticoids, stress hormones produced by the body to maintain homeostasis, regulate the signaling profile of G protein-coupled receptors (GPCRs), the most common target of prescription drugs. Specifically, they found that glucocorticoids act upon GPCRs by up-regulating the gene expression of one signaling protein called beta-arrestin-1, while simultaneously down-regulating the expression of another signaling protein, beta-arrestin-2. These findings may provide the molecular basis for the clinical synergism observed with glucocorticoid/GPCR agonist combination therapies currently used to treat asthma and chronic obstructive pulmonary disease (COPD). Understanding the

mechanisms that make these combinations so powerful may allow the development of other regimens involving glucocorticoids and GPCR-targeted drugs that will be more safe and efficacious.

Oakley RH, Revollo J, Cidlowski JA. Glucocorticoids regulate arrestin gene expression and redirect the signaling profile of G protein-couple receptors. *Proc. Natl. Acad. Sci. USA,* 109: 17591-17596, 2012.

Blood-Brain Barrier Signaling Improves Drug Delivery to the Brain

At the blood-brain barrier, P-glycoprotein, a drug efflux pump is a major impediment to delivering drugs to the brain to treat, for example, tumors, epilepsy and neuroAIDS. We described a signaling pathway within the blood-brain barrier's capillary endothelium that rapidly reduces the transport activity of P-glycoprotein. The pathway involves signaling through a sphingolipid receptor that can also be targeted by drugs currently in use in the clinic. Treating rats with one such drug increased brain uptake of a potent chemotherapeutic five-fold. These findings suggest a new strategy for CNS pharmacotherapy.

Cannon RE, Peart JC, Hawkins BT, Campos CR, Miller DS. Targeting sphingolipid signaling at the blood-brain barrier reduces basal P-glycoprotein activity and improves drug delivery to the brain. *Proc. Natl. Acad. Sci. USA,* 109:15930-15935, 2012.

Recovering Male Infertility with An Estrogen

The female steroid hormone "estrogen" is important in male reproductive function. This study revealed that a specific critical functional domain of the estrogen receptor protein regulates testicular fluid reabsorption. Defects in this process due to loss of estrogen receptor function cause male infertility. This information is useful to possibly evaluate the function of estrogenic endocrine disruptors in male infertility and the role these substances may play in andrology.

Arao Y, Hamilton KJ, Goulding EH, Janardhan KS, Eddy EM, Korach KS. Transactivating function (AF) 2-mediated AF-1 activity of estrogen receptor α is crucial to maintain male reproductive tract function. *Proc. Natl. Acad. Sci. USA.*, 109: 21140-21145, 2012.

Heterotrimeric G protein required for skeletal formation

Mice with a loss-of-function mutation in the heterotrimeric G protein α-subunit gene Gnai3 have fusions of ribs and lumbar vertebrae, indicating a requirement for Gαi (the "inhibitory" class of α-subunits) in somite derivatives. Mice with mutations of Gnai1 or Gnai2 have neither defect, but loss of both Gnai3 and one of the other two genes increases the number and severity of rib fusions without affecting the lumbar fusions. No myotome defects are observed in Gnai3/Gnai1 double-mutant embryos, and crosses with a conditional allele of Gnai2 indicate that Gαi is specifically required in cartilage precursors. These phenotypes reveal a previously unknown role for G protein-coupled signaling pathways in development of the axial skeleton.

Plummer NW, Spicher K, Malphurs J, Akiyama H, Abramowitz J, Nürnberg B, Birnbaumer L. Development of the mammalian axial skeleton requires signaling through the Gα(i) subfamily of heterotrimeric G proteins. *Proc. Natl. Acad. Sci. USA.*, 109: 21366-21371, 2012.

Generation and toleration of chromosome destabilizing lesions following UV irradiation.
DNA damage challenges genome integrity. Bulky DNA lesions, which are subject to nucleotide excision repair, induce chromosome exchanges via homologous recombination. However, since there is no direct generation of double-strand breaks, the underlying mechanism of the damage-induced exchange has been obscure. By investigating UV lesions in the DNA of nondividing budding yeast cells, the processes of repair synthesis past lesions and homologous recombination were found to be redundant repair. A physical assay was developed that detects recombination between circular sister chromatids that revealed that UV-induced recombination is not attributable to double-strand breaks, but instead is directly associated with expanded single-strand gaps and is increased in cells defective in DNA synthesis past lesions. Since yeast has been a model for human studies, the findings have important implications for the role of human repair genes in dealing with environmental agents such as sunlight and various chemicals.

Ma W, Westmoreland J, Resnick MA. Homologous recombination rescues single strand DNA gaps generated by NER and reduced translesion DNA synthesis in yeast G2 cells. *Proc. Natl. Acad. Sci. USA.*, 110: E2895-E2904, 2013.

New mouse model advances study of mitochondrial diseases
Researchers at NIEHS have determined that POLG2, the accessory subunit of the DNA polymerase gamma complex, is necessary for mammalian embryogenesis and mitochondrial DNA (mtDNA) replication. The finding will help scientists better understand disorders caused by mutations in mtDNA or depletion of mtDNA, such as Alpers' disease, an illness that causes dementia, seizures, and liver failure. The DNA polymerase gamma complex is made up of a catalytic subunit and an accessory subunit. While approximately 200 pathogenic mutations in the catalytic subunit, which lead to mitochondrial diseases, have been described, the accessory subunit had not been well characterized. The mouse model developed in this study is the first mammalian model of the Polg2 gene, and allowed the scientists to study the accessory subunit. The researchers monitored mice that were heterozygous for Polg2 for two years and found them to be no different than wild-type mice. However, they determined that a homozygous knockout of Polg2 was embryonic lethal. Further investigation into the knockout revealed a loss of mtDNA, structural defects of the mitochondria, and respiratory-chain failure seen via the lack of cytochrome c oxidase I activity.

Humble MM, Young MJ, Foley JF, Pandiri AR, Travlos GS, Copeland WC. Polg2 is essential for mammalian embryogenesis and is required for mtDNA maintenance. *Hum. Mol. Genet*., 22: 1017-1025, 2013.

Cyclooxygenases and T cell Function
Cyclooxygenase (COX) enzymes are known to be important regulators of Th1, Th2 and Th17 cells in allergic lung disease; however, it is not known whether COX-1– or COX-2–derived eicosanoids regulate Th9 cell function, or the mechanisms involved. This study identifies COX-2 as a key negative regulator of Th9 cell differentiation and function in allergic lung inflammation via an autocrine loop that involves PGD_2 and PGE_2 suppression of IL-17RB through PKA signaling.

Li H, Edin ML, Bradbury JA, Graves JP, DeGraff LM, Gruzdev A, Cheng J, Dackor RT, Wang PM, Bortner CD, Garantziotis S, Jetten AM, Zeldin DC. Cyclooxygenase-2 Inhibits Th9 Differentiation During Allergic Lung Inflammation Via Downregulation of IL-17RB. *Am. J. Respir. Crit. Care. Med.*, 187: 812-822, 2013.

Novel structure of cockroach allergen Bla g 1 has implications for allergenicity and exposure assessment

Sensitization to cockroach allergens is a major risk factor for asthma, especially in lower socioeconomic households. The cockroach allergen Bla g 1 was discovered to form a novel alpha-helical capsule with an internal cavity that binds lipids promiscuously. Lipid binding is a common feature of many allergens and may contribute to sensitizing humans. The structure defines the basic allergen unit, allowing for quantitative environmental exposure measurements. Better standards are needed for this allergen in order to rigorously assess clinical outcomes.

Mueller GA, Lars C. Pedersen LC, Lih FB, Glesner J, Moon AF, Chapman MD, Tomer KB, London RE, Pomés A. Novel structure of cockroach allergen Bla g 1 has implications for allergenicity and exposure assessment. *J. Allergy Clin. Immunol.,* Epub ahead of print, doi: 10.1016/j.jaci.2013.06.014.

Clinical and Serologic Phenotypes of Juvenile Myositis are Distinct and Similar to Corresponding Adult Myositis Phenotypes.

It was determined that juvenile dermatomyositis was characterized by characteristic skin rashes, as well as other photosensitive and vasculopathic skin rashes. Juvenile polymyositis, the form without characteristic skin rashes, was characterized by more severe muscle involvement and more frequent cardiac disease. Patients with overlap myositis, who met criteria for myositis and also another autoimmune disease, more frequently had lung disease, joint involvement, and other distinct clinical features. Mortality was highest in patients with overlap myositis, whereas hospitalizations and wheelchair use were highest in patients with juvenile polymyositis. A related study examined the distinct features of subgroups based on the presence of specific autoantibodies seen only in myositis patients, known as the myositis autoantibodies. Anti-p155/140 autoantibodies were the most frequent subgroup represented in the children with myositis, present in 32% of patients, and this group was characterized by frequent photosensitive skin rashes and a chronic illness course. Anti-MJ autoantibodies was the second most common subgroup, present in 20% of patients, and distinguishing features included muscle cramps, dysphonia, and a shorter disease course. Patients with antisynthetase autoantibodies had higher frequencies of interstitial lung disease, joint pain, and ‘‘mechanic’s hands,’’ and had an older age at diagnosis. The anti-signal recognition particle group, which had exclusively juvenile polymyositis patients without characteristic skin rashes, was characterized by high frequencies of black race, severe muscle involvement, frequent cardiac involvement, a chronic disease course, frequent hospitalization, and wheelchair use. Characteristic features of the anti-Mi-2 subgroup included Hispanic ethnicity, classic dermatomyositis and very low mortality. Several demographic and clinical features were shared between the juvenile and adult myositis subgroups, but several features differed.

Shah M, Mamyrova G, Targoff IN, Huber AM, Malley JD, Rice MM, Miller FW, Rider LG, with the Childhood Myositis Heterogeneity Collaborative Study Group. The Clinical

Phenotypes of the Juvenile Idiopathic Inflammatory Myopathies. *Medicine,* 92: 25-41, 2013.

Rider LG, Shah M, Mamyrova G, Huber AM, Rice MM, Targoff IN, Miller FW, with the Childhood Myositis Heterogeneity Collaborative Study Group. The Autoantibody Phenotypes of the Juvenile Idiopathic Inflammatory Myopathies. *Medicine*, 92: 223-243, 2013.

Enhanced cell killing through PARP inhibition

The effect of inhibitors of the catalytic activity of the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) on cell viability is an important topic in cancer chemotherapy research. This is because the inhibitors kill cancers with DNA repair deficiencies, whereas normal cell are resistant. Nevertheless, there is wide variability in the efficiency of cell killing by PARP inhibitors, and factors modifying the cell killing phenotype are largely unknown. The group and collaborators recently discovered that the stability of a protein complex of base excision repair factors is important in the PARP inhibitor-based cell killing phenotype. Strategic mutations in one of the proteins in the complex (XRCC1) revealed that the protein is a modifier of the cell killing phenotype and that the protein is sensitive to the redox status of the cell.

Horton JK, Stefanick DF, Gassman NR, Williams JG, Gabel SA, Cuneo MJ, Prasad R, Kedar PS, Derose EF, Hou EW, London RE, Wilson SH. Preventing oxidation of cellular XRCC1 affects PARP-mediated DNA damage responses. *DNA Repair*, 12: 774-778, 2013.

Horton JK, Wilson SH. Predicting enhanced cell killing through PARP inhibition. *Mol. Cancer Res.*, 11: 13-18, 2013.

Understanding the genomic impact of the tumor suppressor p53 in cancer cells.

The p53 transcription factor suppresses tumor formation and maintains genome stability by modulating the expression of many genes that deal with environmental stresses. Binding to a target DNA sequence is an essential precursor for the activation or repression of transcription. But, how specific p53 binding sites are selected in response to different stress-inducing agents from the many consensus target sequences in the human genome is poorly understood. Using a genome-wide, high-throughput sequencing approach, new rules of engagement of p53 across the genome were revealed, including binding to half-sites. This is the first complete, in-depth genome analysis that takes into account p53 levels, binding, expression and chromatin changes under diverse stress and non-stress conditions. Conditions were identified that separate binding from changes in gene expression, suggesting a two-step process in which some agents might serve to prime activation by other treatments to provide more effective combined therapies. Importantly, this genome-wide approach also led to the identification of several potential new p53 target genes that are relevant to tumor suppression as well as cancer-associated mutations revealing potential new targets for cancer therapy.

Menendez D, Nguyen TA, Freudenberg JM, Mathew V J, Anderson CW, Jothi R, Resnick MA. Diverse stresses dramatically alter genome-wide p53 binding and transactivation landscape in human cancer cells. *Nucleic Acids Res.*, Epub ahead of print, doi: 10.1093/nar/gkt504

Phthalate Exposure and Allergy
Environmental exposures to phthalates, particularly high molecular weight (HMW) phthalates, are suspected to contribute to allergy. We used data on urinary phthalate metabolites, allergic symptoms (hay fever, rhinitis, allergy, wheeze, asthma) and sensitization from participants 6 years and older in the National Health and Nutrition Examination Survey (NHANES) 2005-2006. The HMW phthalate metabolite mono-benzyl phthalate (MBzP) was the only metabolite positively associated with current allergic symptoms in adults (wheeze, asthma, hay fever, and rhinitis). Mono-(3-carboxypropyl) phthalate and the sum of diethylhexyl phthalate metabolites (both representing HMW phthalate exposures) were positively associated with allergic sensitization in adults. Conversely, in children, HMW phthalate metabolites were inversely associated with asthma and hay fever. Of the low molecular weight phthalate metabolites, mono-ethyl phthalate was inversely associated with allergic sensitization in adults. Thus, in this cross-sectional analysis of a nationally representative sample, HMW phthalate metabolites, particularly MBzP, were positively associated with allergic symptoms and sensitization in adults, but there was no strong evidence for associations between phthalates and allergy in children aged 6-17 years.

Hoppin JA, Jaramillo R, London SJ, Bertelsen RJ, Salo PM, Sandler DP, Zeldin DC. Phthalate Exposure and Allergy in the US Population: Results from NHANES 2005-2006. *Environ. Health Perspect.*, Epub ahead of print doi: 10.1289/ehp.1206211

The herbicide paraquat increases risk of Parkinson's disease in genetically susceptible individuals.
Parkinson's disease results from a combination of genetic changes and environmental exposures. In a study of farmers who applied pesticides, it was found that risk of Parkinson's disease was slightly increased in individuals who used the herbicide paraquat. There was also a slight increase in risk in individuals with a genetic mutation leading to absence of one form of the enzyme glutathione-S-transferase, which helps the body respond to oxidative stress. However, risk of Parkinson's disease was increased 11-fold in individuals with the mutation who also used paraquat. These results help to clarify the causes of Parkinson's disease and may suggest approaches to treatment.

Goldman SM, Kamel F, Ross GW, Bhudhikanok GS, Hoppin JA, Korell M, Marras C, Meng C, Umbach DM, Kasten M, Chade AR, Comyns K, Richards MB, Sandler DP, Blair A, Langston JW, Tanner CM. Genetic modification of the association of paraquat and Parkinson's Disease. *Mov. Disord.*, 27: 1562-1568, 2012.

Exposure to an anti-bacterial agent frequently used by the general population is associated with increased risk of allergic sensitization in children.
Triclosan interferes with cell wall metabolism in bacteria, and is a component of many hand sanitizers. Triclosan is detectable in the urine of a large proportion of the general population in developed countries. This study and another recent study in the U.S. both found that exposure to triclosan, as measured by urine concentration, was associated with increased allergic sensitization in children.

Bertelsen RJ, Longnecker MP, Løvik M, Calafat AM, Carlsen KH, London SJ, Lødrup Carlsen KC. Triclosan exposure and allergic sensitization in Norwegian children. *Allergy*, 68: 84-91, 2013.

Persistent organic pollutants found not to be obesogens.
A great deal of research attention has recently been focused on whether exposure to certain environmental contaminants early in life causes increased obesity. Some early, small studies on this topic suggested that background-level exposure to persistent organic pollutants such at DDT and polychlorinated biphenyls (PCBs) were obesogens. In the present study, which was much larger than previous studies and conducted in a population with much higher levels of exposure than before, no link between persistent organic pollutants and obesity was found.

Cupul-Uicab LA, Klebanoff MA, Brock JW, Longnecker MP. Prenatal Exposure to Persistent Organochlorines and Childhood Obesity in the U.S. Collaborative Perinatal Project. *Environ. Health Perspect*., Epub ahead of print, doi: 10.1289/ehp.1205901.

Promoter Pol II is Pause-itively engaged in RNA synthesis
The pausing of RNA polymerase II (Pol II) during early transcription elongation has emerged as a widespread regulatory phenomenon in metazoan gene expression. Although the phenomenon has recently gained acceptance as a core feature of transcription in higher eukaryotes, the nature of Pol II at the pause site has remained ambiguous. A recent collaboration between the scientists at NIEHS and Cornell University confirms that the vast majority of promoter-associated polymerase is in a transcriptionally engaged state that is competent to resume RNA synthesis. This work solidly establishes the paused Pol II complex as an essential, tunable element of gene regulation.

Core LJ, Waterfall JJ, Gilchrist DA, Fargo DC, Kwak H, Adelman K, Lis JT. Defining the status of RNA polymerase at promoters. *Cell Reports*, 2: 1025-1035, 2012.

How cells achieve high accuracy of chromosomal DNA replication
The accuracy of DNA replication is a crucial factor for the processes by which organisms undergo mutation. To gain understanding in this area NIEHS scientists are studying the accuracy (fidelity) of DNA replication in the bacterium Escherichia coli, which is a simplified but useful model system for these questions. The bacterial chromosome is replicated by the DNA polymerase III holoenzyme (HE), whose accuracy has been studied in detail. But in addition, other DNA polymerases play a role (E. coli has five such accessory DNA polymerases) and they can affect the overall error rate. These studies have shown that two accessory DNA polymerases (Pol II and Pol IV) directly contribute to the chromosomal error rate, reducing or increasing replication errors, respectively, while DNA Pol I fulfills an indirect role through the error-free filling of the Okazaki fragment gaps. These studies have also demonstrated the important role of the 5'-deoxynucleoside-triphosphates (dNTPs), the building blocks used by the polymerases for synthesizing DNA, and how cells must control the dNTP levels to keep replication accurate.

Ahluwalia D, Bienstock RJ, Schaaper RM. Novel mutator mutants of E. coli nrdAB ribonucleotide reductase: insight into allosteric regulation and control of mutation rates. *DNA Repair*, 11: 480-487, 2012.

Conte E, Vincelli G, Schaaper RM, Bressanin D, Stefan A, Dal Piaz F, Hochkoeppler A. Stabilization of the Escherichia coli DNA polymerase III ε subunit by the θ subunit favors in vivo assembly of the Pol III catalytic core. *Arch. Biochem. Biophys*., 523: 135-143, 2012.

Schaaper RM, Mathews CK. Mutational consequences of dNTP pool imbalances in E. coli. *DNA Repair*, 12: 73-79, 2013.

Taira K, Kaneto S, Nakano K, Watanabe S, Takahashi E, Arimoto S, Okamoto K, Schaaper RM, Negishi K, Negishi T. Distinct pathways for repairing mutagenic lesions induced by methylating and ethylating agents. *Mutagenesis*, 28: 341-350, 2013.

Mechanism of DNA synthesis by DNA polymerases

Understanding the chemical mechanism of DNA synthesis by the class of enzymes termed DNA polymerases has been a topic of long standing interest in biological research. By making use of a novel interdisciplinary approach involving X-ray crystallography, enzyme kinetics and computational studies, NIRHS scientists recently identified the mechanism of the chemical reaction. A strategic mutation was engineered into the model DNA polymerase, human pol beta, and the altered enzyme was examined. The results revealed the first understanding of the mechanism of activation of the chemical reaction of nucleotide insertion into DNA.

Batra VK, Perera L, Lin P, Shock DD, Beard WA, Pedersen LC, Pedersen LG, Wilson SH. Amino acid substitution in the active site of DNA polymerase β explains the energy barrier of the nucleotidyl transfer reaction. *J. Am. Chem. Soc.,* 135: 8078-8088, 2013.

Folate Deficiency Impairs Critical Blood-Brain Barrier Functions

Folate deficiency is associated with multiple clinical phenotypes, including neural tube defects during embryogenesis and neurological disorders in children and adults. The proton-coupled folate transporter (PCFT)-null mouse, is a model for systemic folate deficiency. Brain capillaries from PCFT-null mice exhibited multiple defects, including reduced expression of P-glycoprotein and several tight junction proteins and loss of ability to increase transporter expression in response to drugs and environmental pollutants. Treating the mice with S-folinic acid restored some elements of normal blood-brain barrier function. Thus, folate deficiency disrupts blood-brain barrier function by targeting transporters and tight junctions. This may contribute to the development of neurological disorders in folate deficient individuals.

Wang X, Cabrera RM, Li Y, Miller DS, Finnell RH. Functional regulation of P-glycoprotein at the blood-brain barrier in proton-coupled folate transporter (PCFT) mutant mice. *FASEB J.*, 27: 1167-1175, 2013.

Self-renewal by stem cells in the testis is controlled by the microenvironment of the stem cell niche

Spermatogonia sit in a compartment on the basal lamina of the seminiferous tubule, separated from other germ cells by Sertoli cells and from the interstitium by peritubular myoid cells.

Because other spermatogonia are present within the region of the niche, the question becomes how are spermatogonial stem cell (SSC) daughter cells influenced to decide between becoming self-renewing spermatogonia that enter the differentiation pathway of spermatogenesis? These studies demonstrate that population dynamics and interactions of SSCs and their descendants in and around the spermatogonial niche determine their fate.

Eddy EM, Chen L-Y. Location, Location, Location: How does a spermatogonium know it is a spermatogonial stem cell (SSC)? *Biol. Reprod.*, 88: 132, 1-2, 2013.

An important factor that contributes to normal lung and immune development, and protects against lung injury in neonatal mice.

Nrf2 is an essential transcription factor for protection against oxidant disorders; however, its role in organ development and neonatal disease has received little attention. This study is the first to use lung transcriptomic and signaling pathway analyses to understand the role of Nrf2 in the molecular events during saccular-to-alveolar stage transition. Transcriptome analysis of lungs from *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice also supported a functional role for Nrf2 and related downstream effector mechanisms (e.g., *Gpx2* and *Marco*) in the pathogenesis of hyperoxia-induced lung injury in neonates, a model of bronchopulmonary dysplasia (BPD). Results collectively provide insights into Nrf2-driven host defense mechanisms in developing lung, and suggest a therapeutic potential of specific Nrf2 activators in BPD and other neonatal diseases associated with oxidative stress (e.g., respiratory syncytial virus disease).

Cho HY, van Houten B, Wang X, Miller-DeGraff L, Fostel J, Gladwell W, Perrow L, Kobzik L, Yamamoto M, Bell DA, Kleeberger SR. Targeted deletion of *Nrf2* impairs lung development and oxidant injury in neonatal mice. *Antoxid. Redox. Signal.,* 17: 1066-1082, 2012.

A new mechanism for the allergic response to sulfite

Sulfite is widely used in the food industry and as a medical ingredient because of its antioxidant and antimicrobial properties. The prevalence of sulfite toxicity in the lung is relatively high, and it has been associated with allergic reactions characterized by sulfite sensitivity, asthma, and anaphylactic shock. Myeloperoxidase (MPO) is an abundant heme protein secreted from activated human neutrophils that catalyzes the formation of cytotoxic oxidants implicated in asthma and allergic inflammatory disorders. NIEHS investigators found that MPO uses sulfite as a substrate and oxidizes it to a free radical metabolite. A mechanism of MPO-dependent oxidative damage and tissue injury in sulfite-exacerbated inflammatory disorders is proposed. Individuals who develop sensitivity to sulfite may have low levels of sulfite oxidase activity or low concentrations of (pseudo) halides, physiological states conducive to sulfite-dependent protein radical formation and subsequent oxidative protein modification. Novel oxidized proteins may serve as antigens or in some other way initiate an allergic response.

Ranguelova K, Rice AB, Lardinois OM, Triquigneaux M, Steinckwich N, Deterding LJ, Garantziotis S, Mason RP. Sulfite-mediated oxidation of myeloperoxidase to a free radical: immuno-spin trapping detection in human neutrophils. *Free Radic. Biol. Med.*, 60: 98-106, 2013.

Novel actions of an inositol phosphate suggest a new therapeutic direction to counter vascular remodeling in cardiovascular disease.
Multifactorial gene–environment interactions promote the aberrant proliferation and migration of vascular smooth muscle cells (VSMC) that underlies hypertension and atherosclerosis. The identification and functional characterization of the mechanisms of VSMC dysfunction can assist therapeutic intervention in vascular remodeling. The current study demonstrates that VSMC migration is regulated by complex interactions between an ion channel, a channel activator (a protein kinase) and a channel inhibitor (an inositol phosphate signal, IP4). Data indicating that IP4 inhibits cell migration suggests the inositol phosphate is a potential lead compound for synthesizing a drug to inhibit vascular remodeling.

Ganapathi SB, Wei SG, Zaremba A, Lamb FS, Shears SB. Functional regulation of ClC-3 in the migration of vascular smooth muscle cells. *Hypertension*, 61:174-179, 2013.

Metal and ligand binding to the HIV-RNase H active site are remotely monitored by Ile556
HIV-1 reverse transcriptase (RT), a critical enzyme in the life cycle of the human immunodeficiency virus, contains a C-terminal ribonuclease H (RH) domain. Recent studies of Ribonuclease H enzymes demonstrate that substrate binding is required for formation of the enzyme active site. In the absence of substrate active site residues, including those on the C-terminal helix E of the RT RNase H domain are dynamic, lacking a unique structure. Nuclear magnetic resonance studies of the RH domain of RT demonstrate that Ile556, a residue located near the C-terminus of the RH domain, undergoes a conformational change when the active site of the enzyme is formed, either in the presence of substrate or active site-directed inhibitors. This conformational change is readily measured by NMR and facilitates the evaluation of how various ligands influence formation of the RH domain active site. Thus, NMR can be used to screen for ligands that influence active site structure of the RT RH domain.

Zheng X, Mueller GA, DeRose EF, London RE. Metal and ligand binding to the HIV-RNase H active site are remotely monitored by Ile556. *Nucleic Acids Res.*, 40: 10543-10553, 2012.

Coordination in the repair of the ends of a chromosome break.
DNA double-strand breaks can cause genome instability and cancer. While repair can occur through recombination, coincident events at both ends have not been directly addressable at unique or random damage-induced DSBs. NIEHS investigators describe an electrophoresis approach that for the first time distinguishes processing at 0, 1 or both ends of a double-strand break. Resection, an early step in double-strand break end-processing, was found to be efficiently initiated at both ends of random, radiation-induced breaks in wild type budding yeast and cells deficient in late steps of recombinational repair. However, 0- and 1-end resections predominate in several end-processing mutants, indicating new roles for the human cancer-related proteins in repair, namely, efficient and coincident resection at both ends of a break. The structural features of the MRX complex (MRN in humans) responsible for the resection suggest that it directly coordinate resections through interactions with both ends of break. Surprisingly, coincident resection at a clean enzyme-induced differed from resection at radiation-induced breaks suggesting additional requirements in the repair of damage-induced breaks.

Westmoreland J, Resnick MA. Coincident resection at both ends of random, γ−induced double-strand breaks requires MRX (MRN), Sae2 (Ctp1) and Mre11-nuclease. *PLoS Genet.*, 9: e1003420, 2013.

Human mitochondrial DNA polymerase ineffectively repairs acrolein-induced adducts. Researchers from NIEHS, in collaboration with scientists from Oregon Health and Science University, report that acrolein–induced adducts to mitochondrial DNA are bypassed by human mitochondrial DNA polymerase gamma at low fidelity, causing errors in DNA synthesis. Since these errors have the possibility of becoming mutations that could then lead to human diseases, such as neurodegenerative disorders, the research has implications for public health. Acrolein is a mutagenic aldehyde produced by biological processes and by combustion of organic materials, including tobacco smoke. Acrolein reacts with bases on DNA to form adducts that block DNA synthesis. In the nucleus, DNA adducts are repaired by multiple translesion synthesis polymerases that don't exist in animal cell mitochondria. The researchers utilized single nucleotide incorporation and primer extension analyses to assess if mitochondrial polymerase gamma was able to overcome acrolein-induced DNA adducts in mitochondrial DNA. They found adenosine adducts were correctly and efficiently repaired. However, repair of minor groove guanine adducts, although able to be bypassed by pol gamma, exhibited reduced efficiency and low fidelity with a preference for incorporation of opposite-adduct purines.

Kasiviswanathan R, Minko IG, Lloyd RS, Copeland WC. Translesion synthesis past acrolein-derived DNA adducts by human mitochondrial DNA polymerase gamma. *J. Biol. Chem.*, 288: 14247-14255, 2013.

Glycolysis is essential for sperm function
Sperm require a constant source of energy to travel through the female reproductive tract and to fertilize an egg. Lactate dehydrogenase (LDH) is an enzyme involved in the conversion of glucose to energy and a novel version of this enzyme (LDHC) is present in mammalian sperm. NIEHS investigators have shown that LDHC is essential for sperm function and that chemicals and genetic mutations that disrupt its function cause male infertility. Enolase is another glycolytic enzyme and these investigators determined that the enolase (ENO4) in sperm is encoded by a novel gene and that male mice lacking ENO4 are infertile and their sperm have structural and functional defects.

Odet F, Gabel S, London RE, Goldberg E, Eddy EM. Glycolysis and mitochondrial respiration in mouse LDHC null sperm. *Biol. Reprod.*, 88: 95, 1-7, 2013.
Nakamura N, Dai Q, Williams J, Goulding EH, Willis WD, Brown P, Eddy EM. Disruption of a spermatogenic cell-specific mouse enolase 4 (Eno4) gene causes sperm structural defects and male infertility. *Biol. Reprod.*, 88: 90, 1-12, 2013.

Discovery of novel detoxification and mutation prevention mechanisms
The base analog N6-hydroxylaminopurine (HAP), which is an analog of the normal DNA and RNA constituent Adenine, is an extremely potent mutagen in all organisms from bacteria to man. NIEHS investigators have discovered a novel detoxification system for this compound in the bacterium E. coli, and have shown that this system requires the Molybdenum Cofactor. They

have defined the genes as well as the proteins responsible for this activity. These proteins constitute a novel family of Molybdoproteins. These proteins may play a broader role in the detoxification of N-hydroxylated compounds.

Kozmin SG, Schaaper RM. Genetic characterization of moaB mutants of Escherichia coli. *Res. Microbiol.*, 164: 689-694, 2013.

Kozmin SG, Stepchenkova EI, Schaaper RM. TusA (YhhP) and IscS are required for molybdenum-cofactor-dependent base-analogue detoxification. *Microbiol. Open*, doi: 10.1002/mbo3.108, 2013.

Moonlighting: increasing biological complexity through gene pleiotropy.
Craniofacial disorders are a primary cause of infant mortality and have devastating functional, esthetic, and social consequences. One contributory factor is in utero breakdown of spatio-temporal control over synthesis of ribosomal RNA (rRNA). The current study demonstrates that IP5K - a kinase that synthesizes intracellular signals - also "moonlights" as a molecular glue for the assembly of the nucleolus; the latter is the multiprotein complex that synthesizes rRNA. The data show that disruption of IP5K gene function prevents assembly of the nucleolus, inhibiting the rRNA synthesis that is fundamental to the life and destiny of every cell. The work offers new directions for understanding defects in craniofacial development such as those that characterize Treacher-Collins syndrome. Moreover, the new example of how the biological sophistication of higher organisms can arise from gene products acquiring multiple functions (i.e. pleiotropy), promises to increase the reliability of genetic risk-profiling.

Brehm MA, Wundenberg T, Williams J, Mayr GW, Shears SB. A non-catalytic role for inositol 1,3,4,5,6-pentakisphosphate 2-kinase in the synthesis of ribosomal RNA *J. Cell. Sci*,. 126: 437-444, 2013.

New signaling mechanisms for controlling inflammatory responses in the brain
Many neurodegenerative diseases are exacerbated by runaway inflammatory responses by microglial cells in the brain to neuronal degeneration, so understanding how microglial activation, migration, and deactivation are regulated and how they are disrupted by environmental toxicants will be essential for treating and reducing the incidence of neurodegenerative disease. DIR and NTP scientists have discovered that the hormones which regulate microglial cell movements also regulate their potassium channels. Potassium channels are known to be important for cell fate and functions in the immune system by altering calcium levels inside the cell, but how the hormones regulate potassium channel activity was unknown. Here the scientists show that some of those effects are mediated by Rho GTPases.

Muessel MJ, Harry GJ, Armstrong DL, Storey NM. SDF-1a and LPA Modulate Microglia Potassium Channels Through Rho GTPases to Regulate Cell Morphology. *Glia*, Epub ahead of print, doi: 10.1002/glia.22543.

Role of TGF-β activated kinase-1 (Tak1, Map3k7) signaling in the craniofacial neural crest development
While the importance of TGF-β superfamily signaling in craniofacial growth and patterning is well established, the precise details of its signaling mechanisms are still poorly understood. This

study demonstrates that mouse embryos lacking Tak1 (a mediator of TGF-β superfamily signaling) in the neural crest display craniofacial hypoplasia and cleft palate. Tak1 regulates agonist-induced TGF-β superfamily signaling in craniofacial mesenchymal cells, and may play a specific regulator role during craniofacial development. Understanding of detailed mechanisms of TGF-β superfamily signaling contributes to knowledge of pathogenetic mechanisms behind the common craniofacial birth defects in humans.

Yumoto K, Thomas PS, Lane J, Matsuzaki K, Inagaki M, Ninomiya-Tsuji J, Scott GJ, Ray MK, Ishii M, Maxson R, Mishina Y, Kaartinen V. Tak1 mediates agonist-induced rSmad activation and linker region phosphorylation in embryonic craniofacial neural crest-derived ecto-mesenchyme. *J. Biol. Chem.,* 288: 13467-13480, 2013.

Two heads are better than one: a new mode of coincidence detection for regulating cell function.

Cell behavior is governed by sensitive signaling systems that respond to specific external and internal stimuli - but sensitivity carries enormous risk; it increases the possibility that a cell "self-activates" inappropriately, with harmful consequences, purely due to the randomness of biochemical reactions. Such stochastic activation must be suppressed, yet without impeding biological control. One solution is coincidence detection: two signaling events that occur simultaneously. A novel example of this phenomenon is demonstrated in the current study. NIEHS scientists show that cellular responses to a phospholipid (PIP3)-driven signaling cascade relies not just on stimulus-dependent activation of PIP3 synthesis (as had been thought), but also the simultaneous recruitment of a signaling enzyme (PPIP5K1) to de-activate an endogenous inhibitor of PIP3 actions. As perturbations to the PIP3 pathway drive cancer and metabolic diseases such as diabetes, our study has important human health implications.

Gokhale NA, Zaremba A, Janoshazi AK, Weaver JD, Shears SB. PPIP5K1 modulates ligand competition between diphosphoinositol polyphosphates and PtdIns(3,4,5)P3 for polyphosphoinositide-binding domains. *Biochem. J.*, 453: 413-426, 2013.

Protein-Mediated Antagonism between HIV Reverse Transcriptase Ligands Nevirapine and MgATP.

HIV reverse transcriptase (RT), the enzyme responsible for converting viral genomic RNA into proviral doublestranded DNA, is a primary target for drug intervention. There are currently two types of drugs that interfere with RT activity: nucleoside and non-nucleoside inhibitors. The latter appear to be associated with lower long-term toxicity, but their mechanisms are incompletely understood. These new NMR studies demonstrate strong, protein-mediated antagonism between the binding of MgATP and the non-nucleoside inhibitor nevirapine (NVP). This antagonism involves changes in the orientation of the "thumb" subdomain of the reverse transcrptase. Since the structural changes of the active site produced by MgATP are similar to those observed in a catalytic complex, these results provide insight into the basis for inhibition of RT activity by nevirapine and other non-nucleoside inhibitors.

Zheng X, Mueller GA, Derose EF, London RE. Protein-Mediated Antagonism between HIV Reverse Transcriptase Ligands Nevirapine and MgATP. *Biophys. J.,* 104: 2695-2705, 2013.

Deletions in mitochondrial DNA suppressed by mitochondrial polymerases proofreading ability.
Mitochondrial DNA deletions are known to increase in aging and thought to participate in the aging process. Mitochondrial DNA is replicated by the mitochondrial DNA polymerase, which has an intrinsic exonuclease to proofread errors, which allows for high fidelity DNA replication. Researchers at the NIEHS show that disruption this exonuclease activity increases mtDNA deletions by 160-fold. However, disease-associated polymerase variants that reside within this part of the protein did not affect the rate of deletions. Collectively, these results suggest that the exonuclease activity which proofreads single base errors by the polymerase is vital to avoid deletions during mtDNA replication.

Stumpf JD, Copeland WC. The Exonuclease Activity of the Yeast Mitochondrial DNA Polymerase γ Suppresses Mitochondrial DNA Deletions Between Short Direct Repeats in Saccharomyces cerevisiae. *Genetics*, 194: 519-522, 2013.

Enzyme kinetics reveals defects in mitochondrial disease due to *POLG* disease mutations
Human mitochondrial DNA polymerase γ (pol γ) is solely responsible for the replication and repair of the mitochondrial genome. Unsurprisingly, alterations in pol γ activity have been associated with mitochondrial diseases such as Alpers syndrome and progressive external ophthalmoplegia. Thus far, predicting the severity of mitochondrial disease based the magnitude of deficiency in pol γ activity has been difficult. In order to understand the relationship between disease severity in patients and enzymatic defects in vitro, researchers at the NIEHS and Yale characterized the molecular mechanisms of four pol γ mutations, A957P, A957S, R1096C and R1096H, which have been found in patients suffering from aggressive Alpers syndrome to mild progressive external ophthalmoplegia. The A957P mutant showed the most striking deficiencies in the incorporation efficiency of a correct deoxyribonucleotide triphosphate (dNTP) relative to wild-type pol γ, with less, but still significant incorporation efficiency defects seen in R1096H and R1096C, and only a small decrease in incorporation efficiency observed for A957S. Importantly, this trend matches the disease severity observed in patients very well (approximated as A957P $\gg$ R1096C $\geq$ R1096H $\gg$ A957S, from most severe disease to least severe). Further, the A957P mutation conferred a two orders of magnitude loss of fidelity relative to wild-type pol γ, indicating that a buildup of mitochondrial genomic mutations may contribute to the death in infancy seen with these patients.

Sohl CD, Kasiviswanathan R, Copeland WC, Anderson KS. Mutations in human DNA polymerase γ confer unique mechanisms of catalytic deficiency that mirror the disease severity in mitochondrial disorder patients. *Hum. Mol. Genet.*, 22: 1074-1085, 2013.

CD44 adipose inflammation, hepatic steatosis, and insulin resistance.
CD44 is a multifunctional membrane receptor implicated in the regulation of several biological processes, including inflammation. Mice deficient in CD44 were considerably more insulin sensitive and glucose tolerant than WT(HFD) mice and exhibited lower blood insulin levels. These findings indicate that CD44 plays a critical role in regulating several aspects of metabolic syndrome and may provide a new therapeutic target in the management of insulin resistance.

Kang HS, Liao G, DeGraff LM, Gerrish K, Bortner CD, Garantziotis S, Jetten AM. CD44 plays a critical role in regulating diet-induced adipose inflammation, hepatic steatosis, and insulin resistance. *PLoS ONE* 8: e58417, 2013.

MicroRNAs in cancer: The tie that binds

Deficiencies in the ATM gene are the underlying cause for ataxia telangiectasia, a syndrome characterized by neurological, motor and immunological defects, and a predisposition to cancer. MicroRNAs (miRNAs) are useful tools for cancer profiling and prediction of therapeutic responses to clinical regimens. NIEHS scientists investigated the consequences of ATM deficiency on miRNA expression and associated gene expression in normal human mammary epithelial cells (HME-CCs). This study provides preliminary data for defining miRNA profiles that may be used as prognostic or predictive biomarkers for breast cancer. The integrated analysis of miRNA and mRNA expression allowed a better understanding of the signaling involved in breast cancer predisposition and suggests a mechanism for the breast cancer-prone phenotype seen in ATM-deficient patients.

Hesse JE, Liu L, Innes CL, Cui Y, Palii SS, Paules RS. Genome-wide small RNA sequencing and gene expression analysis reveals a microRNA profile of cancer susceptibility in ATM-deficient human mammary epithelial cells. *PLoS One*, 8: e64779, 2013.

Towards understanding how innate immune responses protect us from environmental pathogens.

Cells of the innate immune system are constantly alert for threats from environmental pathogens. As the mechanisms of innate immunity in animals are so tightly conserved, the fruit fly is a useful model system for understanding immune responses in humans. Knowledge of immunity in insects also offers new opportunities for controlling proliferation of those species that impact human health. The current study uses an insect cell model to show that dGBP, a recently discovered immunomodulatory agent from the fruit fly, acts by regulating the inositol phosphate / Ca2+ cell-signaling cascade. This increased insight into the mechanisms of dGBP action opens up new directions for immune research.

Zhou Y, Wu S, Wang H, Hayakawa Y, Bird GS, Shears SB. Activation of PLC by an endogenous cytokine (GBP) in Drosophila S3 cells and its application as a model for studying inositol phosphate signalling through ITPK1. *Biochem. J.,* 448: 273-283, 2012.

Prospero-related homeobox 1 (Prox1) is a novel co-repressor of retinoic acid-related orphan receptors

NIEHS investigators identified Prospero-related homeobox 1 (Prox1) as a novel co-repressor of the retinoic acid-related orphan receptors, RORα and RORγ. Their data suggests that Prox1 is part of a feedback loop that negatively regulates the transcriptional control of clock and metabolic networks by RORs.

Takeda Y, Jetten AM. Prospero-related homeobox 1 (Prox1) functions as a novel modulator of retinoic acid-related orphan receptors α- and γ-mediated transactivation. *Nucleic Acids Res.,* 41: 6992-7008, 2013.

Role for serotonergic pathways in pathophysiology of commonly occurring chronic facial pain condition, temporomandibular disorder (TMD).
Anatomically localized and generalized pain represent clinically meaningful subgroups of TMD and have distinct molecular profiles with correspondingly-distinct genetic backgrounds. A distinct role for serotonergic pathways in pathophysiology of TMD was identified by a new analytic approach that searches for combinations of usually small genetic influences on known cellular signaling pathways. Potentially, this major cellular pathway contributing to development of localized TMD might be effectively treated using serotonin receptor 2 selective ligands. Furthermore, our strategy of genetic modeling using pathway analysis could hold promise for studies of other pain conditions where widespread pain is a significant feature, such as headache, back pain, and fibromyalgia.

Slade GD, Smith SB, Zaykin DV, Tchivileva IE, Gibson DG, Yuryev A, Mazo I, Maixner W, Diatchenko L 2013 Facial pain with localized and widespread manifestations: separate pathways of vulnerability. *Pain*, Epub ahead of print, doi: 10.1016/j.pain.2013.07.009.

Phenotypic characterization of a single-nucleotide variant in a regulatory gene that is predictive of esophagitis and esophageal carcinoma.
New DNA sequencing technologies are beginning to have a significant impact on the detection, management and treatment of diseases, such as inflammation and cancer, that result from interactions between the environment and the genome. Previously, esophagitis and esophageal carcinoma was found to be associated with a single A-to-G nucleotide substitution (or SNP) in the gene encoding PLCε, a protein that normally regulates cellular homeostasis. In the current study, we demonstrate that this SNP is associated with uncontrolled, elevated PLCε activity. Those observations not only rationalize the development of the inflammatory phenotype, but they also validate the predictive reliability of the SNP, and suggest new therapeutic approaches.

Wang LD, Bi X, Song X, Pohl NM, Cheng Y, Zhou Y, Shears SB, Ansong E, Xing M, Wang S, Xu XC, Huang P, Xu L, Wang L, Fan Z, Zhao X, Dong H, Meltzer SJ, Ding I, Yang W. A Sequence Variant in the Phospholipase C epsilon C2 Domain Is Associated with Esophageal Carcinoma and Esophagitis. *Mol. Carcinogen.*, in press

Enhanced Bone morphogenetic proteins (BMP) signaling through BMP type IA receptor in cranial neural crest cells, causes premature suture fusion in mice.
Craniosynostosis describes conditions in which one or more sutures of the infant skull are prematurely fused, resulting in facial deformity and delayed brain development. Approximately 20% of human craniosynostoses are thought to result from gene mutations altering growth factor signaling. This study shows that enhanced bone morphogenetic protein (BMP) signaling through the BMP type IA receptor (BMPR1A) in cranial neural crest cells, but not in osteoblasts, causes premature suture fusion in mice. The finding that relatively modest augmentation of Smad-dependent BMP signaling leads to premature cranial suture fusion suggests an important contribution of dysregulated BMP signaling to syndromic craniosynostoses, and strategies for early intervention can potentially be mitigated by pharmacologic blockade early in their genesis.

Komatsu Y, Yu PB, Kamiya N, Pan H, Fukuda T, Scott GJ, Ray MK, Yamamura K, Mishina

Y. Augmentation of Smad-dependent BMP signaling through a type I receptor BMPR1A in cranial neural crests causes craniosynostosis. *J. Bone Miner. Res.*, 28: 1422-1433, 2013.

Characterization of constitutive CTCF/cohesion loci.
CTCF is a key regulator of mammalian genome. While CTCF has been intensively studied, many functions of CTCF remain unclear. This study systemically investigated the CTCF sites across multiple cell types with respect to their genomic and epi-genomic context and found that CTCF could play a role in maintaining genome domain structure.

Li Y, Huang W, Niu L, Umbach DM, Covo S, Li L. Characterization of constitutive CTCF/cohesion loci: a possible role in establishing topological domains in mammalian genomes. *BMC Genomics*, in press.

New study design to improve the accuracy, reproductability and clarity of studies evaluating the estrogenic activity of endocrine disruptor compounds.
The potential adverse health effects of endocrine-disrupting compounds (EDC) are of great concern globally. A literature overview indicated that low-dose bisphenol A (BPA) animal studies reported inconsistent results and that factors contributing to this inconsistency are the use of high-phytoestrogen diets and the subcutaneous route of exposure. In 44% of all reports, rodents were exposed to BPA via the subcutaneous route and only 20% reported using a phytoestrogen reduced diet. Recommendations are offered regarding the design and conduct of rodent studies evaluating the various effects of EDC. First, experimental diets for studies of potential EDC should contain low or no phytoestrogens, to reduce extraneous variability in estrogen-responsive endpoints. Ideally, an estrogen-free diet, bedding, caging, and water bottles should be used for studies where the primary goal is to determine the estrogenic activity of BPA or other EDC. Second, for animal studies that will provide data for human risk assessment, the route of exposure used in the study should be the same as that for human exposure to the EDC of interest. Third, both the aglycone (active) and conjugated (inactive) BPA contents in serum, urine, feces, and any other relevant tissues should be measured when assessing the effects of BPA in rodent or human studies.

Thigpen JE, Setchell K, Kissling GE, Locklear J, Caviness GF, Whiteside T, Belcher SM, Brown NM, Collins BJ, Lih FB, Tomer KB, Padilla-Banks E, Camacho L, Adsit FG, Grant M. The Estrogenic Content of Rodent Diets, Bedding, Cages, and Water Bottles and It's Effect on Bisphenol A Studies. *J. Am. Assoc. Lab. Anim. Sci*., 52: 130-141, 2013.

Powerful new approach for screening for reproductive toxicity in humans
Exposures to reproductively toxic chemicals can impair human fertility, but measuring exposures to those chemicals can be prohibitively expensive, limiting research in this area. Biostatisticians at NIEHS have developed an approach that calls for pooling biospecimens from sets of participants prior to assay, in studies that screen for toxic effects that prolong time to conception. This new pooling approach can reduce costs considerably while improving statistical power by allowing the inclusion of more couples. By requiring less depletion of nonrenewable bio-specimens it also enables inclusion of more chemicals in the analysis.

Saha-Chaudhuri P, Weinberg CR. Specimen pooling for efficient use of bio-specimens in studies of time to a common event. *Am. J. Epidemiol*., 178: 126-135, 2013.

TRAINING AND MENTORING

The Fellows Award for Research Excellence
The Fellows Award for Research Excellence (FARE) program was started in 1995 to recognize scientific excellence among intramural trainees at all NIH Institutes and Centers. Trainees submit an abstract of their research, which is peer reviewed. The FARE award program is sponsored by the Scientific Directors, the Office of Research on Women's Health, and the Office of Education. Each winner received a $1000 travel award to attend a meeting in the United States at which they presented their abstract, either as a poster or a seminar. FARE winners will be invited also to present their work at one of the FARE poster sessions that will follow each of the Wednesday Afternoon Lecture Seminars in Bethesda, and to serve as a judge for the FARE competition next year.

The NIEHS had 19 winners of FARE awards:

FARE Awardee	Mentor	Fare Abstract Title
Christopher R. Campos, Ph.D	David Miller	Transient barrier disruption increases transport function at the blood-brain barrier
Senthilkumar Cinghu, Ph.D.	Raja Jothi	Meta-analysis identifies key determinants of embryonic stem cell identity and homeostasis
Huaixin Dang, Ph.D.	Anton Jetten	TAK1/TR4 regulates cold induced thermogenesis by inhibiting CREB-PGC1a pathway
Neal A. Englert, Ph.D.	Joyce Goldstein	Epigenetic Modification of Histone (H3) and CYP2C9 Regulation: Involvement of Med25 as the Key Regulator
Bret D. Freudenthal, Ph.D.	Samuel Wilson	The Polymerase Reaction Exposed: Observing a DNA Polymerase Choose Right from Wrong
George J. Fromm, Jr., Ph.D.	Karen Adelman	Pausing of RNA Polymerase II Regulates Mammalian Developmental Potential
Bonnie R. Joubert, Ph.D.	Stephanie London	Maternal smoking and DNA methylation in newborns: An in utero effect or epigenetic inheritance?
Mahita Kadmiel, Ph.D.	John Cidlowski	Glucocorticoid receptor action at the interface with the environment
Nevzat Kazgan, Ph.D.	Xiaoling Li	Intestine-specific deletion of SIRT1 alters systemic lipid and bile acid homeostasis in mice
YuanYuan Li, Ph.D.	Leping Li	T-KDE: A method for analyzing genome-wide protein binding patterns from ChIP-seq data
Kristin N. Lichti-Kaiser, Ph.D.	Anton Jetten	Transcription Factor Glis3 Plays a Critical Role in the Development of Functional Pancreatic beta-cells and Diabetes

Ngome L. Makia, Ph.D.	Joyce Goldstein	Activator Protein 1 Regulation of Human CYP2C9 Expression by Electrophilic Stress Involves MAPK Activation and DNA Looping
Stela Palii, Ph.D.	Richard Paules	Combined disruption of ATM and CHK1 functionalities reveals redundancies in the DNA damage response pathways and results in synthetic growth inhibition following gamma-irradiation
Sabrina E. Robertson, Ph.D.	Patricia Jensen	Developmental origins of central norepinephrine neuron diversity
Lindsay K. Smith, Ph.D.	David Miller	Glucocorticoid Receptor Regulation of P-glycoprotein at the Blood-Brain and Blood-Spinal Cord Barriers
Erica K. Ungewitter, Ph.D.	Humphrey Yao	GLI-similar 3 Maintains Sexually Dimorphic Germ Cell Development in Mouse Embryos
Qingshan Wang, M.D.	Jau-Shyong Hong	Endogenous substance P regulates microglial density in substantia nigra through neurokinin-1 receptor/NADPH oxidase axis-mediated chemotaxis
Jeremy D. Weaver, Ph.D.	Stephen Shears	Kinetic evaluation of an inositol pyrophosphate kinase reveals its signaling credentials.
Gary T. ZeRuth, Ph.D.	Anton Jetten	The Krüppel-like protein Gli-similar 3 (Glis3) functions as a key regulator of insulin transcription

The NIH Pathway to Independence Award (K99/R00)

The Pathway to Independence (PI) Award Program is designed to facilitate receiving an R01 award earlier in an investigator's research career. The primary, long-term goal of the PI Award Program is to increase and maintain a strong cohort of new and talented, NIH-supported independent investigators. The PI Award will provide up to five years of support consisting of two phases. The initial phase will provide 1-2 years of mentored support for highly promising, postdoctoral research scientists. This phase will be followed by up to 3 years of independent support contingent on securing an independent research position. Award recipients will be expected to compete successfully for independent R01 support from the NIH during the career transition award period. The PI Award is limited to postdoctoral trainees who propose research relevant to the mission of one or more of the participating NIH Institutes and Centers.

Mathew Young, Ph.D., received a K99/R00 grant for his proposal entitled "The Roles of Polymerase Gamma Accessory Subunit Gene Mutations in Human Disease." Dr. Young will train in the Laboratory of Molecular Genetics under the mentorship of Dr. William Copeland.

Steven Roberts, Ph.D., received a K99/R00 grant for his proposal entitled "Environmentally-modulated cytosine deamination in genome instability and cancer." Dr.

Roberts will train in the Laboratory of Molecular Genetics under the mentorship of Drs. Michael Resnick and Dmitry Gordenin.

Kathrine Burns, Ph.D, received a K99/R00 grant for her proposal entitled "Endometriosis and Environmental Endocrine Disrupting Chemical Exposure." Dr. Burns will train in the Laboratory of Reproductive and Developmental Toxicology under the mentorship of Dr. Kenneth Korach.

Summer Internship Program Best Poster Awards

NIEHS takes a leadership role in science research and education. Scientists at NIEHS are committed to sharing with students the intensity, excitement, sense of discipline, and tremendous satisfaction that careers in science can impart to those who pursue them. To this end, the DIR established the Summer Internship Program for which internships are given to outstanding high school and college undergraduate students interested in pursuing careers in the biomedical/biological sciences. Participants are selected by intramural scientific mentors and spend between 8 to 12 weeks (during May through September) working on individual research projects that bring them exposure to the latest biochemical, molecular, and analytical techniques. There is a poster session at the end of the summer where participants display the results of their research efforts and respond to questions as though they were participating in a national scientific society meeting. This year the poster session was held on Thursday, July 25, and awards were presented for Best Poster in two categories, High School Interns and Undergraduate Interns. At the Awards Ceremony the following awards were presented:

Best Poster by High School Interns

Forrest Ashworth attends the North Carolina School of Science and Mathematics, Durham, NC, and worked in the Laboratory of Neurobiology under the direction of Dr. Patricia Jensen. Poster: "Isolating the Function of Galanin-Expressing Noradrenergic Neurons of the Locus Coeruleus"

Best Poster by Undergraduate Interns

Spencer Lacy attends North Carolina State University, Raleigh, NC, and worked in the Laboratory of Respiratory Biology under the direction of Dr. Stephanie London. Poster: "*Htr4-/-* Mice Exhibit Altered Lung Function Following Bleomycin-Induced Fibrosis"

Trainee Awards at the Annual Society for the Study of Reproduction Meeting

Five trainees from the Reproductive Developmental Biology Group, under the mentorship of Dr. Humphrey Yao, in the Laboratory of Reproductive and Developmental Toxicology were recognized at the 46th annual meeting for the Society for the Study of Reproduction (SSR) held in Montreal, Canada, July 22-26, 2013. Pre-doctoral trainee Chang Liu won the first place Trainee Research Award for the oral presentation category and postdoctoral fellow Erica Ungewitter won the second place award for the poster presentation category. In addition, Drs. Heather Franco, Chang Liu, and Erica Ungewitter in the group received the Lalor Foundation Merit Award for their abstracts.

The selection process for the Trainee Research Award at the SSR annual meeting is very stringent. Around 900 abstracts from the trainees (pre- and post-doctoral fellows) were initially ranked by external reviewers for each of the thirty research categories. The top 200 abstracts were then chosen by the members of the Award Committee for the second review. Among these 200 abstracts, the Award Committee selected six finalists for the oral presentation and six finalists for the poster presentation for the final round of competition at the meeting. Each of the six finalists for the oral presentation gave a 15 min talk at the opening ceremony of the meeting, in which about one thousand members attended. The posters of the six finalists for the poster presentation competition were shown individually in the front of the poster hall and evaluated by a panel of judges. The judging criteria include merit of the study, presentation format, delivery, visual aids, and response to questions during discussion.